Experimental Design and Investigation of How Cosmetic Proteins Affect Bacteria Found on the Skin

By

Hannah E. Salvage

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Cardiff School of Biosciences
Sir Martin Evans Building
Museum Avenue
Cardiff
CF10 3AX

Abstract

Skin care companies have started to invest in products which are microbe friendly or promote and support 'good' microbes. Cosmetic proteins are proteolysates used in cosmetic formulations for their hydration and moisturization properties. This thesis investigates how cosmetic proteins could affect skin bacteria and the steps required to take the research from *in vitro* to *in vivo*. The preservatives in the cosmetic proteins had varied inhibitory effects on the three chosen species, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Corynebacterium jeikeium*. The three species were also able to utilize the peptides and amino acids found in the cosmetic proteins to aid in both planktonic growth and formation of biofilms. The low pH of the cosmetic proteins controlled *S. aureus* from dominating when in a mixed biofilm culture model. Once skin cells were included in the experiments, it was clear how *S. aureus* elicited an immune response, significantly greater than any of the cosmetic protein were able to elicit. *S. epidermidis* however, was able to reduce the amount of inflammation and stop over proliferation of *S. aureus*. Planning future experiments *in vivo* required investigations of different swab types and buffers for collection of the skin microbiota, as well as choosing suitable swab sites for bacterial studies. The research also found that past cosmetic skin study protocols may not be appropriate for microbial investigations, as the differences in bacterial communities and mass across different skin sites had not been previously considered. In conclusion, in vitro, cosmetic proteins are ingredients which can both promote and harm the bacteria that live on our skin. The amino acids can provide nutrients, while the preservatives have a bacteriostatic or bactericidal effects. Using dry cosmetic proteins would remove the need for preservatives, however this has the potential to cause over-proliferation of undesirable species. Further work using skin panel testing, launching from the final chapters of this thesis, would help conclude whether the answers from the *in vitro* work would be repeated in the *in vivo* experiments.

Contents

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1 Introduction to The Skin, Skin Inhabitants and Cosmetics

1.1 Human Skin

Adult human skin has an average weight of 3.6 kg and covers an area of up to 2 m^2, making skin, not the largest (Sontheimer 2014), but one of the largest organs of the human body (Gravitz 2018). The skin is not only a barrier to physical trauma, but is the first line of defence against pathogenic microorganisms, UV damage and exogenous toxins (Proksch 2008). Other roles that the skin provides are osmotic and temperature regulation, waste disposal and vitamin D synthesis (Tanowitz et al. 1990).

1.2 pH and Temperature

The average pH for skin is around 5 (Lambers et al. 2006), however, the skin is made up of dry, moist or oily regions which causes the skin pH to vary dependent on location (Blank 1938). The most acidic regions of the skin are the scalp and face, these oily areas can have a pH range of 4.2-5.9 (Ali and Yosipov 2013). Moist areas of the skin, including perineal and axilla, are often found to have a pH at the upper end of the scale of around pH 6-8. The dry areas of skin, including the arms, hands, legs and buttocks, tend to stay within the pH range of 5-5.5 (Boer et al. 2016). The varied figures are also reflected in the range of temperatures found on human skin, exposed regions can be as low as 29.5°C, while covered areas and genitals can reach approximately 36°C. On a whole the average temperature of human skin is around 34°C, 3 degrees lower than internal organs (Figure 1) (Wilson 2004).

1.3 Skin Layers and Structure

The three main layers of skin are the top level epidermis, the middle dermis and the lower subcutaneous fatty hypodermis (Figure 2) (Yousef et al. 2020). While these three skin layers are always present, the thickness of skin varies at different locations. The thickest part of the skin is found at the palms of hands, buttocks and at the soles of the feet where the epidermis can reach up to 4 mm thick. The thinnest parts are located on the scrotum and eyelids, where the epidermis is a very thin 0.4 mm (Betts et al. 2016).

The hypodermis is the adipose fat layer which insulates the body, it is also home to large blood vessels and nerves (Wong et al. 2015). The thickness of this fatty layer is mostly dependent on a person's nutritional and fitness levels, once carbohydrate produced energy is used up, the body metabolises into the adipose layer (Lumen 2020). Gender also influences the thickness of these fat layers, with men having a thicker fat level around the stomach and shoulders, while women having the thickest fat layers at the hips, thighs and buttocks (Karastergiou et al. 2012). As an individual ages, this hypodermal layer loses fat which reduces the support of the skin causing drooping. This loss of fat also means that the elderly find it harder to stay warm in the cold, and reduces the ability to sweat so it is also more difficult to stay cool in the warmer weather (Caso et al. 2013).

The dermis lies above the subcutaneous level and is split into two layers, the reticular and

papillary dermis. The lower reticular dermis forms most of the dermis. This layer contains a thicker meshwork of elastic fibres and collagen wads allowing for the flexibility and strength of the skin. Damage to the reticular dermis would cause deeper and longer scarring (Rippa et al. 2019). The papillary is the thinner layer of the dermis, its structure is similar to that of the reticular, but is less bulky and is used structurally to connect to the bottom of the epidermal layer above (Vapniarsky et al. 2015). These two layers are connected throughout by intertwined elastin and collagen created by fibroblasts. This dermal layer also is home to capillaries, smaller nerves, hair follicles and sweat glands (Nafisi and Maibach 2018).

The epidermis itself has four layers; the stratum basale, stratum spinosule, stratum granulosum and stratum corneum (Figure 2) (Kennedy and Wendelschafer-Crabb 1993). Thicker areas of skin have an extra layer below the stratum corneum; the stratum lucidum (Tuomanen 2005).

The stratum basale is the bottom layer of the epidermis, to which the dermis is attached. The single layer is made up of basal cells, the precursor to the keratin producing keratinocytes. These migrate upwards and form 90% of the cells of the epidermal layers. The stratum basale is also home to Merkel cells, which are the touch sensory receptors, and melanocytes, the cell which produce the black/brown pigment melanin. Melanin absorbs UV light and protects the skin from sun exposure (Mo et al. 2019). The stratum spinosum is the next layer of the epidermis and is also referred to as the prickle cell layer. Desmosomes connect keratinocytes and these tight bridges appear ‘prickly’. Langerhans cells are also present at this level, they only make up 5% of the epidermis, but they stretch and cover 25%. Involved in the immunology of the skin, they obtain antigens which are transported to the local lymph nodes and presented to lymphocytes (Mauldin et al. 2016). At the stratum granulosum keratinocytes begin to lose cell structures and organelles, ready to be released as squames at the stratum corneum. In thicker areas of the skin, the stratum lucidum is a layer which helps reduce friction. The final epidermal layer, the stratum corneum, is largely made up of interconnecting dead cells, the final differentiation of keratinocytes, called corneocytes or squames (Tuomanen 2005). These hardy cells are less permeable than their previous form and have a range of uses. They act as an extra layer to protect DNA below the skin from being damaged by UV light. Sebum production at this site is reduced and while many commensals can survive on the minimal nutrients provided by the stratum corneum, oil-loving microbes would not survive for long (van der Krieken et al. 2016). This uppermost layer is shed almost daily, this stops pathogens from successfully attaching, replicating, invading and causing infection (Egelrud 1995).

1.4 Pores and Glands

The surface of the epidermis is scattered with pilosebaceous units. Also known as hair follicles, one of their functions is to produce hair and anchor them deep into the dermal layer (Treister and

Lio 2018). Mammalian hairs are used for thermal regulation, when the body is too cold, hairs trap air against the skin creating an insulating layer (Arens and Zhang 2006). More specialised hair such as eyelashes and eyebrows protect the eyes from dust fragments. Growth of hairs on the human body is an indication of the beginning of sexual maturity (Yu et al. 2014). Many of these hair follicles contain sebaceous glands, which are common across the body, especially concentrated on the scalp and forehead, only absent from palms and the soles of the feet. Sebaceous glands secrete sebum, this lipid rich substance prevents excess moisture loss from both the skin and scalp (Picardo et al. 2009).

The other pores found on the skin are the sudoriferous glands which release sweat. The two types of sudoriferous glands are the abundant eccrine and less common apocrine. The eccrine glands open directly onto the skin through the sweat pore (Tanowitz et al. 1990). The apocrine glands are attached to hair follicles through a duct and are inactive until puberty, it is thought they release sex-related steroids and pheromones. They are only found in the axillae and perineal regions. The hypotonic solution sweat regulates body temperature, disposes of waste and carries mediators of the immune response (Patel et al. 2019).

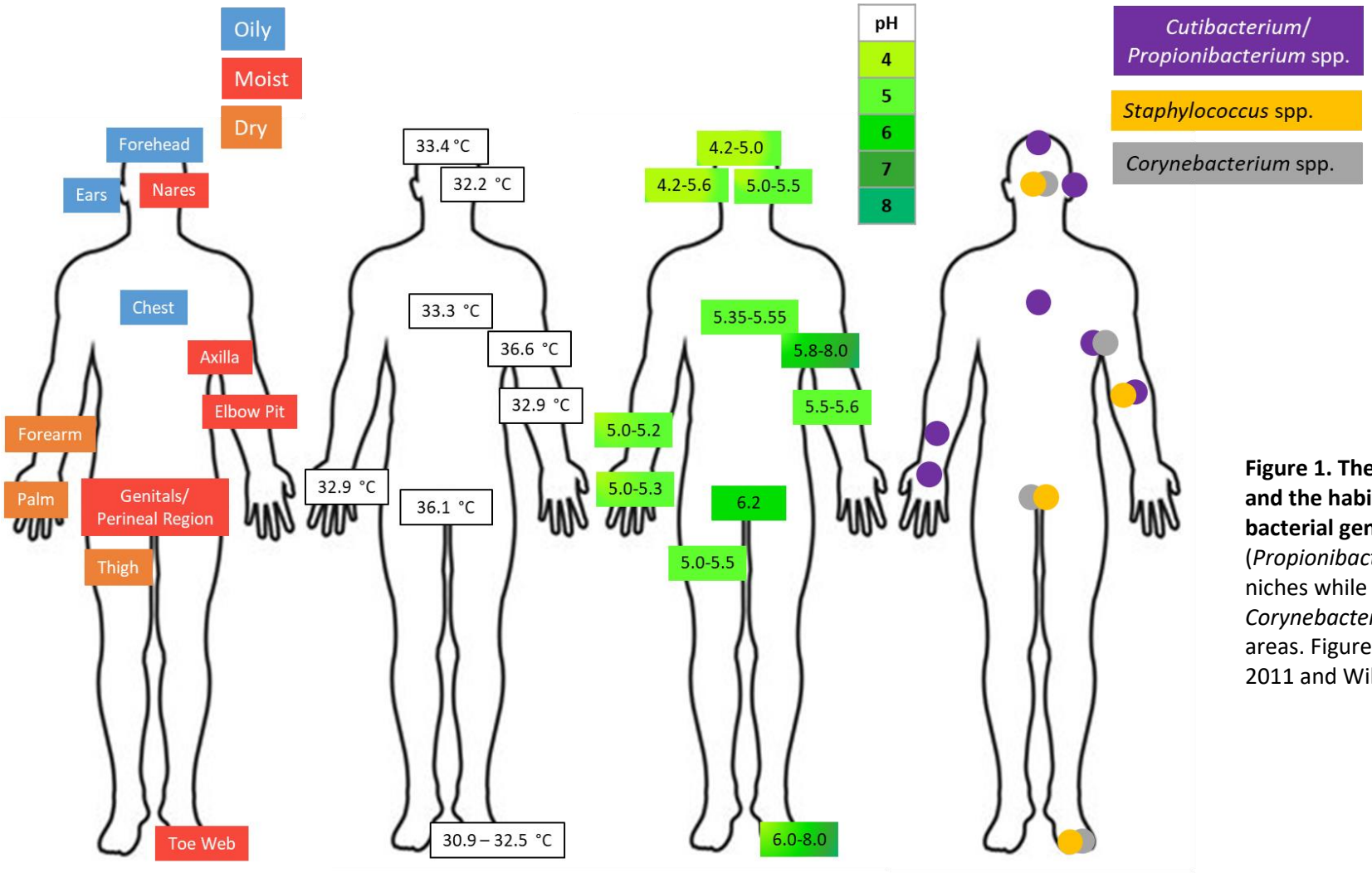


Figure 1. The skin type, temperature, pH, and the habitats of three common bacterial genera. *Cutibacterium* (*Propionibacterium*) occupy the most niches while both *Staphylococcus* spp. and *Corynebacterium* spp. inhabit mostly moist areas. Figure adapted from Grice and Segre, 2011 and Wilson, 2005.

1.5 Skin Immunity

As the first barrier for the body, the skin can suffer serious traumas including wounds, cancers, and pathogenic invasion. Only in the last 30 years has research uncovered that the skin has its own line of defence to handle these stresses (Matejuk 2018). For any type of stress, skin immunity acts in three steps, firstly detection of the problem, secondly destruction of said problem and thirdly, remembering how to handle the problem should it arise again (Pandya et al. 2016).

1.6 Repairing Skin Damage

Wound healing is the regeneration of tissue resulting from an action which has torn the physical skin barrier. The process itself involves many immune factors working together and overlapping phases to ensure that the tissue is repaired, if this is unsuccessful it can lead to potentially fatal infections (Gonzalez et al. 2016).

Macrophages are involved in wound healing; these phagocytic cells release cytokines which start the inflammatory response. To halt the inflammatory stage, these cells activate apoptosis and remove the dead cells (Guo and DiPietro 2010). Peptides such as LL-37, which proliferate at the margins of wounds, activate keratinocytes and their subsequent growth factors to start the wound closure. LL-37 also is involved in angiogenesis and the restoration of blood vessels to the wound (Ramos et al. 2011).

1.7 Dealing with Skin Cancer

White skin is highly vulnerable to both basal and squamous skin cancer and is 50 times more likely to develop these types of cancers than darker skinned ethnicities. Ozone depletion and the subsequent exposure to ultraviolet light is one of the many reasons for the sharp increase in incidences (Diepgen and Mahler 2002). However, without the immune system, the prevalence of these cancers would be even higher (Pandya et al. 2016).

Precancerous cells are often dealt with before being able to proliferate (Pandya et al. 2016), the elimination stage. This stage relies heavily on T-cells being able to identity tumour antigens. If these antigens are not detected then the cancerous cells grow and the immune system can enter the equilibrium state in which harsher measures are needed to rid these cells (Mittal et al. 2014). However, if this stage is bypassed, the cancer can evade the immune system and in fact become tolerated by it (Pandya et al. 2016).

1.8 Defence against Microbes

Immune system specialised cells live throughout the dermal layers. Keratinocytes, which make up 90% of the epidermis, are key to the skin's immunity. These cells have the ability to differentiate between non-pathogenic or pathogenic microbes (Albanesi et al. 2005). Pathogenic microorganisms have numerous infection molecules which come under the umbrella term 'pathogen associated molecular patterns' or PAMPS (Richmond and Harris 2014). Pattern

recognition receptors such as Toll-like receptors (TLR) attach to PAMPS, and this step creates an immune response to the invading pathogen. These receptors work by triggering T-helper cells, which in turn activate lymphocytes. Keratinocytes possess numerous TLRs on their surface, including TLR7 which targets double stranded RNA viruses by stimulating interferons which upregulate the anti-viral cellular defences (Novak et al. 2008). These cells can also produce numerous antimicrobial peptides (AMPs) including cathelicidin, which is effective against a broad number of pathogens (Schittek et al. 2008).

1.9 Skin Microbial Transplants and Bacteriotherapy

Bacteriotherapy treatments are the transference of bacteria from a healthy individual to a patient in the hopes that the microbial composition becomes more akin to a healthy person (Huovinen 2001). This differs from probiotics as these often introduce bacteria which are not normally localised to the original habitat (Shi et al. 2016). Faecal transplants are used commonly to treat recurring antibiotic resistant infections caused by *Clostridiodes difficile* (Mullish et al. 2018). Faecal matter removed from a healthy donor patient can be transferred either via the naso-duodenal route, colonoscopy or ingested in a freeze-dried capsule (Borody et al. 2013; Hecker et al. 2016). Repairing the microbial gut community restores healthy immune-bacterial interactions (Huus et al. 2020). Future potential for this method could be used in the treatment of vastly different diseases, including Type II diabetes (Mullish et al. 2019) and dementia symptoms (Hazan 2020).

The principle behind faecal transplants could therefore be repeated with regards to skin diseases. However, bacterial abundance on the skin is significantly less than that of the gut (Quigley 2013; Coates et al. 2019), and culture methods would be needed to increase the organic material. Adjustment from nutrient rich media to the unnourishing skin environment may prove the transplant to be unsuccessful (Callewaert et al. 2021). When (Perin et al. 2019) attempted to transfer skin from the arm to the back, many of the species that were identified via 16S rRNA gene analysis could not be cultured and therefore were not successfully transferred.

Movement of skin bacteria could cause detrimental results, accidental transferring of pathogenic or unwelcome bacteria. Bacteria originating from the armpit were successfully transferred to the forearm, however the malodour associated with the armpits were now also produced on the arm (Leyden et al. 1981). The reverse of this transfer has also been carried out, where non-smell producing siblings have donated bacteria to the armpits of malodourous siblings and a significant odour reduction did occur (Callewaert et al. 2021).

Like bacteriotherapy, probiotics have been used to introduce ‘good’ bacteria to skin areas populated by pathogenic species. Applications *in vitro* of the lysates of *Vitreoscilia filiformis* (Hendricks et al. 2019) and *Lactobacillus plantarum* (Kim et al. 2015a) to combat atopic dermatitis

have been shown to reduce the symptoms of the diseased areas, although in clinical practice the route is changed to oral administration.

Table 1. Skin Diseases and the Microbes Involved

Disease	Symptoms	Microbes Involved
		Bacteria
Impetigo	Yellow crusty lesions on the skin, very contagious.	[1]*Staphylococcus aureus, Streptococcus pyogenes* (Aly 1996)
Erythrasma	Patches of skin discolouration in areas such as arm pit, groin, and toe webs. Patches turn from red to brown. Skin may be itchy or uncomfortable.	*Corynebacterium minitissium* (Forouzan and Cohen 2020)
Acne Vulgaris	Blocked pilosebaceous units causing inflamed comedones and cysts	*Cutibacterium acnes,* [2]*Staphylococcus epidermidis* (Claudel et al. 2019)
Rosacea	Redness around the cheeks and nose. Little pink or red bumps may appear. Visible Blood Vessels.	*Bacillus oleronius* (Jarmuda et al.)
		Fungi
Seborrheic dermatitis	Scaly patches of dandruff on scalp. Red flaky patches in oily areas of the skin.	*Malessezia furfur*
Tinea Pedis (Athlete's foot)	Scaly, itchy, and burning rash between the toes and on the soles of feet.	*Trichophyton rubrum, Trichophyton tonsurans*
Candidiasis	Redness and intense itching at site. Commonly causes infections in the mouth, vagina and on the skin.	*Candida albicans* (White et al. 2014)
		Viruses
Herpes Labialis (cold sores)	Red blistering around the mouth	[3]*Human alphaherpesvirus 1*
Varicella zoster (Chicken pox and shingles)	Chickenpox – Mainly a childhood disease. Small itchy blisters which start from the trunk and face which then spread to the rest of the body Shingles – Disease usually found in adults who have previously suffered from chickenpox. A rash of blisters is usually localised to one side of the face or body.	*Human alphaherpesvirus 3* (Wu et al. 2020a)
Hand, foot, and mouth disease	Discoloured spots and bumps which may open into sores and blister – Common in children	*Enterovirus A* (Guerra et al. 2022)

[1] Both *S. aureus* and *S. pyogenes* can cause impetigo. Bullous impetigo, which is characterised by large pus-filled blisters is caused by *S. aureus* alone.

[2] *S. epidermidis* has been found in the pores of acne lesions, however little is known about their involvement

[3] While Herpes labialis can be caused by *Human alphaherpesvirus 2*, this species mostly causes genital herpes

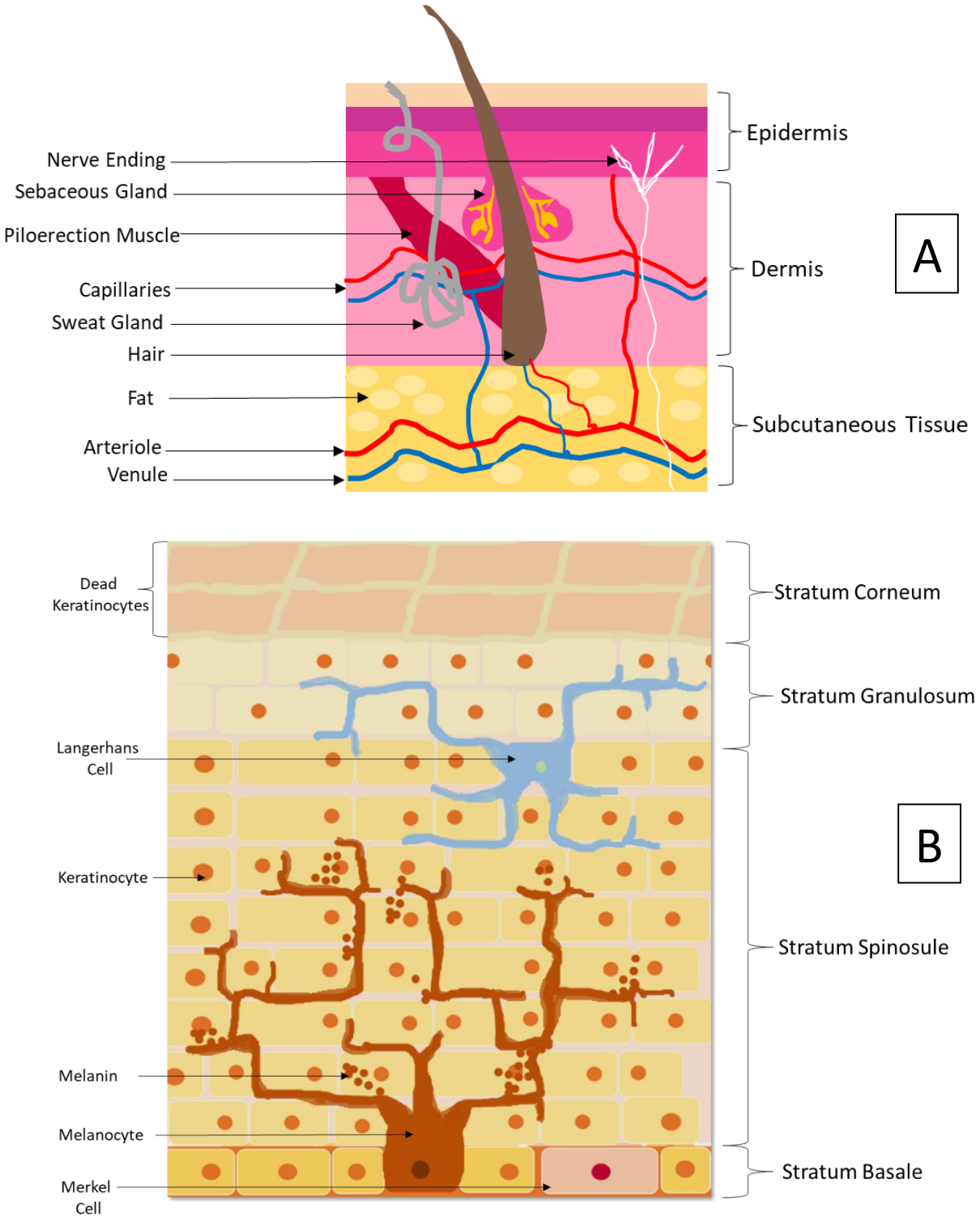


Figure 2. 2A) Layers of the Skin. The skin is made of many layers and components. The subcutaneous tissue layer contains the insulating fat and the blood vessels that supply the capillaries of the skin. The dermis is home to the capillaries and the bottom of the pores, including the hair follicle and sweat gland. The upper most layer is the epidermis which is divided into more layers as seen in 2B. **2B) Layers of the Epidermis.** Keratinocytes migrate upwards through the layers of the epidermis. Starting at the stratum basale where Merkel cells also reside. Merkel cells lay close to nerves and are involved in relaying touch stimuli. Melanocytes start here and entwine through the upper layers. In the stratum spinosule melanocytes release the pigment melanin. Langerhans cells mostly reside here and outstretch through the epidermal layers. These cells are involved in immune presentation of antimicrobial agents. As keratinocytes move through the stratum granulosum, they lose their nuclei and organelles, ready to be shed at the stratum corneum.

1.10 The Skin and Microbes

While the skin has numerous mechanisms to aid the immune system in evading microbial pathogens from occupying and invading the body, the epidermis is home to many microbes who simply reside commensally (Coates et al. 2018). The skin is an ecosystem inhabited by a diverse range of organisms, inclusive of bacteria, viruses, fungi and mites (Grice and Segre 2011). These microbes reside mainly on the surface of the skin and the upper portion of the follicles, but some do extend into the deeper skin layers and sebaceous glands (Zeeuwen et al. 2012). Many of these organisms are classed as commensal species; they reside on our skin with potentially beneficial attributes and only become pathogenic when opportunity presents (Cogen et al. 2008). Colonisation is dependent on various aspects including pH, topography, exogenous and endogenous factors and the immune system response (Grice et al. 2008).

1.11 Mites

Humans are the only mammalian host for two ectoparasitic mites from the genus *Demodex*. The larger of these two species, *D. folliculorum*, are approximately 0.3-0.4 mm long and reside in the base of lashes. The smaller, around 0.15-0.2 mm long, *D. brevis*, in the pilosebaceous unit (Rather and Hassan 2014). These mites feed off dead skin cells and can be problematic when found in high numbers or the immune system is weakened. Facial demodicosis is an uncommon infection that is caused by these parasites. The infection can present with many different symptoms including red skin, raised spots and an itchy rash (Kaur et al. 2012). While there are many speculations as to how these organisms cause such infections such as pore blocking, skin shedding and faecal droppings, the inconsistency in affected individuals suggests that these mites may act as a vector for the rosacea causing bacteria, *Bacillus oleronius* (Gunn and Pitt 2012; Zhu et al. 2018). However, the majority of people living with these parasites on their skin do not have any problems and do not even realise they share their skin with them (Fromstein et al. 2018).

1.12 Viruses

Viruses are not as easy as other microbes to detect on our skin. Microbiological techniques, both culture and culture independent, used to isolate bacteria from the skin are unable to isolate viruses with ease (Kong and Segre 2012). To identify viruses on the skin, transcriptomics and metagenomics sequencing are used as well as imaging of viral-like particles by EM.

However, due to the ever-increasing problem of antibiotic resistant bacterial species, more research is emerging into bacteriophages (bacteria infecting viruses) (Bragg et al. 2014) . Bacteriophages work by entering a host cell and using the bacteria's own DNA and RNA replication system to create more virus instead. The replicated viruses then diffuse out of the host cell, causing lysis and death to the bacteria (Clokie et al. 2011). The virus can also integrate into the chromosome of the cell and become a lysogenic phage. Replicating and passing on to

daughter cells without destroying the host (Kasman and Porter 2020). Many of these species which are becoming less sensitive to antibiotics are skin commensals (Chabi and Momtaz 2019), and therefore bacteriophages which target these species are also likely to be found on skin. Prior work, 'bacteriophage mining' on skin, have revealed that there are virus families present, Siphoviridae, Podoviridae and Myoviridae, which target *Staphylococcus* spp. (Valente et al. 2021). Other bacteriophages identified on skin are known to target *Samonella* spp., *Rhodoferax* spp. and *Mycobacterium* spp. (Wang et al. 2020). However, bacteriophages may collect host genetic material, including any resistance genes, and may aid in transfer of these genes into the next bacterial host. Therefore, these viruses also have the potential to spread antibiotic resistance throughout a community (Principi et al. 2019)

Byrd (et al., 2018) identified four viruses found on human skin, human papillomavirus (HPV), Merkel cell polyomavirus, Polyomavirus HPγV7 and RD-114 retrovirus. HPV is a common human virus as it is associated with cervical cancers. This double-stranded DNA virus is passed through skin-to-skin contact, with around 50% of individuals having contracted the virus at some point in their lives. However, it is often asymptomatic and most immune systems are able to clear the virus (Brianti et al. 2017). Merkel cell polyomavirus is a double-stranded DNA virus that has only come into prominence in the last decade. Humans are commonly infected with the virus where it is most likely to reside on the skin. This type of virus has associations with a rare neuroendocrine cancer (Spurgeon and Lambert 2013). HPγV7 is another common double-stranded DNA virus which is part of the skin microbiota on healthy individuals. This virus is mostly asymptomatic but individuals who have undergone organ transplants have been found to have itchy rashes in which the virus was discovered (Ho et al. 2015). While RD-114 is a feline endogenous retrovirus (Miyazawa et al. 2016), the microbes found on human skin can be influenced by the animals that we live with (Song et al. 2013; Trinh et al. 2018).

1.13 Fungal Residents of the Skin

The slightly acidic pH of the skin allows colonisation by fungal species (Vylkova 2017). The hair and scalp are regions with more genus diversity, but distinct differences exist between healthy individuals and those with skin ailments. In a group of healthy individuals, genera such as *Cryptococcus*, *Acermonium* and *Didymella* were present in high numbers (Findley et al. 2013). However, in scalps which are affected by dandruff, significant quantities of the genera *Penicillium*, *Eupenicillium* and *Filobasidium* were seen (Park et al. 2012).

Fungal infections of the feet are common, one of the most prevalent being Tinea Pedis (Athlete's Foot). This highly infectious fungal disease causes itchy, flaky rashes on the top, soles and toe webs of the feet, but the infection can spread to other areas of the body (Crawford 2009). Three

species of dermatophytes are thought to be involved in the infection, *Trichophyton rubrum, Trichophyton mentagrophytes,* and *Epidermophyton floccosum* (Gupta et al. 2018). However, the feet have some of the greatest fungal diversity with over 11 different genera having been isolated from healthy skin (Findley et al. 2013).

While moist or oily areas have the most varied fungal diversity, the dry and core areas are dominated by one genus (Byrd et al. 2018). *Malassezia* spp. constitute up to 80% of the adult skin fungal species in both culture and culture independent methods (Grice and Segre 2011; Findley et al. 2013). Malassezia is a genus of dimorphic fungi, with more than 90% of the species being lipophilic (Inamadar and Palit 2003). Lipophilic species feed from the fatty acids on the skin and therefore are mostly found in the seborrheic regions of the upper core, hair and face (Grice and Segre 2011). This genus is mostly commensal but can cause superficial skin problems if sebum levels get too high and the organism is allowed to proliferate. The only disease, in which the genus has been conclusively linked to, is Pityriasis versicolor (PV). *M. furfur* is the main pathogen responsible (Young et al. 2015), but other *Malassezia* spp. have been isolated from the infection sites. PV is an infection which discolour patches of skin on the upper core (Inamadar and Palit 2003). Individuals effected mostly live in humid or tropical countries and are typically younger adults who may have excessive sebum or do not wear breathable clothes (Karray and McKinney 2020). Other infections associated with the genus include seborrheic dermatitis and *Malassezia* folliculitis (Thayikkannu et al. 2015). While this genus can cause nasty skin infections, the commensal microbe has some potential benefits. Pityriacitrin is a tryptophan induced alkaloid which reduces the sensitivity of the yeast formations to ultraviolet. While the yeasts can cause PV, (Saunte et al. 2020) and reduce melanin production, these skin sites were also shown to have reduced susceptibility to UV damage (Mayser et al. 2002). Suggesting that pityriacitrin is protecting the skin, not just the organism. (Limon et al. 2017)

1.14 Dominant Skin Bacteria - Phyla and Genera

The most abundant kingdom found on skin is Bacteria (Byrd et al. 2018). The majority of the resident bacteria found on the skin are Gram-positive species, while most Gram-negative bacteria are only present transiently and rarely become part of the resident microbiota (Roth and James 1988). Phyla diversity is low, the three most abundant being Actinobacteria, Firmicutes and Proteobacteria. Contrariwise, the species diversity found at specific skin locations is very high (Grice and Segre 2011). As with any ecosystem, when these bacterial species cohabit peacefully, the skin is healthy. However, if any of the species proliferate and an imbalance occurs, ecosystems become susceptible to bacterial infections (Sanford and Gallo 2013).

Each species of bacteria found on skin occupies a niche and therefore the make-up of the community is dependent on the habitat; these include dry, moist and sebaceous areas of the skin

(Schommer and Gallo 2013). High species diversity is found at the dry areas of the skin, while moist areas are habituated by more specific species such as *Staphylococcus aureus*, *Corynebacterium jeikeium* (Costello et al. 2009). While most of the residents on the skin are relatively benign, some have beneficial attributes. The production of AMPs and antibiotics limit the adhesion and colonisation of pathogens (Gallo 2015). Some bacteria also have the ability to interact with the host immune system, especially regarding inflammation and expression of AMPs and lymphocytes (Cogen et al. 2008). The most prominent species that reside on the skin typically belong to three genera, *Staphylococcus*, *Corynebacterium* and *Cutibacterium* (*Propionibacterium)* (Figure 1)(Dréno et al. 2018).

1.15 Commensal and Pathogenic *Staphylococcus* spp.

Numerous *Staphylococcus* species are found to reside on the skin as part of the natural microbiota (Foster 1996). *Staphylococcus* spp. are Gram-positive, non-motile, facultative anaerobes that form grape-like clusters (Figure 3). Pigmentation is dependent on species, but are most notably yellow or white (Madigan et al. 2014). The two most common species found on skin include the commensal, *Staphylococcus epidermidis* and the pathogenic *Staphylococcus aureus*. *S. epidermidis* can be isolated from almost all body sites, while *S. aureus* resides mainly in and around the nose (Otto 2010), axillae and perineum (Wertheim et al. 2005). Other well-known skin commensal *Staphylococcus* spp. include the 'honeymoon cystitis' causing *S. saprophyticus* (Hovelius and Mardh 1984) and the body odour producing *S. hominis* (Lam et al. 2018).

The golden pigmented *Staphylococcus aureus* (Lowy 1998) is one of the only two species of *Staphylococcus* which produces the extracellular protein coagulase (Foster 1996). The other being *S. intermedius*, an animal facial skin microbe which very rarely infects humans (Kelesidis and Tsiodras 2010). *S. aureus* is carried by approximately 50% of the population; 20% carry the microbe constantly while the other 30% are inhabited transiently (Wertheim et al. 2005). The organism is an opportunist pathogen and resides peacefully with the commensal species when an individually is healthy (Krismer et al. 2017). However, in the immunosuppressed, *S. aureus* can cause several skin ailments (Kobayashi et al. 2015). Individuals can be colonised by both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant (MRSA). MRSA strains are mostly found on oily or moist skin sites, infected wounds and up the nose (Albrecht et al. 2015; Sakr et al. 2018). *S. aureus* is still classed mostly on the side of pathogenic rather than commensal, due to its array of virulence factors which can cause diseases in otherwise healthy individuals (Newstead et al. 2020).

To colonise the skin, *S. aureus* uses fibronectin-binding proteins to adhere to the stratum corneum (Cho et al. 2001). The pathogen also can create pores by binding to the lipid layers in skin and expressing exotoxins (Bhakdi and Tranum-Jensen 1991). Once adhered, *S. aureus* must

avoid the host defence mechanisms. Protein A is a cell wall surface protein which aides *S. aureus* in evading the immune system by binding to the FC region of antibodies, this stops the bacteria being marked for phagocytosis (Kobayashi and DeLeo 2013). Other factors that hinder the immune response include γ-toxin and leucocidin, separate toxins which work together to impair host membranes. γ-toxin produces weak leukotoxins, while leucocidins produce a much more potent form (Foster et al. 2014).

Infections caused by *S. aureus* can be mild and treatable, these include common skin ailments such as impetigo, atopic dermatitis, and abscesses (Leyden et al. 1974; Foster 1996; Ghazvini P et al. 2017). However, complications arising from these can lead to life threatening diseases (Corey 2009). When causing as infection, *S. aureus* produces α-haemolysin, an extremely aggressive toxin which, through mechanisms unknown, can cause sepsis induced organ failure (Bhakdi and Tranum-Jensen 1991; Surewaard et al. 2018). However, *S. aureus* can have beneficial attributes, some produce bacteriocins which are effective against other pathogenic Gram-positives (Gagliano and Hinsdill 1970).

Staphylococcus epidermidis is the non-pigmented (Madigan et al. 2014), commensal cousin of *Staphylococcus aureus* (Otto 2009). It is part of the collective coagulase negative *Staphylococcus* spp. (Otto 2009). *S. epidermidis* is a highly resilient organism which can tolerate environments with both low water activity and high salt concentrations. This bacterium can also grow in a wide range of temperatures, 10°C – 45°C , and at pHs of between 4 – 9 (Wilson 2004). These qualities aide in the colonisation of the skin, *S. epidermidis* is actually the most commonly isolated bacterium from the stratum corneum (Cogen et al. 2008)

Beneficial attributes of *S. epidermidis* include the production of antimicrobials which reduce competition from and colonisation of pathogenic Gram-positive species; these lantibiotics include epidermin and Pep5 (Otto 2010). The lantibiotics primarily work against Gram-positive bacteria, but some activity has been noted against Gram negative bacteria too. These antimicrobial agents work by producing openings in the cytoplasmic membrane, which disrupts the proton motive force (McAuliffe et al. 2001). This disruption prevents adenosine triphosphate (ATP) synthesis (Divakaruni et al. 2014) and therefore reduces the target bacteria's ability to produce energy (McAuliffe et al. 2001).

S. epidermidis is often referred to as a beneficial skin microbe (Nakatsuji et al. 2018), however, the common commensal can cause a plethora of nosocomial infections (Otto 2009). This opportunistic pathogen can cause ailments in the immunocompromised, the microbes low virulence, antibiotic resistance and ability to form biofilms on implanted medical devices make it hard to treat (Blum and Rodvold 1987; Ziebuhr et al. 2006) Planktonic *S. epidermidis* adhere to hydrophobic surfaces, facilitated by adhesins such as AtlE and biofilm-associated pili Ebp. The size

of the biofilm increases through aggregation of cells and becomes three-dimensional as it undergoes maturation (Otto 2009). It is unclear the processes in which *S. epidermidis* biofilm go through maturation, but as with other bacteria it is highly likely quorum sensing is involved (Trautner and Darouiche 2004; Kostakioti et al. 2013). Catheterisation often results in a bacterial infection possible by a range of organisms (Ziebuhr et al. 2006). *Staphylococcus epidermidis* releases urease enzymes which break down urea to ammonium hydroxide and increases the pH of urine. The increased pH causes the release of minerals which crystallise and cause a blockage (Nickel and Costerton 1992). While non-motile itself, *S. epidermidis* can migrate through to the bladder with motile species capable of swarming, such as *Proteus mirabilis* (Sabbuba et al. 2008).

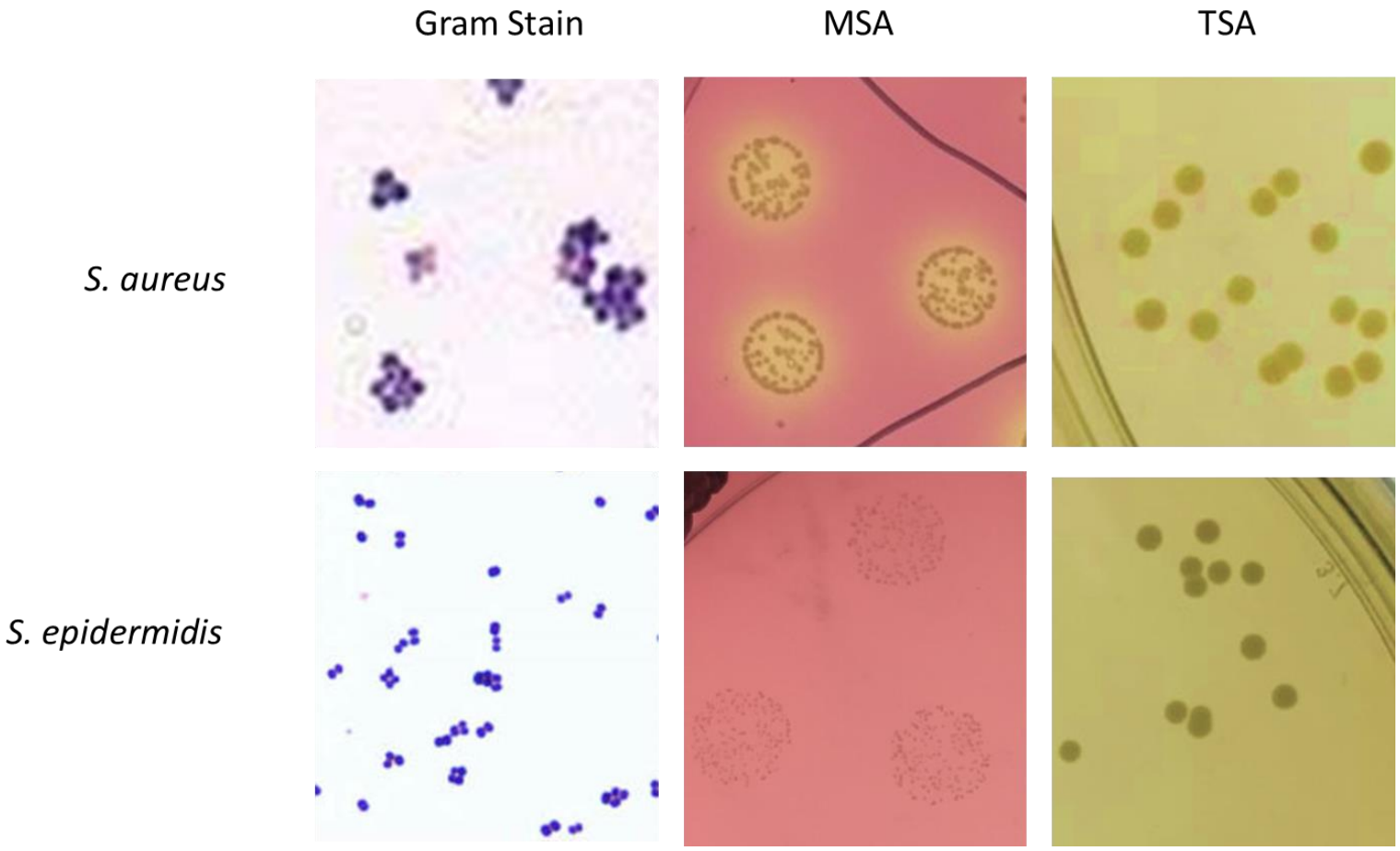


Figure 3. The Gram-Stain of *S. aureus* and *S. epidermidis*, plus Colonies of each on Mannitol Salt Agar and Tryptone Soy Agar. *As Staphylococcus* spp. are gram-positive, they stain purple. They both form clustered groups made up of individual round colonies. While near impossible to tell apart when stained under the microscope, they appear very different when grown on different media types. Both species grow on the mannitol salt agar surface, but only *S. aureus* utilises mannitol, so yellow halos appear around *S. aureus* alone. When grown on TSA, *S. aureus* displays its namesakes colouring of large gold colonies. While *S. epidermidis* colonies are smaller and white.

1.16 Skin Dwelling Coryneform

Coryneform is a term which encompasses non-acid fast, non-spore forming, non-branching, Gram-positive, bacillus-shaped bacteria (Funke et al. 1997; Wilson 2004). They also have pleomorphic abilities (Brabb et al. 2012) which allows them to change their shape or size in response to their habitat (Wainwright 1997). However, the genus *Corynebacterium*, covers bacteria with the aforementioned morphological properties along with other attributes such as being non-motile, catalase-positive (Brabb et al. 2012), arabinogalactan containing cell walls and having a guanine-cytosine content of 46-74 mol% (Wilson 2004). There are over 80 known species of Corynebacterium, although only a small few are part of the skin commensals including *Corynebacterium amycolatum*, *Corynebacterium jeikeium* and a group of cutaneous lipophilic *Corynebacterium* spp. (Bernard 2012). These skin species are all halophiles, except *C. jeikeium*, and can be facultative or obligate aerobes (Riegel 1998; Ventosa et al. 1998; Wilson 2004). *Corynebacterium* spp. occupy the moist areas of the body and are thought to be involved in breaking down sweat and releasing the subsequent odour (Grice and Segre 2011; Troccaz et al. 2015). *Corynebacterium* spp. require amino acids as a nitrogen and energy source but lack extracellular proteases necessary to hydrolyse proteins. It is likely that these bacteria utilise the by-products from other skin bacteria breaking down larger proteins (Kwaszewska et al. 2014).

While not part of the skin microbiota, the pathogen *Corynebacterium diphtheriae* releases an exotoxin which causes the respiratory and skin disease Diphtheria. It is often isolated from abscesses formed on alcoholics, intravenous drug users and individuals who live in poor areas with limited access to hygiene (Murphy 1996). A vaccine has successfully reduced outbreaks in first world countries, but the disease is still prevalent in the developing world (Cogen et al. 2008).

Corynebacterium jeikeium is a species which is commonly isolated from the skin (Cartwright et al. 1993; Cogen et al. 2008). Unlike other *Corynebacterium* spp., *C. jeikeium* is an obligate aerobe and is unlikely to be found in pores or follicles due to the competition for the reduced oxygen (Ifantidou et al. 2010). It is a species that is lipophilic and requires a fat source to grow, when the organism is cultured, a lipid substitute is added to the media, Tween 80 is commonly used between 0.1 and 1% v/v (Kim et al. 2015b). *C. jeikeium* is another well-known opportunistic pathogen that often infects the immunocompromised. Like *Staphylococcus epidermidis*, *Corynebacterium jeikeium* can be highly problematic in a clinical setting and can form antibiotic resistant biofilms, often on medically implanted devices (Soriano et al. 1993; Ziebuhr et al. 2006). Complications arising from these biofilms can lead to sepsis and endocarditis (vanderLelie et al. 1995). *C. jeikeium* also has some beneficial attributes; the bacterium acquires manganese to protect itself from superoxide free radicals and in the process may protect the host from them too (Tauch et al. 2005; Balamurugan 2019). Although under-researched, *Corynebacterium*

jeikeium release compounds which are not too dissimilar from known bacteriocins to which pathogens are susceptible (Tauch et al. 2005; Cogen et al. 2008).

1.17 New Classification for the old *Propionibacterium* spp.

Propionibacterium was a genus in which four species were commonly found on the skin, *P. acnes, P. granulosum, P. avidum* and *P. propionicum* (Gharamti and Kanafani 2017; Corvec 2018; Suzuki et al. 2019). However, whole genome sequencing highlighted the inaccuracies of the previous 16S rRNA gene sequencing and thus three of the species were placed into the new genus, *Cutibacterium* and the fourth into another new genus *Pseudopropionibacterium* (Scholz and Kilian 2016).

The *Cutibacterium* gen. are a group of non-motile, non-sporing, fermentative, Gram-positive rods. These species range from obligate and facultative anaerobes to microaerophilic; explaining why they can be found both on the surface and within pores of the skin (Wilson 2004). Both *Cutibacterium granulosum* and *Cutibacterium acnes* are follicle dwellers, while *Cutibacterium avidum* is found in the axillary regions, where sweat is abundantly produced (Corvec 2018). While commonly found on adult skin *Cutibacterium* spp. are rarely seen on prepubescent children (Chomnawang et al. 2005). The *Cutibacterium* genus adapt to living on the skin by growing at a range of pHs, optimally between 5.5 and 6. They also produce numerous lipases which release fatty acids from lipids to use as carbon and energy sources (Brzuszkiewicz et al. 2011).

The most well-studied species, *Cutibacterium acnes,* was isolated from an acne vulgaris region over 100 years ago and has been associated with disease ever since (Bojar and Holland 2004). Acne vulgaris is a very common skin ailment and a large proportion of the population will have had it in their teenage years (Bhate and Williams 2013). The condition results in numerous closed (whiteheads) and open (blackheads) comedones caused from blockages of the pilosebaceous units (Izumi et al. 1970). While this ailment is not life threatening, it can make sufferers feels embarrassed and self-conscious (Kellett and Gawkrodger 1999) and extreme cases can lead to facial scarring (Fabbrocini et al. 2010). The cause is still not clear, but it is thought that as puberty onsets, an influx of sebum in the follicles causes a proliferation of *C. acnes*. The immune system responds to the proliferation and the pores inflame and fill with white blood cells (Vary 2015). It is disputed that *C. acnes* is not the sole cause and that other species which co-occupy acne lesions, including antibiotic resistant strains of *S. epidermidis*, may be involved (Nishijima et al. 2000).

Although *C. acnes* has pathogenic potential, and has been the target of antibiotics for acne treatment (Leyden and Del Rosso 2011), it is in fact a commensal and has a range of advantageous properties. The species stimulates the sebaceous glands into releasing sebum, *C. acnes* then break down the sebum which releases free fatty acids onto the skin thus lowering the pH of the skin

(McLaughlin et al. 2019). This lower pH stops colonisation from pathogenic species which often prefer more neutral pHs (Grice and Segre 2011).

Multiple strains of *C. acnes* also release an enzyme which converts linoleic acid into conjugated linoleic acid (CLA) (Liavonchanka et al. 2006). These isomers have been shown to have some capabilities in reducing tumour formation by triggering apoptosis (Lee et al. 2005). Many *C. acnes* strains also release bactericidal chemicals such as acnecin which inhibits other species and strains of *C. acnes* without the acnecin producing gene (Fujimura and Nakamura 1978).

1.18 Splitting of the *Micrococcus* Genus

Micrococcus is a Gram-positive genus that is associated with the human skin, these catalase-positive, non-sporing, cocci are not unalike in appearance to *Staphylococcus* spp., but are genetically dissimilar (Becker et al. 2015) and unlike *Staphylococcus* spp., *Micrococcus* spp. are resistant to the bacterial enzyme lysostaphin (Geary and Stevens 1986). *Micrococcus luteus* is the most clinically important of the species and has been isolated from the head, legs, and arms (Kloos and Musselwhite 1975). The small, bright, yellow colonies are a frequent contamination from the skin and surroundings when aseptic techniques are poor (Hall and Lyman 2006; Hetem et al. 2017). The species is tolerant to a range of temperatures and high salt concentrations (Chan and Leung 1979), this tolerance and their ability to create nutrients from bacterial metabolic waste, make them ideal skin dwellers (Wilson 2004). While not an issue for healthy people, *Micrococcus luteus* can cause problems in the immunocompromised and is often the source of cutaneous infections in HIV infected individuals (Smith et al. 1999). Teichuronic acids found in the cell walls of *M. luteus* are involved in activating immune responses, including tumour necrosis factors (Yang et al. 2001) which assign cell death (Chu 2013). In 1995, *Micrococcus* was split into five separate genera after 16S rRNA gene analysis and chemotaxonomic tests highlighted differences (Ekkelenkamp et al. 2010).

Three previous *Micrococcus* spp. have now been placed into the genus *Kocuria* (Stackebrandt et al. 1995). *Kocuria rosea* (formally *M. rosea*) is another classic species which resides peacefully on the skin until given an opportunity to become pathogenic (Altuntas et al. 2004) As with other commensal Gram-positives, this *K. rosea* can cause serious illness in individuals with underlying health problems or people who have had implanted medical devices (Dunn et al. 2011). Although unlike other opportunistic skin pathogens, *K. rosea* has not been noted with the ability to produce biofilms and therefore it is suggested that this species causes illness by entering the bloodstream and causing chronic bacteraemia (Purty et al. 2013). *Kocuria varians* is the most frequented skin isolate from this genus and have been noted to be able to cause relapsing infections deep into the tissue (Dotis et al. 2015). Two strains, isolated from meat in a salami factory, have been shown to produce heat and pH resistant antimicrobial chemicals which are effective against a broad range

of Gram-positives (Gálvez et al. 2007). Although not skin isolates, it is likely that they will produce similar bacteriocins to protect themselves.

1.19 Do Gram-negative skin commensals exist?

The debate as to whether Gram-negative bacteria are skin commensals or transient visitors is ongoing, but their numbers are significantly less than Gram-positive species (Evans et al. 1950; Wilson 2004; Grice and Segre 2011; Myles et al. 2016). Resident Gram-negative bacteria are only found in the moist, more pH neutral areas such as the toe web or axillae (Davis 1996). The combination of dry and acidic proves the skin too harsh of an environment for the majority of Gram-negative bacteria (Chiller et al. 2001). However, culture-independent techniques have revealed that skin dwelling Gram-negative species may be more prevalent than originally proposed (Conlan et al. 2012). More thoroughly developed culturing methods for these species have been established for future works (Myles et al. 2016). The prevalent skin genera are the enteric, *Enterobacter*, *Klebsiella*, *Escherichia*, *Proteus* and soil dwelling, *Acinetobacter* (Davis 1996). The common gut residents are also regularly found around the anal regions, where there is a possible a lingering faecal veneer (Ki and Rotstein 2008). However, Gram-negative bacteria can be highly prevalent on people with poor hygiene or skin infections (Wassilew 1989; WHO 2009; Tang et al. 2020). Over treatment of chronic skin infections may also lead to over-growth by Gram-negative bacteria and the prevalence of harsher skin ailments (Böni and Nehrhoff 2003)

1.20 Commensal or Pathogen? It Isn't Straightforward

Bacteria that reside on the skin can be placed into beneficial, commensal, or pathogenic categories. In reality the beneficial or commensal species have the potential to cause skin infections when given the opportunity (Grice and Segre 2011). Equally, species which are classed as problematic can live harmlessly on the skin with other microbes and can alert the immune system to invasion from more pathogenic organisms (Krismer et al. 2017).

1.21 Changes in Skin Bacteria from Birth

Initial colonisation starts pre-birth. Microbes originally thought to be from the uterus, placenta, umbilical cord, and amniotic fluids have been isolated from new-borns. However, the mass colonisation starts with delivery and is dependent on both method and whether the baby has reached term (Dunn et al. 2017). Immediately after birth, babies delivered by vaginal delivery are covered by *Lactobacillus* spp., the genera responsible for maintaining a healthy vagina (Zapata and Quagliarello 2015). C-Section babies, however, are dominated by *Staphylococcus* and *Corynebacterium* spp., as would be common on their mother's skin and in hospital settings (Neu and Rushing 2011). The difference in skin microbiota, aided with an under-developed immune system (Shao et al. 2019), may explain why C-Section babies are more likely to acquire nosocomial infections (Miller et al. 2020). Preterm babies differ from full term babies, they possess large abundancies of *Staphylococcus* spp. and gut associated Gram-negative bacteria (Younge et al. 2018). At around three months of age, birthing route differences in skin microbiota begin to dissipate, skin species are no longer present all over the body, but start to become site-specific (Zhu et al. 2019; Skowron et al. 2021).

The skin microbiome of children is vastly different to those of adults, with the most significant difference being around oily areas like the nose (Oh et al. 2012). However, Zhu (et al., 2019) found that both the mode of delivery and the mother's own bacterial community do have an influence on the facial microbes in children even as old as ten. *Streptococcus mitis* and *Streptococcus pseudopneumoniae* are two species found on children's skin which are not commonly part of the adult commensal microbiota (Oh et al. 2012) Once the body starts to undergo puberty however, the increased production in sweat and sebum causes a proliferation in more adult skin organisms, such as *Cutibacterium* spp. (Bhatia et al. 2004; Ellis et al. 2019). This increase in sebum aides in pH reduction of the skin, thus inhibiting neutral pH loving staphylococcal and streptococcal spp., genera which are found more abundantly on children (Oh et al. 2012).

When adulthood is reached, the microbes on the skin reach an equilibrium tailored to that individual (Oh et al. 2012), although hormonal changes and lifestyle choices like smoking or drinking alcohol, can disrupt the equilibrium and cause microbial imbalances (Oh et al. 2012; Wallen Russell 2018; Bagatin et al. 2019; Dimitriu et al. 2019). For instance, cigarette smoke may even be able to promote and increase antibiotic resistance in MRSA by stressing *S. aureus* into more frequent mutations (Lacoma et al. 2019). While *Corynebacterium* spp. could be used as a biomarker for obesity related diseases (Brandwein et al. 2019).

Like children, the elderly produce less oil and sweat than adults (Wu et al. 2020b) and their lack of microbial diversity reflects this (Rodrigues Hoffmann 2017). The microbial skin colonisation of elderly people is dependent on numerous factors including home residence (Grice and Segre

2011). Many of the older society live in care homes, where full showers are less frequent and bed baths are a common way of washing. (Groven et al. 2017). Therefore the skin of people residing in nursing homes are more likely to harbour gut residing Gram-negative bacteria, drug resistant microbes and an abundance of bacteria responsible for nosocomial infections (Roghmann et al. 2017) .

1.22 Skin Microbial Diversity of the Western World

In the modern Western world there has been an increase in cases of skin diseases, which had been much less prevalent in the previous centuries (Chu et al. 2017). Antibiotics have been the saviour of humans and cured a multitude of infectious diseases, and allowed many surgical procedures to be performed, including bone marrow and solid organ transplants (Adedeji 2016). Preservatives have been very useful in preventing products in both the food and cosmetic industry from spoiling (Parke and Lewis 1992; Halla et al. 2018). However, compliant use and the broad range of these antibiotics and preservatives could have reduced the number of helpful bacteria that reside on our skin (Llor and Bjerrum 2014; Langdon et al. 2016). Another possibility includes changes in the way westerners go about their daily lives. Sedentary jobs in the cities are now more common than physical jobs in agriculture or the fishing industry (Uhlig 2003; Seafarers 2018; Harrison 2019; Trussler 2019).

This theory that our western lifestyles have contributed to a change of residential skin microbiota is highlighted by studies in which the skin community of isolated rural human populations was analysed. Communities in which hunting and gathering are still the means to collect food and whose bodies have been untouched by modern day antibiotics, possess skin bacterial species unseen on urbanised populations (Blaser et al. 2013). Societies in which agriculture is still a key part of life also, unsurprisingly, have been found to harbour higher levels of soil dwelling associated bacteria on their hands. Even in western communities, individuals exposed to a farming or rural lifestyle possess differing microbial skin communities than those brought up in a more urban existence (Parajuli et al. 2018; Steiman et al. 2018)

All of these studies tie in with the ‘hygiene hypothesis’, in which our extra clean lifestyles may have abetted changing the microbes which reside on, and in our bodies (Okada et al. 2010).

1.23 Battle of the Sexes

Women and Men have differences between the topographical, physiological, hormonal and the pH of their skin (Giacomoni et al. 2009) and the differences in their skin microbiota reflect this. However, many studies are contradictory as to which gender has the most microbial skin diversity (Fierer et al. 2008; Song et al. 2013; Ma and Li 2019). Women have been found to harbour less bacterial diversity than men in several studies. The most significant difference being around the thighs, groin, and axilla (Marples 1982; Ma and Li 2019).

Fierer (et al., 2008) found that women's hands had a more diverse microbial content than men. This study stated that women's higher pH may allow for more species colonisation. While genera on the hands of males and females are not significantly different, the abundance of these genera is. Women tend to have a higher proportion of gram-negative bacteria associated with their genitalia, while men have more 'classic' skin genera like *Corynebacterium* and *Cutibacterium.* Song (et al., 2013) echoed Fierer, and found that women have a higher microbial diversity. They also found that cohabiting couples of different sexes have similar microbiota and that the couples could be correctly identified by simply examining their microbial communities.
Any differences between men and women could be down to numerous causes (Giacomoni et al. 2009; Wallen Russell 2018). The way that individuals of the same gender have significantly different microbial skin communities (Leung et al. 2015) ensures that it is near impossible to pinpoint one reason alone (Grice and Segre 2011).

1.24 Cosmetics

Cosmetics have been used in many cultures throughout the ages. The ancient Egyptians used cosmetics to satisfy their extremely hygienic ethos and wore cosmetic make-up at celebrations (Hardy and Rollinson 2012). Until relatively recently, cosmetics were a luxury that the upper classes could afford. Modern day cosmetics are mostly used for hair removal, personal hygiene, improvement of skin health and appearance, inhibiting the signs of aging and enhancing or diminishing features. These products include, but are not limited to, deodorants, moisturisers, hair washing and styling, body washes, make-up and perfumes (Draelos 2014). As these products are designed to be used on or near the body, so the formulations and ingredients are vigorously tested to ensure that they are not harmful or damaging to the skin (Walker et al. 1996). However, only recently have cosmetics been tested on how they interact with skin microbiota. Many ingredients found in cosmetic products have the potential to either enrich or cause stress to the microbes (Dobler et al. 2019).

1.25 Probiotics

Past studies of cosmetics and microbes have focused on the use of probiotics and prebiotics. Probiotics are used to introduce helpful microorganisms into an environment, while prebiotics are used to promote 'good' microbes which already reside (Kechagia et al. 2013). Products which claim to possess probiotic and/or prebiotic properties are mostly 'leave-on' formulations targeting the face (Warming et al. 2018), although skin probiotics are also often ingested (Kober and Bowe 2015). These kinds of products have been met with scepticism in the past (Reid 2005). For example, *Lactobacillus* is a genus which is often endorsed as an excellent all round body probiotic (Rossi et al. 2019). Research into *L. rhamnosus* lysates usage have reported that tight junction proteins are further expressed and that skin sensitivity to surfactants have been reduced (Jung et al. 2019). However the additional promotion of phagocytes, in turn, could actually induce skin

diseases (Ouwehand et al. 2010). However, there has been studies into compounds commonly found in cosmetics and their interactions with a range of microbes.

1.26 Emulsifiers and Surfactants

Emulsifiers are used as stabilisers in cosmetic formulations to stop separation of oil and water. Polysorbates, or Tween are a derivative of ethoxylated sorbitan. They are common emulsifiers and act as non-ionic surfactants in formulations as they are mild on skin. Previous studies have shown how polysorbates affect a range of bacterial species. Polysorbate 80 and 20 have been show to disperse *in vitro* biofilms of common gut bacteria (Sloup et al. 2016). However, the opposite is true for *S. aureus*, in which the addition of Tween 80 to the growth media causes increased biomass for biofilm formation (Nielsen et al. 2016). These polysorbate groups are a common addition to growth media to allow lipid loving bacteria to have a fat source, including common skin species *Cutibacterium acnes* and *Corynebacterium jeikeium* (Ray and Kellum 1970; Chevalier et al. 1987). Another widely used emulsifier/surfactant used in cosmetics is lecithin (Figure 4) and hydrogenated lecithin (Fiume 2001). The amphiphile is found in all living organisms, but plant-sourced lecithin is in higher demand, mostly due to the diminished role of animal derivatives in cosmetic formulations (Kabene and Baadel 2019; Partridge et al. 2019). The stabilising agent can form viscous gels which can easily deliver formulations onto the skin (Attar Nasseri et al. 2010; Zhou et al. 2010; Raut et al. 2012). However, previous *in vitro* studies have noted that using 2% purified bovine lecithin in media can support the growth of *Cutibacterium acnes*, while showing inhibitory effects to *Staphylococcus aureus* (Kabongo et al. 1982).

1.27 Preserving Measures

Liquid ingredients or formulations, require protection from microbial spoilage to increase the lifespan of the cosmetics (Panico et al. 2019). Preservatives are used throughout the cosmetic and food industry to prevent contamination without causing unpleasant tastes or harm to the consumer or user (Halla et al. 2018). Many of the organisms that cause bacterial spoilage are also bacteria that are naturally found on skin, thus simply lowering the pH of the formulation is not always sufficient (Varvaresou et al. 2009; Halla et al. 2018).

Other tactics include reducing the water activity (aw) by the addition of sugars, salts, or proteolysates (Hahn-Hägerdal 1986). Most organisms require a water activity of 0.9 or above to be able to grow, reducing the water activity limits the bacterial spoilage (Table 2) (FDA 1984-2014). However, the addition of too many sugars or salts will affect the taste of food, and water activity is key component in cosmetic products. This type of preservation also opens up contamination by halophilic or osmophilic microorganisms (Todar 2015). Another common way of preserving foods or cosmetics is the addition of either natural or synthetic compounds which have specific antimicrobial properties (Juhász and Marmur 2014; Pandey et al. 2016). Synthetic

preservatives cover a range of chemical types including, but not limited to, organic acids, formaldehyde releasers and aromatic alcohols (Halla et al. 2018) (See Table 3 for examples and mechanisms). Although all possess antimicrobial activity, using two or more together increases the spectrum of spoilage targets. Many preservatives are also often paired with the chelating agent Ethylenediaminetetraacetic acid (EDTA) (Izzat and Bennett 1979). EDTA is used to improve the efficacy of preservatives by reducing the integrity of the cell membrane (Finnegan and Percival 2015).

Societal pressures are now causing cosmetic companies to step away from synthetically produced preservatives and be replaced with naturally derived antimicrobial ingredients (Vergis et al. 2015). Essential oils are secondary metabolites produced by aromatic plants (Guerriero et al. 2018). Originally used for cosmetic properties, these oils are packed full of antimicrobial terpenes, hydrocarbons, lipids and phenyls (Swamy et al. 2016). Although they are antimicrobial, studies have shown that essential oils' preserving properties are only comparable to synthetic compounds when used in high concentrations (Dreger and Wielgus 2013).

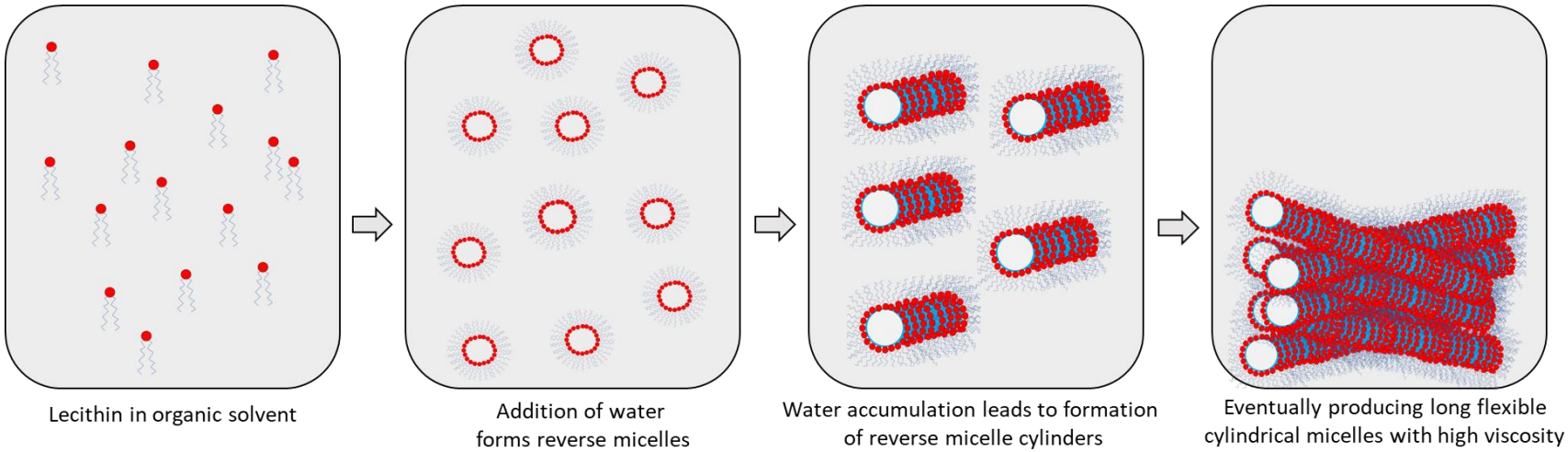


Figure 4. Formation of Lecithin Gel. The addition of water to free lecithin in organic solvent causes the formation of reverse micelles. Further addition of water leads to cylindrical reverse micelles, eventually leading to long flexible cylinders forming a compound trapping gel.

1.28 Cosmetic Proteins

Highly adept at binding water to the skin, cosmetic proteins are used for their moisturising properties and often marketed for their 'regenerating' and 'rejuvenating' qualities (Prokopowicz and Różycki 2017). Acid hydrolysis is commonly used to split the insoluble native protein into mixture of soluble amino acids and peptides (Secchi and Teglia 1999). This type of hydrolysis, however, can cause the loss of amino acids cysteine and tryptophan as well as increasing the chances of racemisation (Burnett et al. 2014). An alkaline process for hydrolysis utilises hydroxide variants at high temperatures to split the protein, however this method can cause a high percentage loss of amino acids (Hou et al. 2017). Both acid and alkaline hydrolysis are cheap ways to split proteins, but amino acid return can be poor. Enzymatic hydrolysis can use individual or mixed enzymes to cleave proteins into peptides and amino acids, thermal denaturation of the enzymes are often required to limit further cleavage and damage of the desired product (Verzeaux 2021). Powdered cosmetic proteins have negligible water activity, but liquid protein complexes (lpcs) are vulnerable to spoilage and therefore are preserved with synthetic preservatives (Scibisz et al. 2008).

The hydrolysed proteins can come from numerous animal or plant sources, and less commonly algae or fungi (Secchi 2008). Cosmetic proteins originating from animal sources include collagen, keratin, elastin and silk derivatives (Secchi and Teglia 1999). These can provide amino acids, such as proline, which is often marketed to aid in the healing of skin (Trookman et al. 2009; Szpak 2011). However, many cosmetic companies are moving away from using animal products and so the use of animal derived proteolysates are running out of favour (Secchi 2008). Plant proteins are concentrated in the seeds (Nehete et al. 2013). The amino acid make-up of plant proteins differs significantly from animal proteins. Therefore, these hydrolysed proteins can deliver exogenous amino acids which humans cannot synthesize, but can lack other useful amino acids, such as proline (Scibisz et al. 2008).

As the composition of these cosmetic proteins include amino acids and/or peptides, the use of cosmetic proteins could both benefit or hinder the natural microbiota found on skin (Holland and Bojar 2002). Amino acids are used by bacteria as a nutritional source of nitrogen and for protein synthesis. The addition of amino acids may go further than simply providing sustenance. Introduction of proline onto the skin could be valuable to bacteria undergoing osmotic or saline imbalances, the stocking of this amino acid protects cells from these physical stresses (Liang et al. 2013). The uptake of amino acids by *Staphylococcus aureus* and subsequent synthesis of ammonia offsets any pH decreases, thus allowing formation and sustaining of biofilms (Ammons et al. 2014). Bacteria enveloped by keratinocytes may have the potential to steal and uptake essential amino acids to extend their life before apoptosis occurs. While beneficial for the bacteria, the

amino acids now unavailable to skin cells could cause disruption to the host cell's metabolism pathways (Chi et al. 2013; Arimoto et al. 2017)

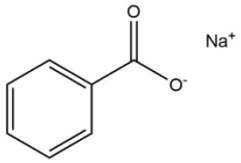
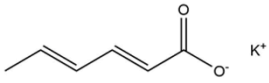
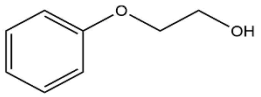
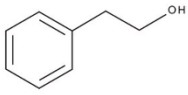
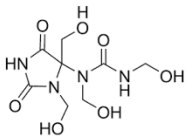
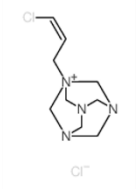
In contrast, the use of cosmetic proteins could negatively affect the resident bacteria and their virulence factors. Several D-amino acids, have been shown to be able to reduce the attachment and biofilm formation of bacteria, including *S. aureus* (Kolodkin-Gal et al. 2010; Hochbaum et al. 2011; Ampornaramveth et al. 2018). Proteolysates reduce the water activity of a formulation, therefore it is possible that this could lower the water activity of skin (lbèr et al. 2014). This could potentially disrupt the balance of the skin microbiota as different species require different water activity levels (FDA 1984-2014; Rahman 2009; Ijabadeniyi and Pillay 2017).

Table 2. The Lowest Water Activity (a_w)[4] Level in which Select Bacteria Species can Grow.

Lowest Water Activity (a_w) for Growth	Bacteria Species
0.97	*Pseudomonas fluorescens*
0.94	*Clostridium botulinum* A
0.93	*Bacillus cereus*
0.91	*Bacillus subtilis*
0.90	*Staphylococcus epidermidis*
0.87	*Staphylococcus aureus*
0.85	No Bacterial Growth

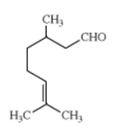
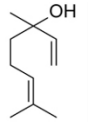
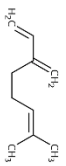
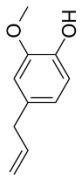
[4] Water activity (aw) is defined as the ratio of the partial pressure of water in the atmosphere in equilibrium with the substrate to that of the atmosphere in equilibrium with pure water at the same temperature and is expressed on a scale of 0 to 1 where 1 is pure water. Most bacteria require a water activity level of above 0.9 to be able to grow.

Table 3. Molecular Structure, Susceptible Species and Mechanisms of Synthetic Preservatives[5]

Preservative and Molecular Structure	Some Known Susceptible Species	Mechanisms
Organic Acids		
Sodium Benzoate 	**Gram Positive** *Staphylococcus aureus* *Bacillus subtilis* *Bacillus mycoides* **Gram Negative** *Escherichia coli* *Pseudomonas aeruginosa* **Fungi** *Aspergillus* spp. *Pencillium citrinum*	Sodium benzoate is bacteriostatic rather than bactericidal. At pH 5 and below benzoic acid is formed. This impedes with the permeability of the cell membrane and energy formed from the electron transport chain. The acid also inhibits the uptake of amino acids and thus restricting access to a nitrogen source.
Potassium Sorbate 	**Gram Positive** *Staphylococcus aureus* *Bacillus cereus* *Clostridium botulinum* **Gram Negative** *Vibrio parahaemolyticus* *Salmononella* spp. **Fungi** *Aspergillus flavus* *Aspergillus parasiticus* *Penicillium cyclopium*	When in solution, sorbic acid can infiltrate the cell wall of an organism and effect the osmotic balance. The ability to form covalent bonds with thiol groups, this hinders a range of enzymatic processes including carbohydrate uptake and energy release. Amino acid uptake is also reduced.
Aromatic Alcohols	**Gram Positive** *Staphylococcus* spp. *Micrococcus* spp.	Phenoxyethanol interrupts the cell membrane and allows potassium ions to pass through, disrupting osmotic balance. The preservative also inhibits the coupling of the electron transport and phosphorylation and stops the synthesis of ATP. It also is involved in stopping both DNA and RNA synthesis. Pairing with a chelating agent improves efficacy.
Phenoxyethanol 	**Gram Negative** *Pseudomonas aeruginosa* *Proteus mirabilis* *Escherichia coli* **Fungi** *Candida albicans* *Aspergillus niger*	
Phenyl Alcohol 	**Gram Positive** *Streptococcus* spp. **Gram Negative** *Shigella* spp. *Escherichia coli* *Pseudomonas aeruginosa* *Salmonella* spp. **Fungi** *Candida albicans* *Candida Africana*	Aromatic alcohols inactivate cell membrane proteins in bacteria. The change in the cell membrane causes some bacteria to go into a 'cold shock response' in which enzyme productivity decreases and there is a reduction in transcription and translation.
Formaldehyde Releasers	**Gram Positive** *Staphylococcus* spp.	These type of preservatives release formaldehyde in solution. Formaldehyde stops the production of the essential amino acid methionine. This in turn halts messenger RNA being translated into proteins. Formaldehyde also has coagulating properties which dehydrates the membranes and dries out the cytoplasm inside the cell.
Diazolidinyl urea 	**Gram Negative** *Klebsiella pneumoniae* *Pseudomonas* spp. *Escherichia coli* **Fungi** *Aspergillus niger* *Candida albicans*	
Quarternium - 15 	**Gram Positive** *Staphylococcus aureus* *Bacillus* spp. **Gram Negative** *Escherichia coli* *Pseudomonas aeruginosa* **Fungi** *Aspergillus niger* *Candida albicans*	

[5] Common synthetic cosmetic preservatives

Table 4. Essential oils and their Antimicrobial Compounds [6]

Essential Oil and Antimicrobial Compounds			Susceptible Species	Mechanisms of Antimicrobial Compounds
Citronella Oil				
Citronellal 	**Linalool** 	**Myrcene** 	**Gram Positive** *Staphylococcus aureus* *Cutibacterium acnes* *Streptococcus* spp. **Gram Negative** *Pseudomonas aeruginosa* *Salmonella* spp. *Campylobacter* spp. **Fungi** *Candida albicans* *Aspergillus niger*	Citronellal, linalool and myrcene are all types of terpenes. The mechanism is not well known but it is believed that these compounds work by disrupting the integrity of the bacterial cell membrane and disrupting it's functions. Linalool not only disrupts the cell membrane of bacteria but the electron transport chain is targeted and damaged. Citronellal disturbs the stability of electron donor components and causes changes to the electrophoretic separation. Myrcene has been shown to increase the antimicrobial effects compounds through limiting microbial resistance mechanisms.
Clove Oil				
Oleic Acid 	**Eugenol** 	**Linoleic Acid** 	**Gram Positive** *Streptococcus pyogenes* *Corynebacterium* spp. *Staphylococcus* spp. **Gram Negative** *Salmonella enteritidis* *Escherichia coli* *Bacteroides fragilis* **Fungi** *Candida albicans* *Trichophyton rubrum*	Linoleic acid and oleic acid are both antimicrobial fatty acids. These are most efficient at penetrating gram-positive cytoplasmic membrane. Gram-negative bacteria posses lipopolysaccharides in the cell membrane which prevent permeation from fatty acids. The disruption the cell membrane eventually causes the cell to lyse. Eugenol is an allylbenzene and has antimicrobial activity due to its hydroxyl group. Eugenol causes an increase in bacterial cell membrane and causes an influx of potassium ions which upsets the osmotic balance. Eugenol also produces excess reactive oxygen species resulting in the inability of cell growth and causes DNA damage. Eugenol also causes similar problems for fungi, bypassing the envelope disrupts cell morphology and permeability, while also disabling the ability for fungi to attach to a host.
Sandalwood Oil				
α-Santalol 	β-Santalol 		**Gram Positive** *Staphylococcus aureus* *Bacillus subtilis* **Gram Negative** *Helicobacter pylori* *Klebsiella pneumoniae* **Fungi** *Candida* spp. *Microsporum* spp.	Santalol is a sesquiterpene found in sandalwood oil. α-Santalol is more abundant than β-Santalol. The ratio of alpha and beta-Santalol, as well as sensitive microbial species, changes dependent on sandalwood species. Along with other essential oil antimicrobial compounds, the mechanisms of sesquiterpenes are faily unknown. However, they have been shown to disrupt the bacterial membranes and reduce biofilm formation of *Staphylococcus* spp.. β-Santalol has been shown to interrupt replication of influenza viruses.

[6] Essential oils are packed with chemicals which have antimicrobial properties

1.29 Reasons for Research

With the rise of urban skin ailments, it is important to note how cosmetics, or their ingredients interact with natural microbiota that reside on our skin. Cosmetic companies are now starting to move away from the once popular antimicrobial tag line in their formulations to making products with claims of promoting the 'good bacteria' that reside on our skin. However, these 'good' bacteria, include species which are common sources of nosocomial infections. These are opportunistic pathogens, which may not be as harmful as other more pathogenic species, can cause nasty infections in the immunocompromised. Even what companies would class as 'bad bacteria' often live peacefully on our skin and cause no problems day-to-day, as well as potentially stopping other pathogens from living on our skin.

1.30 Hypothesis

Cosmetic proteins have the potential to be utilised by the bacteria found on skin, to help with their growth. While the preservatives found in cosmetic proteins are likely to have inhibitory effect against the same bacteria.

1.31 Aims

- Design a minimal medium to produce growth conditions more akin to skin (Section 2)
- To use minimal inhibitory concentration methods to understand the inhibitory effect of the preservatives found in cosmetic proteins (Section 3)
- To use growth kinetics and biofilm formation techniques to identify whether the cosmetic proteins can be utilised by skin bacteria (Section 3)
- To co-culture *Staphylococcus aureus* and *Staphylococcus epidermidis* (Section 4)
- Expose co-cultured *Staphylococcus* spp. to the cosmetic proteins and investigate dominance of species in both planktonic conditions and for biofilm formation (Section 4)
- Use tissue culture to understand the bigger picture by incorporating skin cells and their interactions with cosmetic proteins and skin bacteria (Section 5)
- Start to move this study from the *in vitro* phase to *in vivo*, by investigating retrieval of bacteria from skin using different swab head types and buffer compositions (Section 6)
- Investigate whether current cosmetic protocols for skin tests can be used when incorporating a microbial element (Section 7)
- Find a body site with the highest bacterial return to limit relative background contamination (Section 7)

2 Production of a Minimal Medium to Reflect the Skin's Harsh Environment

2.1 Growth Requirements

To successfully grow microorganisms, the growth medium must reflect the need of the species and the environment in which they naturally live (Boundless 2017). Bacterial respiration occurs when the organism is in contact with the correct balance of gases (Rogers and Kadner 2020). Aerobes, facultative anaerobes, and micro-aerobes, require access to varying levels of oxygen to grow (Morris and Schmidt 2013; Singh 2016). Many aerobic species can grow in micro-aerobic conditions when there is competition for, or low levels of oxygen present (Partridge et al. 2007). While facultative anaerobes can switch between aerobic and anaerobic respiration, when oxygen is reduced or not present (Sanz 2011). Obligate anaerobes, however, can be negatively affected or harmed by the presence of oxygen (Hentges 1996). Cultivations *in vitro* involve atmospheres of either a hydrogen/nitrogen or nitrogen/carbon dioxide combination (Cox and Mangels 1976; Behbehani et al. 1982).

Correct pH is also important for the growth of bacteria. Acidophilic bacteria prefer growing in a pH of between 3-5 (Jin and Kirk 2018), while alkaliphilic species grow at the higher end of the scale between 9 and 11 (Horikoshi 1999; Rampelotto 2013). Neutrophiles are unlikely to proliferate in a pH which is more than two units away from neutral (Keenleyside 2019), but often can adapt to survive at the edges of acidic and alkaline conditions (Pradeep and Sarika 2008).

The cultivation of bacteria requires the ambient temperature to be within the species' range for growth. While most mesophilic bacteria can endure harsher temperatures than larger organisms, they are not adapted to proliferate below 10^{o}C and above 50^{o}C (Bendia et al. 2018). The ideal temperature for these bacteria is between 20-45^{o}C (Schiraldi and De Rosa 2016). At low temperatures, the cell membrane of mesophiles becomes rigid which interferes with transport of ions and waste. Protein production slows and leads to inaccurate folding of the nascent molecules, leading to lack of function. Cell division ceases and the bacteria enter a stasis (D'Amico et al. 2006). While at high temperatures the thermo-sensitive proteins denature, and fluidity of structural lipids cause cell membranes damage (Kumar et al. 2000; Siliakus et al. 2017).

Bacteria utilise compounds in the environment to fuel growth and anabolic (energy required) and catabolic (energy produced) metabolisms (Jurtshuk 1996). The speed of growth, or cell division is dependent on the richness of the nutrient source (Monahan et al. 2014). In ideal conditions, some bacterial species' cells can double in minutes, while in severely depleted environments, it may take decades or more (Berry et al. 2010; Allen and Waclaw 2019). The minimal nutritional requirements for bacterial growth include water, mineral salts, a carbon source, a nitrogen source, phosphorus for adenosine triphosphate (ATP) and sulphur for cysteine and methionine (Fagerbakke et al. 1996). However, some more niche species may also need more specific

supplements (Atlas 2010). Selenium supplements, for example, have been shown to help *Lactobacillus* spp. inhibit the growth of pathogenic bacteria (Kousha et al. 2017).

Bacteria are around 70% water, which means it is vital for their survival (Hobot et al. 2015; Boundless 2021). Bacteria also require access to unbound water to allow for the transference of nutrients into the bacteria and waste products to be removed (Bonnet et al. 2019). Water activity (aw) measures the amount of unbound water of a solution. With the highest water activity of 1, distilled water is the most ideal source of water for the base of growth media as there is no additional salts or chemicals and the composition is therefore consistent (Schultz 2016; Allen 2018). Gram-negative bacteria require more than 0.96 aw to grow, while Gram-positive bacteria require upwards of 0.9 aw (Beuchat 1981; Sperber 1983). Only halophilic bacteria, yeasts and fungi can generally survive below 0.89 aw (Stevenson et al. 2015).

Mineral salts supply trace elements which are vital for synthesis of essential cellular components, nutrition, and cell metabolism. Phosphates are required to produce nucleic acids (Lodish et al. 2000), sulphates in methionine and cysteine production (Kushkevych et al. 2020), while magnesium ions are divalent cations involved in catalysation of ATP decomposition (Ebashi et al. 1973; Matevosyan et al. 2019).The reduction and reoxidation of Copper II ions on the isoenzyme superoxide dismutase are integral to the conversion of oxygen radicals into hydrogen peroxide (Nóbrega et al. 2019) and potassium is a counterion used to maintain cell homeostasis through preserving neutrality (Epstein 2003).

Carbon sources are broken down into smaller molecules such as amino acids or sugars through catabolic metabolism. These are used in various metabolic pathways which can generate proteins, nucleic acids and energy (Cooper 2000a). Autotrophic bacteria can utilise carbon dioxide to produce energy (Baas-Becking and Parks 1927), while heterotrophs rely on carbon sources available in the environment (Kirchman 1994). These carbon sources include various monosaccharides and disaccharides (Wawrik et al. 2005), while polysaccharides require extra enzymatic steps to reduce them to lower molecular weight molecules which will be small enough to enter the cell (Inman 2011). Carbohydrates enter the cellular respiration pathways in which the energy-carrying molecule ATP is produced. In the most ideal conditions, using the carbon source of glucose (Bren et al. 2016), a maximum of 38 molecules of ATP can be produced, two from glycolysis and the Kreb's cycle and upwards of 34 from the electron transport chain (Jurtshuk 1996). In the less efficient anaerobic respiration, ATP production count stands at two (Stojan and Christopher-Stine 2015; Melkonian and Schury 2020)

A source of nitrogen is required to produce nucleic acids and proteins. Organic nitrogen sources, such as amino acids, are more commonly utilised by bacteria than inorganic sources such as ammonium salts. While some bacteria possess extracellular enzymes, which can hydrolyse large

proteins (Wandersman 1989; Dalbey et al. 2012; Garai et al. 2017) and carbohydrates (Rivière et al. 2016), most Gram-negative bacteria prefer smaller peptides, which are easier to transfer through into the cell (Walker and Altman 2005).

Most standard recipes for non-selective media are very nutrient rich and contain abundant amounts of the minimum requirements to try and achieve maximum growth of bacteria. Tryptone soy broth or agar (TSB and TSA respectively) is a commonly used medium, which can grow numerous bacteria species. The composition of TSB includes nitrogen rich casein and soya peptones, the latter which also supplies natural sugars, a carbon source of glucose and an osmotic balancer of sodium chloride and with a suggested neutral pH to finish (SigmaAldrich 2018).

Mueller-Hinton (MH) is a medium used for studying the antimicrobial effects of antibiotics and preservatives on bacteria (Lenczewski et al. 1996). It contains beef extract, casein hydrolysates and starch. While still nutrient rich, there is a lack of an additional sugar source as found in TSB. The addition of starch absorbs the bacterial waste products and stop them interfering with the antibiotics (Mueller and Hinton 1941).

Both media have been designed for a purpose, whether that be optimising growth conditions or testing susceptibility of a species. However, neither reflect the environment in which most bacterial species are found. For example, the human skin is a nutrient depleted niche (Moskovicz et al. 2020) and too acidic for many Gram-negative species to permanently settle (Grice and Segre 2011).

Previous research into bacteria which originated in low nutritional habitats had simply diluted nutritionally rich media. Dilution of the medium enabled the slower growing species to be cultivated. In media of high nutrients, fast growing bacteria use up the nutrients far too quickly and out-compete other, slower growing species (Medina et al. 2017; Chaudhary et al. 2019; Sun et al. 2019). If one wanted to carry out experiments on skin bacteria *in vitro*, using a nutrient rich medium such as TSB would not reflect the conditions which many of the skin dwelling bacteria find themselves and are adapted to overcome.

2.2 Growth Kinetics

The growth pattern of a microorganism is dependent on the environment they are grown in and their access to nutrition. In a batch culture, most go through a recognised staged process (Figure 5). The collective phases of this process are known as the growth curve (Zwietering et al. 1990; Rolfe et al. 2012). These stages of the growth curve start with the adaption of the organism to the medium source and growth space. At this lag phase, while metabolically active, little or no growth is seen (Rolfe et al. 2012). If the medium is complex, with different sugar sources available, there can be multiple lag phases. This is called diauxic growth (Chu and Barnes 2016). The log phase begins as the organism utilises the nutrients available and multiply exponentially (Bruslind 2017). As nutrients and space available decrease or toxins accumulate, growth decelerates, and the

curve enters the stationary phase (Stanbury et al. 2017). If left long enough, the bacteria will enter the final decline or death phase. In a closed batch system, this is where the competition for lack of space or nutrients finally causes the bacteria to die off (Prado Barragán et al. 2016).

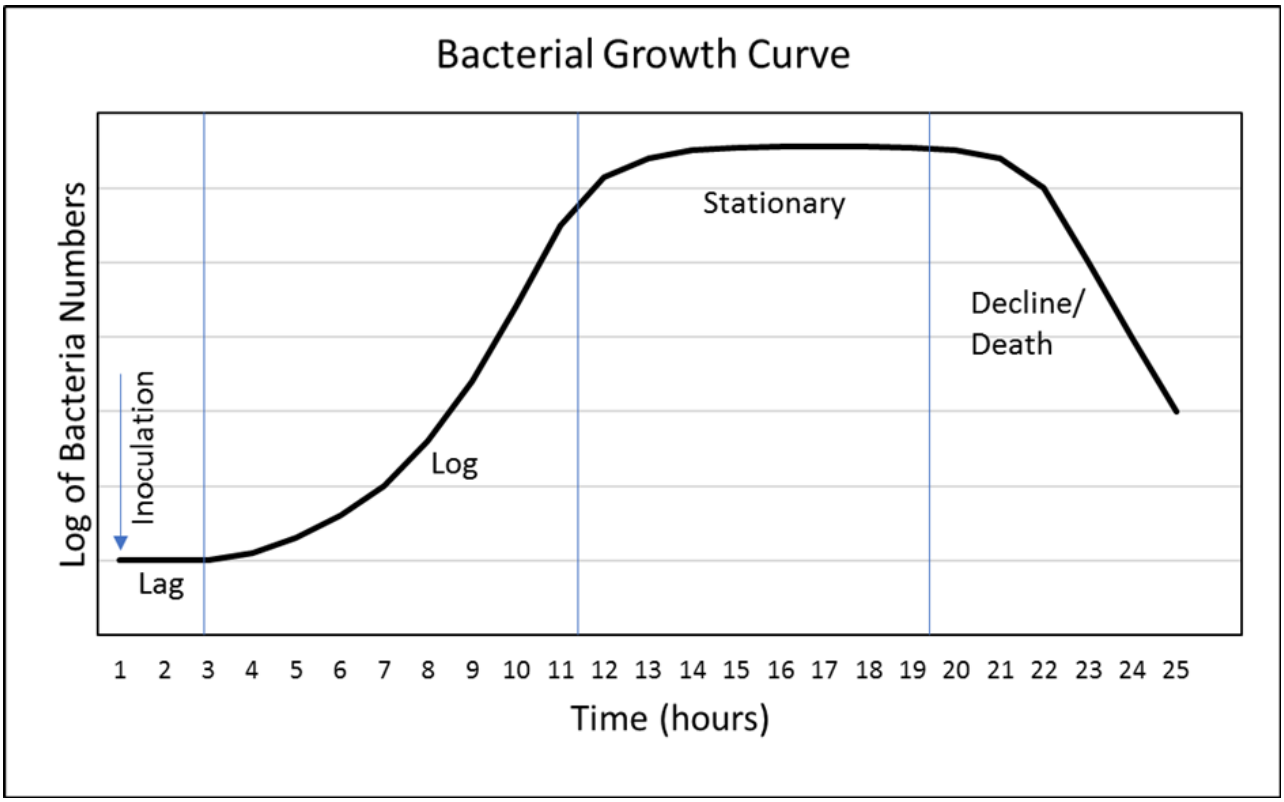
Growth curves and kinetics are a way of measuring how successful a species of bacteria is at utilising components of a given medium source (Budd and Spencer 1968). They can also be used to compare the behaviour of bacteria when grown at different temperatures, pH and when given different substrates (Zwietering et al. 1990). Observations can be made from the length of the lag period, growth rate and total growth yield, among others (Kneifel 2009).

2.3 Null Hypothesis

There would be no difference in growth between the conventional, high nutrition media in comparison to the low nutritional minimal medium.

2.4 Aims

- To produce a medium which reflects the low nutrition and low pH of the skin
- To show that conventional, high nutritional media are unsuitable for skin bacteria



experiments

Figure 5. The Four Phases of a Bacterial Growth Curve in a Closed Batch System. The stages include the lag, log, stationary and decline/death.

2.5 Materials

2.6 Bacteria

The three bacteria species used in this study were all from two common skin genera (Dréno et al. 2018). *Staphylococcus epidermidis* (DSMZ-20044), *Corynebacterium jeikeium* (DSMZ-7171) and a clinical isolate, *Staphylococcus aureus*, taken from the perineal region of a patient. The three species were confirmed using 16S rRNA gene sequencing. These three skin species were chosen as all can grow aerobically and in similar media conditions. Previous works have often compared the pathogenic *S. aureus* with its commensal cousin *S. epidermidis*. However, it was decided to also use a slower growing *Corynebacterium* spp., to compare to the fast-growing *Staphylococc*us spp..

2.7 Media

Dehydrated tryptone soy broth and dehydrated Mueller-Hinton broth were both purchased from Thermofisher Scientific (Waltham). The media were made as per instructions located on the packaging provided.

2.8 Minimal Medium

To reproduce conditions more akin to skin, a minimal medium was required. The Davis minimal broth is a medium which is a mixture of salts and a low concentration of glucose as a single energy source, it was designed to isolate *Escherichia coli* (Atlas 2010). The medium was chosen as it was low nutritional but contained the bare minimum required for microbial growth. A modified version of this minimal medium was created to carry out experiments. Modified Davis medium composition as stated below (Table 5). HCl (1 M) was added to the medium until pH 5 ± 0.2. The medium was autoclaved at 121°C, 15 psi for 15 minutes. Supplements could be added separately as required. A 1-2% w/v agar version of the minimal medium was attempted, but there was either no growth or very small and unusable colonies.

Minimal Medium Composition	
Component	**Per Litre**
Dipotassium phosphate	7.0 g
Potassium phosphate	2.0 g
Ammonium citrate	0.5 g
Ammonium chloride	1.0 g
Magnesium sulphate	0.1 g
Glucose	1.0 g
Supplements	
Casamino acids	0.6% w/v
Tween 80	0.2% v/v

Table 5. Recipe for Modified Davis medium.

2.9 Methods

2.10 Media Growth Curves

The three bacterial species were grown overnight in TSB for 18 hours. The suspension was placed into a 1 ml cuvette and optical density (OD) was measured at 600 nm using a Jenway 6300 spectrophotometer (Cole-Palmer Ltd., Cheshire). The cell suspensions were diluted in either the TSB, Mueller-Hinton or the Minimal Medium, to approximately $1x10^5$ cfu/ml. The cfu/ml was confirmed by serial dilution, incubation and by colony count method of Miles and Misra (Miles et al. 1938) the following day. Growth assessments were carried out by producing growth curves using an Infinite® 200 PRO microplate reader (Tecan, Zurich). An aliquot (100 µl) was plated into a MicroWell™ 96-Well Microplate (Corning, New York) well and placed into the 200 PRO multimode reader platform. Programme specifications were temperature 37 °C and absorbance reading 600 nm. The plates were orbitally shaken at 30-minute intervals before each read at a frequency of 218 rpm for 10 seconds.

2.11 Statistics

Growth curves were illustrated, and kinetics analysed using GraphPad (San Diego) Prism (Version 9.1.2). Comparisons between a group of growth curves were analysed using One-way ANOVA, while comparisons between individual growth curves were analysed using a t-test. Total yield (total OD) was calculated using descriptive statistics of the curve. Growth rate and doubling time were calculated using non-linear regression fit of the curve analysis. Lag and log phase time were taken from observations straight from the growth curves, as there were no analysis options for these.

2.12 Results

2.13 Growth Curves with TSB, Mueller-Hinton and Minimal Medium

All three species were grown in TSB, Mueller-Hinton, and the minimal medium for 20 hours at 37 ^{o}C, however, a Tween 20 supplement of 0.2 % v/v, was required for *C. jeikeium* to utilise as a lipid source. In the main, sigmoid growth curves were observed with clearly defined stages of lag, log, and stationery. However, when the two *Staphylococcus* spp. were grown in Mueller-Hinton, the stationary phase did not follow the traditional pattern. *S. aureus* continued growth never led to a stationary plateau, while *S. epidermidis* did reach a plateau, but at around 20 hours, also following some extra, non-exponential growth. *C. jeikeium* did not reach stationary phase in 20 hours, when grown in Mueller-Hinton (Figure 6, Figure 7). However, there were significant ($P<0.001$) differences between the growth kinetics of each of the species when grown in the three media.

The lag phase of *S. epidermidis* totalled six hours when grown in the minimal medium, while only four hours when grown in TSB or Mueller-Hinton. Exponential growth of *S. epidermidis* lasted 11.5 hours in TSB, 9 hours in Mueller-Hinton and 7 hours in the Minimal medium. *S. epidermidis* had the slowest exponential growth rate when grown in TSB at 0.087 OD/hour compared to 0.092 OD/hour in Mueller-Hinton and 0.149 OD/hour in the Minimal medium. Doubling times were longer for *S. epidermidis* when grown in TSB, 7.9 hours, and MH broth, 7.5 hours, than in the minimal medium, 4.7 hours. The total yield for *S. epidermidis* when grown in the three media differs significantly. The highest total OD was achieved when the bacteria were grown in TSB, with an optical density of 0.769. The final total OD when grown in Mueller-Hinton is slightly less at 0.642, and significantly lower ($P<0.05$). when grown in the minimal medium, only having reached 0.055.

The total lag phase time of *S. aureus*, when grown in all three media was approximately 3 - 3.5 hours. The exponential growth rate is also similar across the board, 0.070 OD/hour in TSB, slightly higher at 0.086 OD/hour in Minimal medium and 0.070 OD/hour in Mueller-Hinton. Doubling time was similar when *S. aureus* was grown in all three media but longer than *S. epidermidis*. Doubling time in TSB was 9.9 hours, in MH broth was 8.0 hours and 9.9 hours in the minimal medium. The log phase of *S. aureus'* growth lasted 5 hours in TSB, 4.5 in Mueller-Hinton and 7.5 hours in the Minimal medium. Again, as seen in *S. epidermidis*, while the exponential growth stopped, non-exponential growth was still observed during what would be the stationary phase in Mueller-Hinton broth. The final OD reached in TSB was 0.780, 0.610 in Mueller-Hinton and, again, the significantly lower ($P<0.05$) 0.041 in the minimal medium.

C. jeikeium had the longest lag phase in two out of the three media lasting approximately 6.7 hours in TSB and 10 hours in Mueller Hinton (Figure 6). The lag phase in minimal medium lasted approximately 3.5 hours, the same length of time as *S. aureus* (Figure 7). Once the exponential

growth had started, both the growth rate was faster than that of the *Staphylococcus* spp. The exponential growth rate of *C. jeikeium* had the most rapid in both TSB (0.118 OD/hour) and Mueller-Hinton Broth (0.190 OD/hour) and slowest in minimal medium at 0.082 OD/hour, slightly quicker than the growth rate of *S. aureus*. The final OD of *C. jeikeium* is less when grown in all three media, though the total yield had not yet been reached after 20 hours in Mueller Hinton. The final OD of *C. jeikeium* in TSB is 0.268, 0.042 in the Minimal medium and, although the growth had not reached stationary phase, 0.557 in Mueller Hinton. Again, doubling time of *C. jeikeium* in all media were shorter than *S. epidermidis* and *S. aureus*. When grown in TSB, the doubling time was 5.9 hours, 3.7 hours in MH, and the slowest in the minimal medium at 8.5 hours.

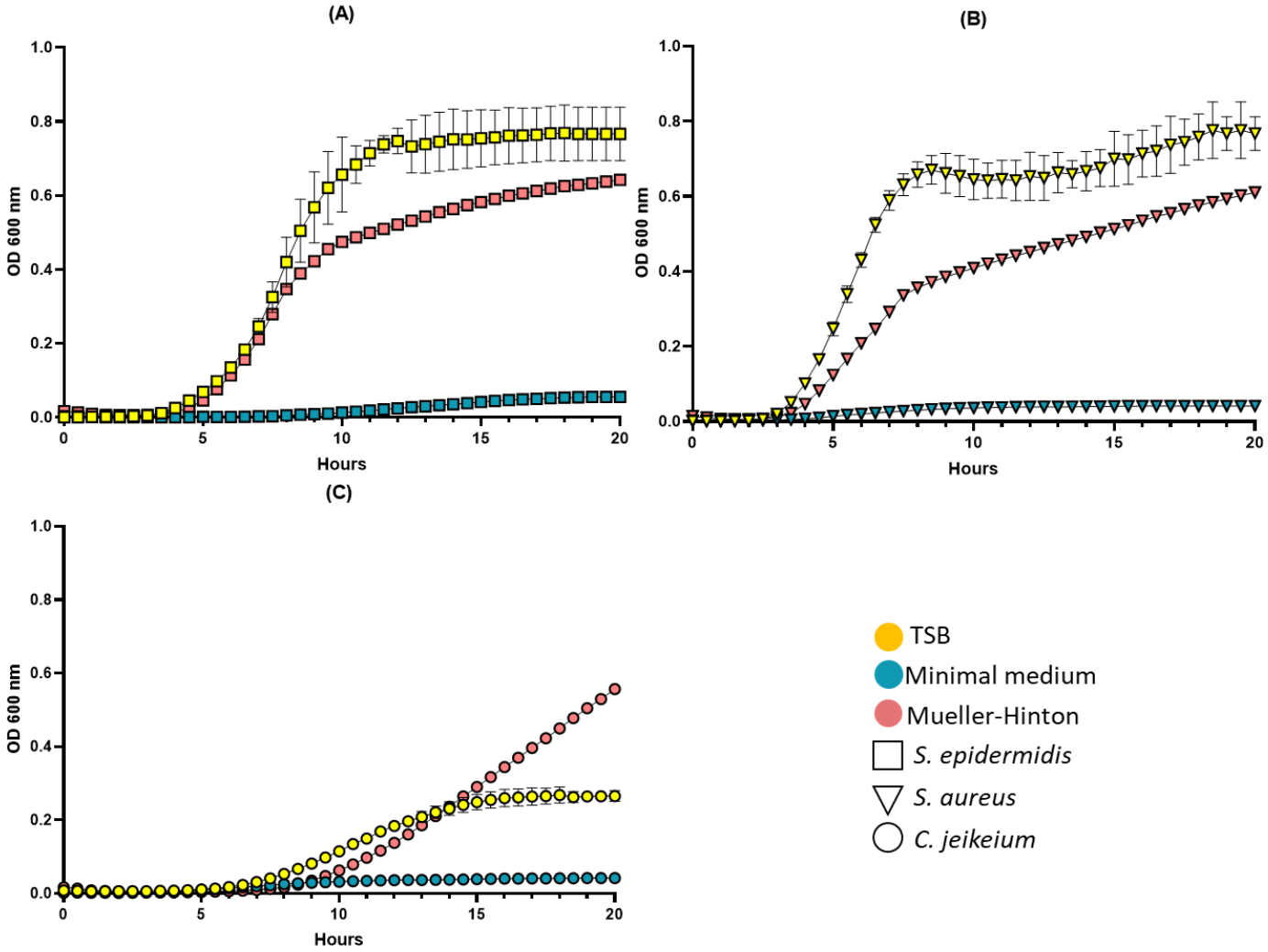


Figure 6. Growth Curves of the Three Skin Bacteria with all Three Media Types. This experiment was performed with three technical replicates and three biological replicates**.** (A) *Staphylococcus epidermidis* grown with TSB and Mueller-Hinton Broth at pH 7 and the Minimal medium at pH 5. (B) *Staphylococcus aureus* grown with TSB and Mueller-Hinton broth at pH 7 and the Minimal medium at pH 5. (C) *Corynebacterium jeikeium* grown with TSB and Mueller-Hinton broth at pH 7 and the Minimal medium at pH 5. Figure 7 shows more clearly the growth curve of all three bacteria and the minimal medium.

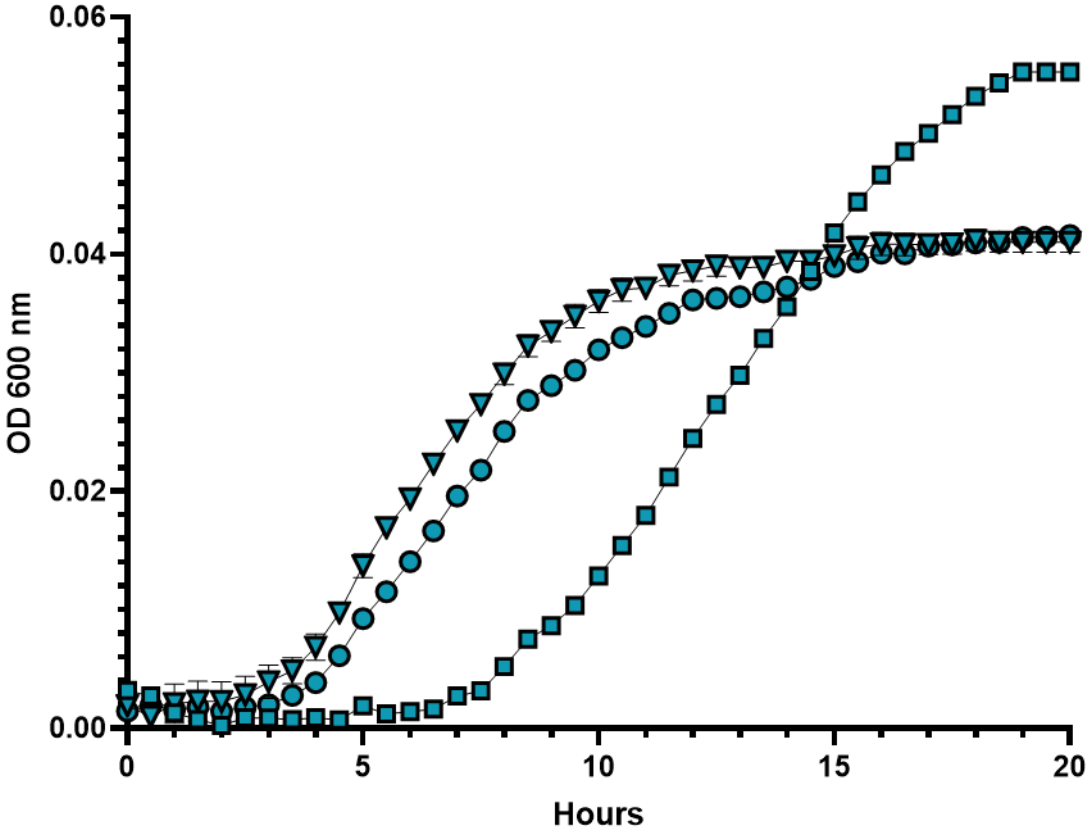


Figure 7. Growth Curves of the Three Skin Species Grown in the Minimal Medium. This experiment was performed with three technical replicates and three biological replicates**.** Growth curves of *S. epidermidis* (□), *S. aureus* (▽) and *C. jeikeium* (○) when grown for 20 hours with the Minimal Medium at pH 5. While exponential growth starts later for *S. epidermidis* than the other two species, total yield is far greater. There was no significant difference between the growth curves of *S. aureus* and *C. jeikeium* (P>0.05).

2.14 Discussion

We can reject the null hypothesis that the three bacteria have the same growth pattern when grown with any of the chosen media.

2.15 Why are Nutrient Rich Media not useful for Skin experiments?

Previous research has highlighted the disadvantages of using high nutritional media when investigating bacteria from low nutritional environments. Using high nutritional medium has meant that many species isolated from non-equivalent conditions have not been accurately represented (Davis et al. 2005). Disproportionate species growth can be seen in the growth curves from Figure 6. Both Staphylococcus spp. started the exponential phase sooner, had faster growth rates and higher final OD than *C. jeikeium* when grown in TSB. Using a depleted medium more akin to the natural environment has previously conveyed a more accurate species representation (Janssen et al. 2002; Davis et al. 2005). Once grown in minimal medium the growth curves patterns were much more alike between the three species, with no significance noted between *S. aureus* and *C. jeikeium.* Therefore, using growth medium designed for optimum growth kinetics would not be representative of the harsh conditions that organisms face while living on skin, it could also give faster growing organisms an advantage when grown in a culture of multiple species. While using a minimal medium can 'level the playing field'.

2.16 Nutrient Rich Media can Provide both Metabolic Advantages and Disadvantages

Using a complex nutrient rich medium could provide metabolic advantages to bacteria which would not be present in their natural surroundings. *S. aureus*, for example, when grown in Mueller-Hinton broth has enhanced membrane integrity compared to other media. The membrane improvement comes from an increased production of the carotenoid virulence factor, staphyloxanthin (Jaśkiewicz et al. 2017). Free amino acids from the casein digest induce higher production of branched-fatty acids composition and staphyloxanthin levels are also subsequently increased (Sen et al. 2016). The improved membrane integrity gives *S. aureus* better coping mechanisms with both oxidative stress and host immune responses (Clauditz et al. 2006). Mueller-Hinton broth also contains starch, which soaks up toxins produced by bacteria into the surrounding medium (Olsen and Scott 1946). Advantages caused by medium composition could be why there is no clear stationary phase when *S. aureus* is grown with MH (Figure 6) the management of oxidative stress or lack of toxin build up could increase the total yield of the bacteria over time.

The growth of *S. epidermidis*, however, produced a more traditional looking growth curve with MH than *S. aureus*, but still exhibited some non-logarithmic growth before finally plateauing at 20 hours. When *S. epidermidis* has previously been grown with Mueller-Hinton broth, the effect on membrane integrity is the opposite to that of *S. aureus*. Staphyloxanthin is not present in the cell membranes of *S. epidermidis*, so that when branched chain fatty acids are increased, the

membrane becomes more flexible. This decreased membrane integrity made *S. epidermidis* more susceptible to surrounding stresses (Tiwari et al. 2020). This may be why there was an eventual stationary phase, the starch removed the toxins which allowed for longer period of growth, but the decreased membrane integrity did eventually mean that *S. epidermidis* could succumb to other growth stresses.

When grown in TSB, *C. jeikeium* reaches stationary phase in 15 hours, with a poor growth rate and low yield to show for it. *C. jeikeium* does not possess extracellular proteases (Kwaszewska et al. 2014), which would mean that when grown in a peptide rich medium such as TSB (Moss and Speck 1966), *C jeikeium* may not be able to uptake enough of a nitrogen source for successful growth. When grown in MH, *C. jeikeium* started the log phase of growth slightly later than when grown in TSB. However, total yield when grown in MH surpassed that of TSB, and, within 20 hours, a stationary phase was never achieved. Mueller-Hinton broth contains a lot of free amino acids, due to the casein digest. This medium therefore suited *C. jeikeium* better, as the free amino acids were utilised without the extra hydrolysis steps.

2.17 Conclusion

Once the resources of these high nutritional media are used up, competition increases and waste accumulates, the initial surge of logarithmic growth and high cell yield can negatively affect the long-term cell survival (Kram and Finkel 2015). In a normal skin environment, there is some competition, but many of the niche organisms simply reside with each other, some even offering other species vital compounds through their by-products or protection against antimicrobials (Kwaszewska et al. 2014).

Unlike the high nutritional media, the minimal medium only provides small quantities, or the bare minimum of the nutritional compounds needed for bacterial growth. The pH was changed to reflect the acidic conditions found on skin. The complex media are designed to have the optimum pH for most culturable bacteria, while the skin has a mostly acidic pH range. While pH can easily be rectified, the compositions of these media are also designed to provide optimum nutrition for metabolic pathways. The complex media are not without their experimental uses. The fast growth and high yield when all three bacteria were grown in TSB means the medium is useful for quickly setting up bacterial experiments. While the addition of starch in MH allows for unaltered sensitivity testing of antimicrobial compounds. However, a medium with an acidic pH and the addition of nutritional compounds that only provide the bare minimum for survival and not provide a metabolic advantage for faster growing organism, would be more reflective to how species survive on skin.

3 Testing Experimental Conditions to Determine whether Bacterial Skin Species Can Utilise Cosmetic Proteins as a Peptide and Amino Acid Source and if Preserving Measures are Effective Against Them

3.1 Introduction

Hydrolysed proteins are a common ingredient in cosmetics to add moisturising and hydrating qualities to the final formulation (Aguirre-Cruz et al. 2020). Cosmetic proteins are used in formulations at a range of desired active levels. The active level signifies the percentage of organic material in the final product. Formulations with cosmetic proteins can deliver amino acids to the surface which claim to benefit the skin health (Sethi et al. 2016). However, there is little research into how this ingredient group interacts with the microorganisms which live there.

3.2 Uptake of Amino Acids

Most bacteria possess genes which enable production of 20 amino acids used in protein synthesis (Amorim Franco and Blanchard 2017; Price et al. 2018; Sanchez et al. 2018). However, exogenous peptides and amino acids are brought into the cell to be used in further metabolic pathways, and can make up to 50% of the amino nitrogen needed by bacteria (Coffin 1989). Only some genera possess extracellular proteases which can break down large protein molecules (Wistreich and Lechtman 1988), which is why protein digests are often added as a nutritional supplement in media (Robinson and Rettger 1918). Lactose-free skimmed milk media can be used to identify bacteria which possess these extracellular proteases able to break down proteins or large peptides (Morris et al. 2012).

Due to differences in their chemical make-up, there are many different routes by which bacteria assimilate amino acids (Gale 1947). Membrane bound proteins are involved in many of these different methods in moving amino acids across the cell membrane (Alberts et al. 2002). Facilitated diffusion uses channel proteins to bypass the phospholipid membrane. There is no energy required, the amino acids move across into the cell by the electrical potential differences between the outside and inside the cytoplasm (Cooper 2000b). ATP-Binding Cassettes (ABCs) are made up of ATP enzymes and membrane bound proteins. The amino acids attach to the outer-membrane, ABCs then use the energy from ATP hydrolysis to alter their shape, which moves the amino acids (Schneider and Hunke 1998). Ion-coupled transport is another way to bring amino acids into the cell. Ions are released from the cell, so the gradient of ions is shifted (Jacquez). Amino acids can enter the cytoplasm as the ions return into the cytoplasm through protein channels (Wilson and Ding 2001). These are mostly hydrogen ions although sodium ions are also commonly used (Heyne et al. 1991).

Individual bacterial species select amino acids to utilise, based on their specific needs (Jiang et al. 2020). These needs include changes in the environment, such as fluctuating salt concentrations (Alreshidi et al. 2019) and surrounding/dissolved gas composition (Sun et al. 2012). There have also been noted differences amino acid usage dependent on whether the bacterial growth is planktonic or sessile (Zhu et al. 2007; Ammons et al. 2014).

3.3 Preservatives

All six cosmetic proteins, manufactured by Croda Europe Ltd., were liquid and therefore had preservatives added to stop microbial spoilage. These preservative measures are found in the product description found on the Croda Europe Ltd. personal care website (Croda 2022). While these preservatives would be diluted in the final formulation, their presence could influence the microbiota that reside on the skin (Pinto et al. 2021). A lot of what would be classed as formulation contaminants, also happen to be species of bacteria found on skin (Flores et al. 1997), so cosmetic preservatives are knowingly effective against them (Campana et al. 2006). Recent research has shown that while these common preservatives are effective against skin species *in vitro*, *in vivo* this is less so (Murphy et al. 2021).

3.4 Studying Growth Kinetics When Switching Nutritional Sources

To examine whether bacteria utilise certain compounds in a media, previous work had switched out the source of nutrition with the test compounds (Fallah et al. 2015; Liu et al. 2020b). Experiments have also used a minimal media with only the bare minimum growth requirements so as not to introduce exogenous interference (Collins and Thune 1996) and to not favour the faster growing organisms. Analysis of growth kinetics have been used to investigate and compare microbial utilisation of various compounds (Meletiadis et al. 2001).

3.5 Biofilms

Many bacteria can have a different form of growth than planktonic; biofilm formations are a type of growth which are especially prevalent in hospital settings (Dincer et al. 2020). While biofilms do not occur on healthy skin, many of the bacteria which reside there are opportunistic pathogens and under the correct circumstances could form one. A simple cut would cause a break in the skin barrier, which could allow opportunistic bacteria to enter and form a biofilm (Percival et al. 2014). A biofilm is a collection of microbials cells, from one or more species, which significantly adhere to a surface (Brandwein et al. 2016). These cells are surrounded by extracellular polymeric substances, which make it difficult to remove either physically or chemically (Donlan 2002; Sharma et al. 2019). Therefore, supplying bacteria with enough nutrition or suitable conditions to be able to create a biofilm could be detrimental for the health of the skin. While no sure links have been found between skin conditions and biofilm formation of skin microbiota, biofilms have long been associated with antibiotic resistant and deadly diseases (Vaishnavi et al. 2019).

3.6 Null Hypothesis

Cosmetic proteins do no provide sufficient nutrition to aid or support bacterial growth of any kind.

3.7 Aims

- To undertake experiments as to whether cosmetic proteins can deliver nutrition to skin bacteria for a) successful growth b) biofilm formation
- To investigate whether the addition of preservatives could have a detrimental effect on skin microbiota

3.8 Materials

3.9 Bacteria

The three bacterial species used were as described in Section 2.6.

3.10 Cosmetic Proteins

The cosmetic proteins were all produced by Croda Europe Ltd., Hydrolysis of the proteins were carried out either by blended acid/alkali or enzymatically. The proteins were all sent at various active levels. All were diluted to 10% active in deionised water and measured to ensure that the pH was 5 ± 0.2. Preservative content can be viewed in Table 6 and the amino acid content of the proteins in Table 7

3.11 Media

The media used were TSB for overnight cultures, pH adjusted Mueller-Hinton broth for MIC and pH adjusted minimal media described in Section 2.9. Both Mueller-Hinton broth and the minimal medium were adjusted to pH 5 using 1 M HCl solution.

3.12 Methods

3.13 Calculating Minimum Inhibitory Concentration

The three bacteria were grown overnight in tryptone soy broth in conditions as stated in Section 2.9 and diluted to OD 1. The bacteria were centrifuged at 4000 g for 10 minutes, and the media removed. The bacterial pellet was resuspended in 2 x Mueller Hinton broth and diluted further to approximately 1 x 10^5 cfu/ml. The preservative combinations (Table 6) were made up to their stated concentrations in pH 5 dH_2O. These were diluted further in pH 5 dH_2O to the concentration at which would be found if the protein was used at 8% active. 200 µl of this '8%' preservative mix were added to three wells on the first line of a 96-Well microplate (Nunc, Denmark) and 100 µl of pH 5 dH_2O was added to the wells proceeding these. 100 µl of the preservatives from the first line were then added to the 100 µl of dH_2O of the second. 100 µl of this mixture was added to the 100 µl of dH_2O below and continued until the sixth row when after mixing 100 µl was discarded to keep consistent volumes. The seventh row was kept as 100 µl of dH_2O. 100 µl of bacterial suspension was added to all the wells apart from the empty eighth row and media controls. The preservative concentrations were at the active levels as seen in Figure 8. To ensure that stationary phase was achieved, a growth curve of each bacterium in Mueller-Hinton broth at pH 5 was also performed (data not shown).

3.14 Lactose-free Skimmed Milk Agar Protease Test

Lactose-free-skimmed milk (LFSM) agar was prepared as according to (Morris et al. 2012). The three species were grown overnight in tryptone soy broth as in Section 2.10. The bacteria were serial diluted in 1xPBS. Each dilution was spread onto the LFSM agar using the spread-plating method (Sanders 2012). The plates were incubated overnight at 37 ˚C. Dilution plates with clear, individual colonies were then examined for protease activity zones surrounding the colonies.

3.15 Growth Curve Kinetics Experiment

Minimal media from previous work was used as a way of producing some conditions which reflect the skin environment. However, to test whether bacteria can utilise the cosmetic proteins, the casamino acid growth supplement was removed from the recipe. Instead, the media was supplemented with the cosmetic protein at active levels in which they may be used in the final formulation 0, 0.125, 0.25, 0.5, 0.75, 1 and 2%.

Bacteria were grown into single colonies on TSA. A single colony was removed and place into 4 ml of TSB, which were then grown for 18 hours at 37 ˚C on a shaker. The optical density (OD) was measured at 600 nm and the cell suspensions were diluted $1x10^5$ cfu/ml in minimal media containing the cosmetic protein. 100 µl was plated into a MicroWell™ 96-Well Microplate well plate (Nunc, Denmark) and placed into the 200 PRO multimode reader platform (Tecan, USA). For the programme specifications, the temperature was set to 37 °C and absorbance was read at 600 nm every 30 minutes, over 21 hours.

3.16 Counting the CFU/ml of The Growth Curves

After incubation for the growth curve data, the cells were diluted and plated onto tryptone soy agar plates and counted using the Miles and Misra method (Miles et al. 1938). If no growth was recorded on the plate reader, the number of colonies would determine as to whether the addition of the cosmetic proteins was bacteriostatic or bactericidal. The use of a neutraliser was decided as unnecessary, as the concentration of preservatives were already low and would be further diluted using this method.

3.17 Biofilm Formations

Bacteria were grown overnight, as stated above, and diluted to $1x10^5$ cfu/ml in minimal media. The media was supplemented with the cosmetic protein at active levels in which they may be used in the final formulation 0, 0.125, 0.25, 0.5, 1, 2% and 4%. 200 µl of bacteria and supplemented minimal media were placed into a flat bottom MicroWell™ 96-Well Microplate well plate (Nunc, Denmark). Flat bottom plates were chosen due to the non-motile nature of the three organisms (O'Toole 2011). The method chosen to quantify biofilm formation was the crystal violet staining method, which measures the bacterial cells and extracellular matrix which forms the biofilm as a whole (Welch et al. 2012). These plates were placed into a 37 ˚C incubator for 24

hours. The biofilms were stained and quantified using the protocol stated by O'Toole (2011). This involved quickly expelling out the cells from the plate and submerging in dH_2O twice, before adding 125 µl of 0.1% w/v crystal violet to each well and incubating at room temperature. After staining the wells were then submerged in dH_2O again and dried overnight. The following day 125 µl of 30% v/v acetic acid was added to the wells to remove the adhered crystal violet, incubated, and transferred to a clean 96 well plate. Measurements were carried out using the 200 PRO multimode reader platform (Tecan, USA) at 550 nm. This process was previously completed using TSB and MH as the media source to confirm that the three species were able to produce biofilms.

3.18 Statistics

All statistics were analysed using GraphPad Prism (Version 9.1.2). Growth curves were illustrated, and kinetics analysed using GraphPad Prism (Version 9.1.2). Comparisons between a group of growth curves were analysed using One-way ANOVA, while comparisons between individual growth curves were analysed using a t-test. Total yield (total OD) was calculated using descriptive statistics of the curve. Growth rate was calculated using non-linear regression fit of the curve analysis. Lag and log phase time were taken from observations straight from the growth curves, as there were no analysis options for these. Both Biofilm and MIC values were both comparison of mean values and were also analysed using t-tests.

Table 6. Table of Information Regarding the Cosmetic Proteins.

Cosmetic Protein	Active Level Supplied	Preservatives	Source	Hydrolysis Method	Molecular Weight (Da)
Crosilk Lpc	12.7	0.5% w/w sodium benzoate + w/w 0.01% disodium EDTA	Silk Noils	Acid/Alkaline	200
Hydroavena HPO	22.4	0.5% w/w sodium benzoate + w/w 0.3% potassium sorbate	Oat	Enzymatic	1000
Hydrolactin Liquid PE	18.3	1% w/w phenoxyethanol + w/w 0.2% disodium EDTA	Milk - Casein	Enzymatic	1800
Hydrosativum P	20.3	1% w/w phenoxyethanol + w/w 0.3% potassium sorbate	Pea	Enzymatic	1500
Hydrosolanum PE	20.8	1% w/w phenoxyethanol + 0.3% w/w potassium sorbate + 0.2% w/w disodium EDTA	Potato	Enzymatic	750
Prolevium	21.3	1% w/w phenoxyethanol + 0.3% w/w potassium sorbate + 0.2% w/w disodium EDTA	Cottonseed	Enzymatic	800

Table 7. Amino Acid Content of the Cosmetic Proteins

w/w %	Crosilk Liquid Protein Complex (LPC)	Hydroavena HPO	Hydrolactin Liquid PE	Hydrosativum P	Hydrosolanum PE	Prolevium
Alanine	28.4	5.1	2.9	4.74	5.0	4.1
Arginine	1.5	5.3	3.6	8.36	5.2	12.2
Aspartic Acid	4.7	7.4	6.5	12.37	12.1	10.3
Cys[7]	0.1	2.5	0.4	1.75	0.6	1.2
Glutamic Acid	4.1	25.8	20.5	18.82	13.6	22.7
Glycine	34.7	5.3	1.8	3.92	4.3	4.2
Histidine	0.8	2.4	2.7	2.88	2.7	5.0
Isoleucine	0.8	3.5	5.8	4.2	4.3	3.1
Leucine	1.2	7.8	8.8	7.91	9.6	5.9
Lysine	1.4	4.4	7	8.52	7.7	4.0
Methionine	0.2	2	2.7	0.98	2.2	1.4
Phenylalanine	0.9	5.4	4.7	3.72	4.7	5.5
Proline	1.2	6.6	10.4	4.31	5.4	3.7
Serine	15.4	6	5.9	6.18	6.1	5.4
Threonine	1.9	4.1	3.8	4.14	6.1	4.0
Tyrosine	0.6	1.2	5.1	2.41	4.9	2.9
Valine[8]	2	5.4	6.5	4.8	5.5	4.5

[7] May be in form of cystine, cysteine, cysteic acid or lanthionine

[8] Tryptophan content was only available for Hydrolactin Liquid PE- found at 1.2%. It is likely that similar tryptophan levels would be found in the enzymatically digested proteins but not found in the acid hydrolysed proteins

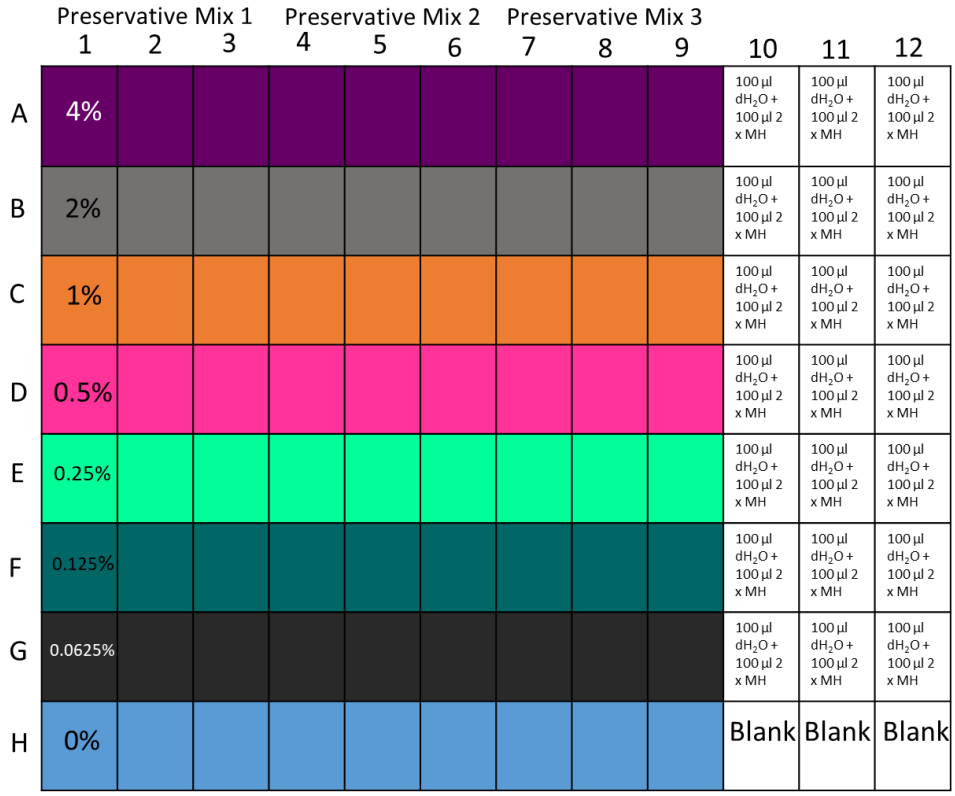


Figure 8. Layout of MIC plate and Preservative Concentrations as Found at Active Levels. Each row was a dilution of 1 in 2 dilution of the previous row, except for the last row which was minimal medium alone.

3.19 Results

3.20 Using Lactose Free Skimmed-Milk Agar to Assess Whether the Bacteria Possess Extracellular Proteases

Both *Staphylococcus* spp. produced hydrolysed clearing zones and therefore tested positive for extracellular proteases. While colonies of *C. jeikeium* grew on the agar, no zones of clearing were observed and therefore do not possess extracellular proteases. See Figure 9 below.

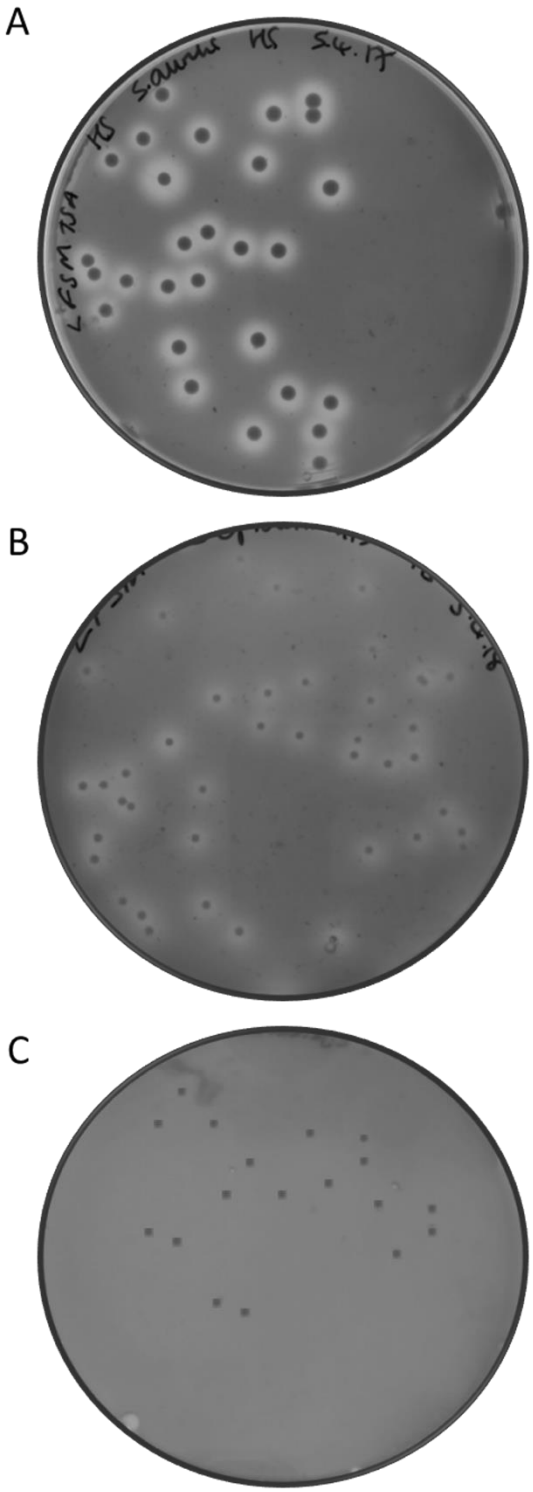


Figure 9. **Extracellular Protease Production from The Three Skin Bacteria.** A) *Staphylococcus aureus* B) *Staphylococcus epidermidis* C) *Corynebacterium jeikeium*. Both *Staphylococcus* spp. display a clear zone of clearance around the colonies and therefore possess extracellular proteases. *C. jeikeium* colonies do not have a clear halo and therefore unlikely to possess these extracellular proteases.

3.21 Do the Preservatives found in the Proteins reach the Minimum Inhibitory Concentration or Limit Bacterial Growth?

To be classed as the minimum inhibitory concentration, 80% of total growth yield must be inhibited by addition of the preservative or preservative combination (Rushton et al. 2013). Both *Staphylococcus* spp. grew to their stationary phase within 24 hours as confirmed by the in-unison growth curves. The stationary phase was not achieved by *C. jeikeium* therefore the MIC could not be estimated. It was decided not to carry on the experiment for more than 24 hours as the proteins would be unlikely to be in contact for longer than that period on the skin. All results from this Section refer to the MIC values found in Figure 10, Figure 11 and Figure 12. All inhibition values and statistical data can be found in Table 16, A, B and C, in the Appendix, in Section 10.1.

3.21.1 Do the Preservatives found in Crosilk Lpc inhibit Visible Growth of Three Common Skin Bacteria?

At the active level in which the protein is originally sold (Section 3, Table 6), Crosilk Lpc was preserved with 0.5% sodium benzoate and 0.01% disodium EDTA. *S. aureus* (Figure 10) was, on average, inhibited 99.7% when grown with 4% active level of Crosilk Lpc and 90.3% inhibited when grown with 2%, however at 1% active level, inhibition was at 43.5%, therefore the MIC would be between 1.8-2% active. Below 1% active level, Crosilk Lpc preservatives inhibited between 15.2 and 2.07% dependent on active level. Similar results were observed with *S. epidermidis* (Figure 11), at 4% active level, the Crosilk Lpc inhibited the growth of *S. epidermidis* 100%, while at 2% the bacteria were inhibited 97.6%. At 1% active level, *S. epidermidis* was only inhibited by 46.3%, which puts the MIC likely between 1.7%-2% active level. *C. jeikeium* (Figure 12) was only inhibited by more than 80% when grown with 4% active level, Crosilk, at 2% or below it was inhibited between 41.4-2.28%. However, Crosilk significantly inhibited more *C. jeikeium* growth than *S. epidermidis* at, 0.5% ($P=0.0214$), 0.25% ($P=0.0054$) and 0.125% ($P=0.0024$) active level. When grown with 0.5% and 0.25% active level of Crosilk Lpc, *C. jeikeium* was significantly more inhibited than *S. aureus* ($P<0.05$). While *S. epidermidis* was significantly more inhibited by Crosilk at 2% than *S. aureus* was ($P<0.001$), at 0.5%, 0.125% and 0.0625% it was vice versa ($P<0.05$).

3.21.2 Do the Preservatives found in Hydroavena HPO inhibit Visible Growth of Three Common Skin Bacteria?

At the active level supplied, Hydroavena HPO was preserved with 0.5% sodium benzoate and 0.3% potassium sorbate. *S. aureus* (Figure 10) growth was inhibited by 93.2% when grown with 4% active level of Hydroavena HPO preservatives, but only 59.4% when grown with 2%. The MIC of Hydroavena preservatives on *S. aureus* would be upwards of 3.2% active level. At 1% active level and below, *S. aureus* was inhibited between 20.2-4.29%. Hydroavena preservatives inhibited

98.3% of *S. epidermidis* (Figure 11) when grown with 4% of the protein, while just 62.9% was inhibited at 2%. Therefore, the MIC was between 3-4% active level. At 1% and below, inhibition ranged from 16.9 to 1.03%. *C. jeikeium* (Figure 12) was inhibited by 87.4% when grown with 4% Hydroavena preservatives, and just 46.4% when grown with 2%. However, even at the lower active levels, most inhibited *C. jeikeium* growth more than either *Staphylococcus* spp.. From 0.0625-1% active levels, Hydroavena preservatives significantly inhibited *C. jeikeium* more than *S. epidermidis* ($P<0.0007$) and *S. aureus* (between $P<0.0001$ and $P=0.01520$). At 4% and 2% active levels, Hydroavena preservatives inhibited significantly more of *S. epidermidis*' growth than *S. aureus* ($P<0.0471$), but at 1% and below this was reversed ($P<0.0103$).

3.21.3 Do the Preservatives found in Hydrolactin Liquid PE inhibit Visible Growth of Three Common Skin Bacteria?

Hydrolactin Liquid PE is preserved with 1% phenoxyethanol and 0.2% disodium EDTA at the active level sold. The 80% inhibition for the MIC was not met against *S. aureus* (Figure 10). Hydrolactin preservatives inhibited *S. aureus* by 70.5% with the equivalent found at 4% active level. At 2% active level and below, the preservatives inhibit *S. aureus* between 0.0-19.3%. Hydrolactin preservatives inhibited *S. epidermidis* (Figure 11) by 97.7% with the 4% active level and 42.4% with the 2% active level. The MIC of the Hydrolactin preservatives would be around the 2.6% active level mark. At 4% and 2% active levels, the Hydrolactin preservatives only inhibited *C. jeikeium* (Figure 12) by 67.3% and 48.3% respectively. However, between 1% and 0.125%, the inhibition of *C. jeikeium* was significantly more than either *S. epidermidis* ($P<0.0016$) or *S. aureus* ($P<0.001$). Apart from a slight significant difference at 2% when *S. aureus* is more inhibited than *S. epidermidis* ($P=0.0152$), there is no significant difference between the two staphylococcal species at any other active level ($P>0.05$).

3.21.4 Do the Preservatives found in Hydrosativum P inhibit Visible Growth of Three Common Skin Bacteria?

Hydrosativum P is preserved with 1% phenoxyethanol and 0.3% potassium sorbate at the active level sold. The minimum inhibitory concentration was never reached for any of the species, both *Staphylococcus* spp. were only inhibited at 18.6% and 21.6% at what would be the highest dose (Figure 10, Figure 11). While there are some overlaps of inhibition percentages at different active levels, they all follow the expected trend. Similar high standard deviations were noted with *C. jeikeium* (Figure 12) but there was significantly greater inhibition between 4% and 0.5% than the other two species ($P<0.0365$).

3.21.5 Do the Preservatives found in Hydrosolanum PE inhibit Visible Growth of Three Common Skin Bacteria?

Hydrosolanum PE is preserved with 1% phenoxyethanol, 0.3% potassium sorbate and 0.2% disodium EDTA at the active level sold. The minimum inhibitory concentration was not reach

when *S. aureus* (Figure 10) was exposed to the preservatives. Between 0.0625% and 4% active levels, *S. aureus* was inhibited by between 0-64.6%. With *S. epidermidis* (Figure 11), the Hydrosolanum preservatives inhibited 94.7% of growth at 4% active level and 44.7% at 2% active level. These results would suggest that the MIC of this preservative set would be upwards of 2.68% against *S. epidermidis*. At these higher preservative concentrations, *S. aureus* is significantly less inhibited than *S. epidermidis* when grown with the preservatives found in Hydrosolanum PE ($P<0.0028$). Again, while the MIC cannot be determined when grown with *C. jeikeium* (Figure 12), the lower preservative concentrations still inhibited significantly more growth than both *Staphylococcus* spp. ($P<0.0221$).

3.21.6 Do the Preservatives found in Prolevium inhibit Visible Growth of Three Common Skin Bacteria?

Prolevium was preserved with 1% phenoxyethanol, 0.3% potassium sorbate and 0.2% disodium EDTA at its sold active level. Again, the MIC was not reached for *S. aureus* (Figure 10), with the preservatives at 4% active level only inhibiting on average 64.9% of growth. Between 0.0625% and 2% active level, *S. aureus* was inhibited 0-21.6%. The growth of *S. epidermidis* however was inhibited 94.3% by the presence of the Prolevium preservatives at 4% active level. As the preservatives at 2% active level inhibited *S. epidermidis* by 47.4%, the MIC would be around 2.68% and above. At 0.5% and below, the susceptibility shifts, and more of *S. aureus* growth is significantly inhibited than *S. epidermidis* (Figure 11). For example, at 0.5% active level, *S. aureus* is inhibited, on average, twice as much and significantly more than *S. epidermidis* ($P=0.0411$). At the higher active levels, the preservatives do not inhibit *C. jeikeium* (Figure 12) to the extent of the other two species, but as with the other preservative measures, at the lower active levels, the preservatives inhibit *C. jeikeium* significantly more than both *Staphylococcus* spp. Inhibition was most significant at 0.125% active level where *C. jeikeium* was inhibited by 13.3% compared to *S. aureus* at 7.8% and *S. epidermidis* at 1.43% ($P<0.02$).

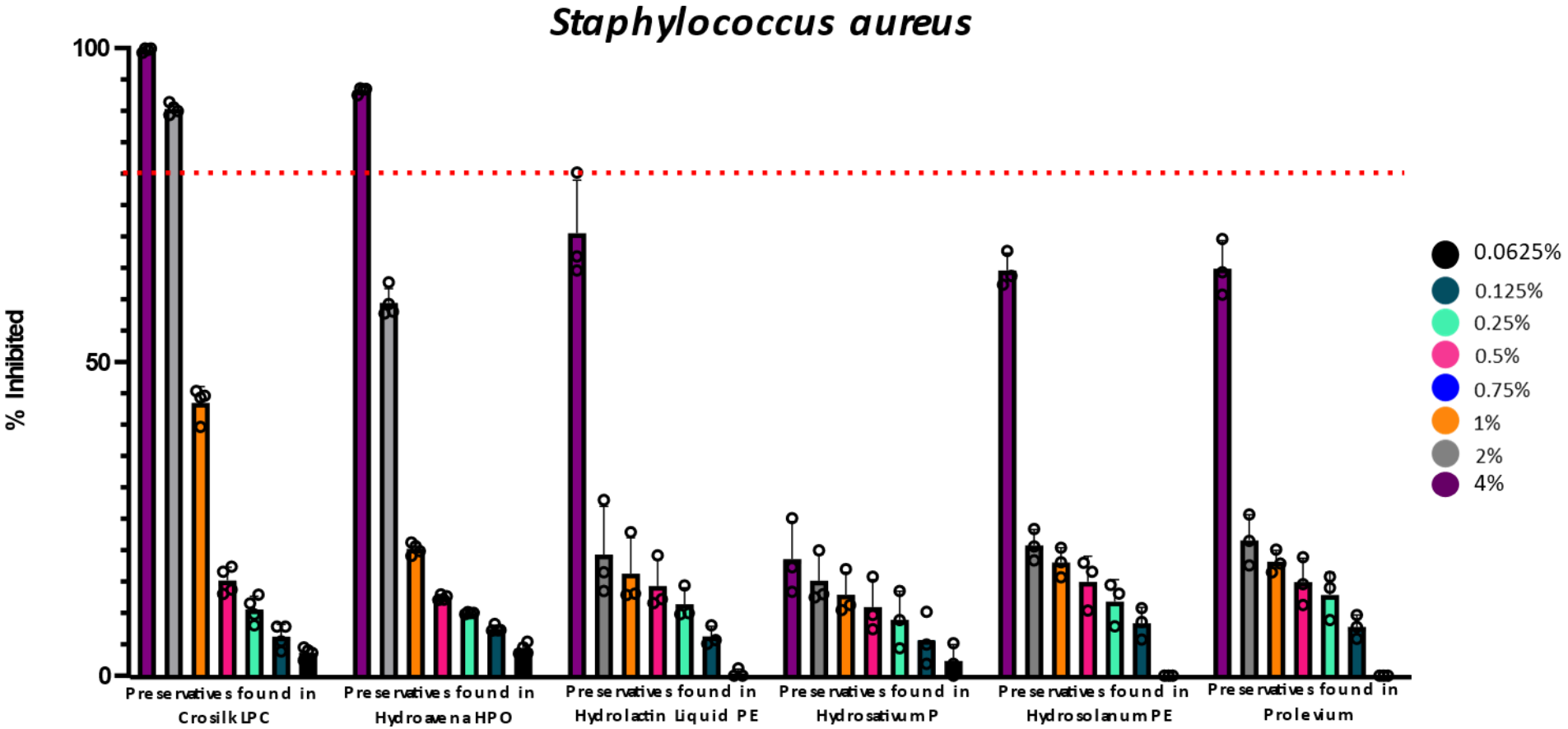


Figure 10. Inhibition of *Staphylococcus aureus* by Preservatives found in Cosmetic Proteins. The experiment was repeated with three technical and biological replicates. The results seen in this Figure are discussed in Section 3.21. The red dotted line indicates the 80% inhibition threshold for the minimum inhibitory concentration. Only two preservative combinations met the inhibition percentage to be classed as the MIC.

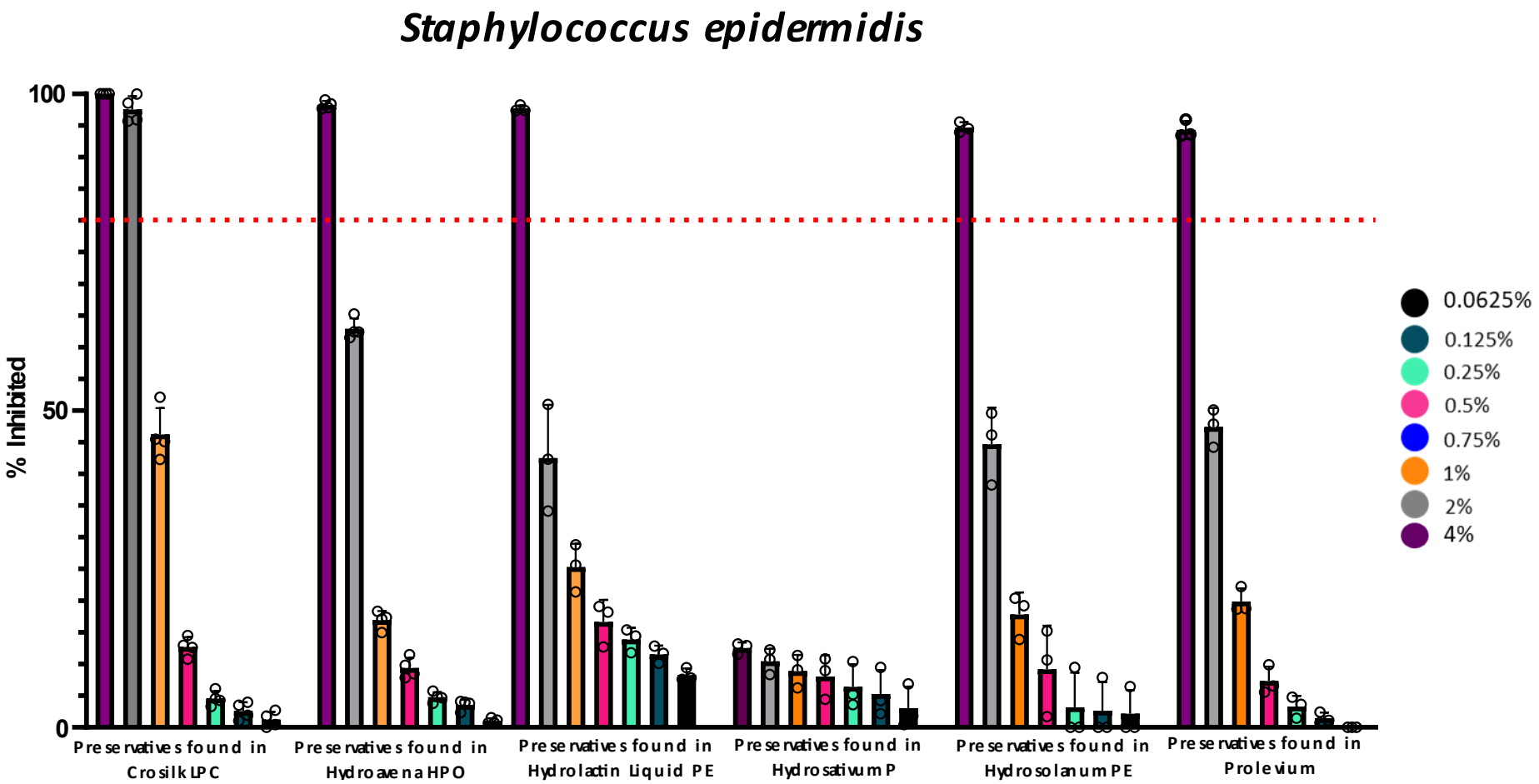


Figure 11. Inhibition of *Staphylococcus epidermidis* by Preservatives found in Cosmetic Proteins. The experiment was repeated with three technical and biological replicates. The results seen in this Figure are discussed in Section 3.21. The red dotted line indicates the 80% inhibition threshold for the minimum inhibitory concentration. Five preservative combinations met the percentage threshold to be classed as the MIC.

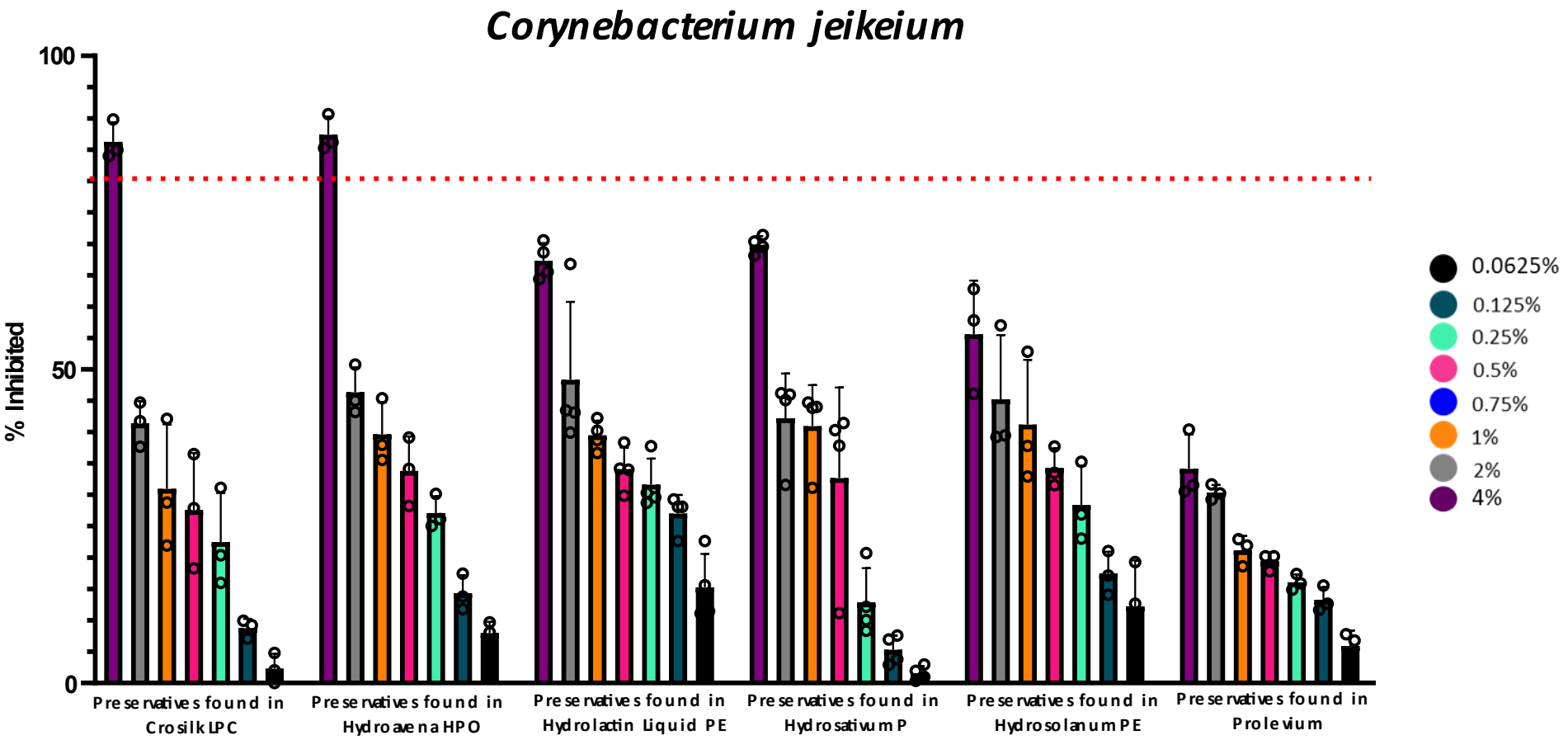


Figure 12. Inhibition of *Corynebacterium jeikeium* by Preservatives found in Cosmetic Proteins. The experiment was repeated with three technical and biological replicates. The results seen in this Figure are discussed in Section 3.21. The red dotted line indicates the 80% inhibition threshold for the minimum inhibitory concentration. Only two preservative combinations met the inhibition percentage to be classed as the MIC.

3.22 Using Growth Curves to Determine Whether Cosmetic Proteins Can Supplement Bacterial Growth

At 0% active level, which was minimal medium without a protein supplement, no growth was recorded for all three bacterial species.

3.22.1 Growth Curves of Three Skin Bacteria Supplemented with Crosilk Lpc

All results from this Section are referring to graphs in Figure 13. When grown with Crosilk, a classic growth curve was only observed at 0.125% active level for all three species. A slight growth with 0.25% active level was started by *C. jeikeium* at approximately 18 hours, but not enough to conduct statistical analysis. The exponential start time for both *Staphylococcus* spp. began at 10.5 hours, while the exponential phase for *C. jeikeium* started 6 hours earlier at 4.5 hours. At 0.125%, *S. aureus* had an exponential growth rate of 0.34 OD/hour, while *S. epidermidis* had a slightly slower rate of 0.31 OD/hour, with the slowest growth rate being *C. jeikeium* at 0.16 OD/hour. Total yield for both *S. aureus* and *S. epidermidis* was an of OD 0.026, but *C. jeikeium* had the highest yield with an OD reading of 0.032. There was no significant difference between the two *Staphylococcus* spp. growth curves at 0.125% active level ($P=0.92$), but both *S. aureus* and *S. epidermidis* had significantly different growth curve patterns than *C. jeikeium* ($P<0.0001$).

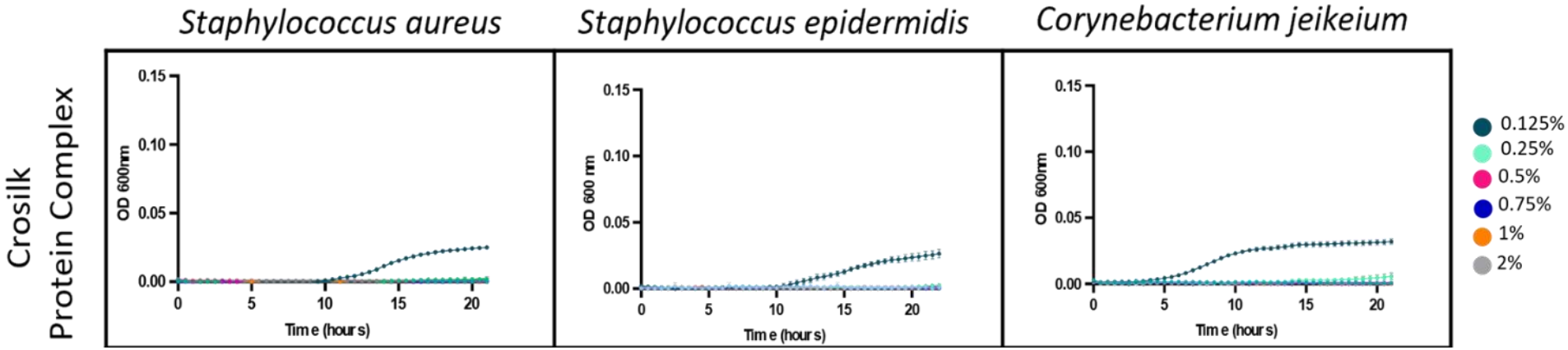


Figure 13. Growth Curves of Three Skin Bacteria Supplemented with Crosilk Lpc. The experiment was performed as three technical replicates and three biological replicates. Results were discussed in Section 3.22.1. A traditional growth curve was observed when all three species were supplemented with 0.125% active level of Crosilk LPC. Neither *Staphylococcus* spp. grew with any other active level supplemented and only a small growth was beginning to be observed when *C. jeikeium* grew with 0.25% Crosilk LPC. There was no significant difference between the growth curve pattern of the *Staphylococcus* spp. ($P=0.96$), but they were both significantly different to the *C. jeikeium* growth curve with 0.125% Crosilk ($P<0.05$).

3.22.2 Growth Curves of Three Skin Bacteria Supplemented with Hydroavena HPO

All results from this Section are referring to graphs in **Figure 14. Growth Curves of Three Skin Bacteria Supplemented with Hydroavena HPO.**Figure 14.The addition of Hydroavena HPO to the minimal medium provided different growth curve patterns for all three species. Both *Staphylococcus* spp. grew with 1% active level Hydroavena HPO, while no growth at any active level was observed of *C. jeikeium* until around 19 hours when the start of some growth at 0.125% and 0.25% active level were observed. The log phase of the 0.125% and 0.25% growth curves started 5.5 hours, for 0.5% and 0.75% the lag phase started at 8 hours, while 1% active level the exponential phase started at 10.5 hours. No growth as observed at 2% active level. The growth rate of *S. aureus* when grown with 0.125% was 0.19 OD/hour, 0.25% was slightly faster at 0.24 OD/hour, at 0.5% the growth rate was 0.32 OD/hour, at 0.75% was 0.38 OD/hour and at 1% active level the growth rate was 0.45 OD/hour. The total OD yield reached for *S. aureus* when grown with 0.125% active level was 0.048, when grown with 0.25% active level was 0.099, 0.5% total yield was 0.110, 0.75% total yield was 0.063 and at 1% active level, the total OD for yield was 0.037. There were no significant differences between the growth of *S. aureus* with 0.125% and 0.5% Hydroavena HPO ($P=0.42$). There were also no significant differences between the growth curves produced by *S. aureus* at 0.25% active level and 0.5% active level ($P>0.05$). However, there were significant differences of the growth of *S. aureus* between all other active levels ($P<0.04$). The lag phase of *S. epidermidis* started at 7.5 hours with both 0.125% and 0.25% active levels, 8.5 hours with 0.5% active level, 14 hours with 0.75% and there was a slight growth starting at 17.5 hours with 1%. Again, no growth was observed with 2% active level Hydroavena HPO. The growth rate for *S. epidermidis* when grown with 0.125% active level Hydroavena HPO was 0.31 OD/hour, 0.32 OD/hour with 0.25%, 0.39 OD/hour with 0.5%, 0.38 OD/hour with 0.75% and 0.22 OD/hour with 1% active level Hydroavena HPO. The total OD yield reached by *S. epidermidis* varied when grown at each different active level, with 0.125% it reached 0.118, at 0.25% the bacteria reached 0.119, at 0.5% the total yield was 0.103, at 0.75% the yield was 0.022 and at 1% the total yield was 0.011. When grown with 2% Hydroavena HPO, *S. epidermidis* did not increase significantly past the starting OD ($P>0.05$). There was no significant difference between the growth curves of *S. epidermidis* when grown with 0.125%. 0.25% and 0.5% active levels Hydroavena HPO ($P>0.10$). There was also no significant difference in the growth of *S. epidermidis* when grown with 0.75% and 1% active levels ($P=0.104$). There was however a statistical difference between the lower active levels (0.125-0.5%) and the upper active levels (0.75%-2%) ($P<0.0001$). The small growth observed by *C. jeikeium* at any active level was not enough to statistically analyse. Until grown with 0.75% active level, there was no statistical difference between the growth of *S. aureus* and *S. epidermidis* ($P>0.145$). When grown with 0.75%, 1% and 2% active level Hydroavena, the two species' growth significantly differs ($P<0.016$). Both *S. epidermidis* and *S. aureus* significantly differ

in growth at all active levels compared to *C. jeikeium* ($P<0.001$).

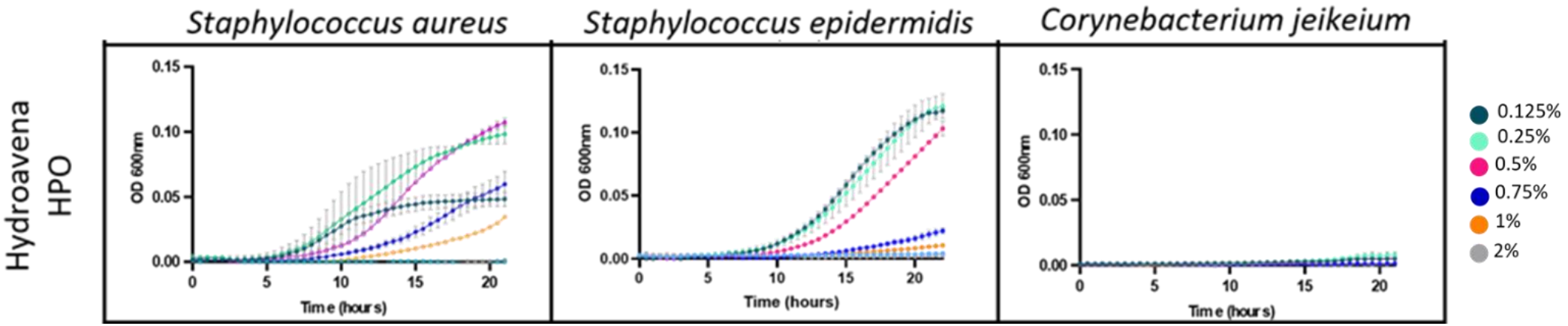


Figure 14. Growth Curves of Three Skin Bacteria Supplemented with Hydroavena HPO. Results from this figure were discussed in Section 3.22.2. The experiment was performed as three technical replicates and three biological replicates. Both *Staphylococcus* species grew with the greatest number of active levels of Hydroavena HPO, while there was barely any growth recorded of *C. jeikeium* with any of the active levels. There was no significant difference between growth cures of *S. aureus* when grown with 0.125% and 0.5%, as well as 0.25% and 0.5% Hydroavena HPO (P>0.05). There was no significant difference between the growth curves of *S. epidermidis* when grown with 0.125%. 0.25% and 0.5% active levels Hydroavena HPO (P>0.10).

3.22.3 Growth Curves of Three Skin Bacteria Supplemented with Hydrolactin Liquid PE

All results from this Section are referring to graphs in Figure 15. *Staphylococcus aureus* did not achieve a high yield with the addition of Hydrolactin Liquid PE, while there was growth at all active levels the total yield was low. The log phase of growth of *S. aureus* started at the same time for all active levels, at 9.5 hours. If allowed to grow exponentially, the growth rate gradually declined as the active levels increased starting at 0.27 OD/hour when grown with 0.125% active level Hydrolactin, 0.26 OD/hour when grown with 0.25%, 0.24 OD/hour with 0.5%. 0.23 OD/hour with 0.75%, 0.21 OD/hour with 1% and 0.19 OD/hour when grown with 2% active level Hydrolactin Liquid PE. The total yield for *S. aureus* when grown with Hydrolactin also decreased as the active levels increased. The total OD yield when *S. aureus* was grown with 0.25% was 0.012, 0.011 with 0.25%, 0.008 with 0.5, 0.75% and 1% and 0.005 when grown with 2% Hydrolactin Liquid PE. Each growth curve is not statistically different from the previous growth curve, so growth at 2% active level is not statistically different from growth at 1%, which in turn is not significantly different from growth at 0.5% and so on ($P>0.05$). There is also no statistical difference between all the growth curves of *S. aureus* when grown with Hydrolactin Liquid PE between 0.125-1% active level. The only outlier is at 2% which is significantly different to 0.75% active level and below ($P<0.0193$). While total yield again was poor when *S. epidermidis* was grown with Hydrolactin Liquid PE, *S. epidermidis* grew at every active level. The start of the log phase for *S. epidermidis* when grown with 0.125% active level Hydrolactin started at 9 hours, with 0.25% exponential growth started at 8.5 hours, while when grown with both 0.5% and 0.75% the log phase started at 6.5 hours. *S. epidermidis* went into the log phase of growth at 5.5 hours when grown with 1% and 2% active level. Unlike previous proteins, the growth rate of *S. epidermidis* quickens as the active levels increase, starting at 0.256 OD/hour with 0.125% active level and ending up at 0.286 OD/hour with 2%. The total OD yield also increased as the active levels increased, at 0.125% the total OD yield was 0.013, which more than doubled when grown with 2% active level which was 0.033. Again, no growth curve was significantly different from the growth curve directly before it ($P>0.05$). There was a significant difference between growth at 2% and 0.5%, 0. 25% and 0.125% ($P<0.05$), as well as at 1% and 0.25% and 0.125% ($P<0.0105$), 0.75% and 0.125% ($P=0.0036$).

C. jeikeium achieved the highest total yield compared to the *Staphylococcus* spp. when grown with Hydrolactin Liquid PE. However, growth was only recorded with 0.125% and 0.25%. The log phase of growth for *C. jeikeium* when growth with both 0.125% and 0.25% Hydrolactin Liquid PE started at 10.5 hours. The growth rate of *C. jeikeium* when grown with 0.125% was 0.24 OD/hour, while growth at 0.25% was slightly slower at 0.20 OD/hour. The total yield for growth at 0.125% was an OD of 0.126 and slightly higher with 0.25% at an OD reading of 0.143. However, there was no statistical difference between the overall growth of *C. jeikeium* when grown with 0.125% or

0.25% active levels of Hydrolactin Liquid PE. Where growth was recorded all other growth curves between all three species were significantly different ($P<0.05$), except when both *Staphylococcus* species were grown with 0.125% active level there was no significant ($P=0.52$) differences between the growth patterns.

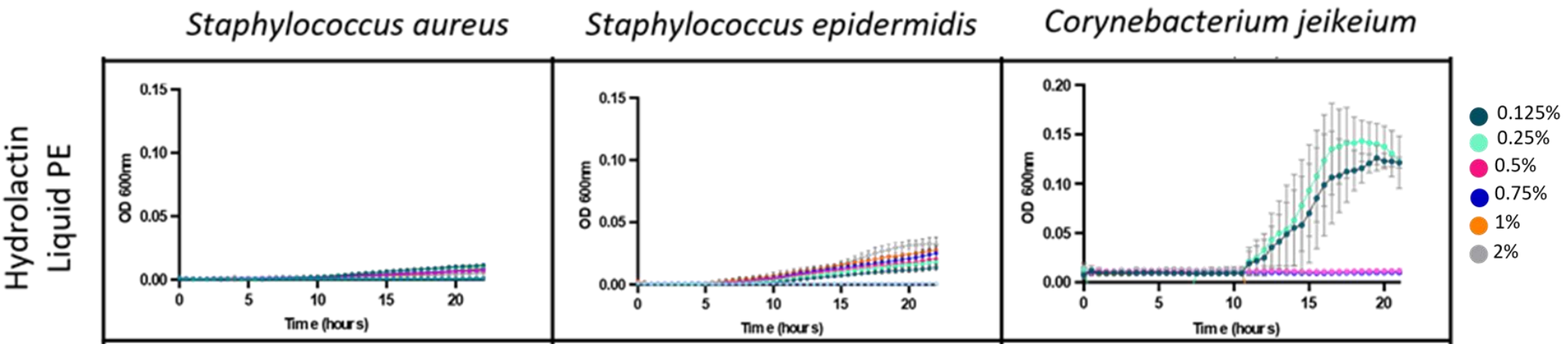


Figure 15. Growth Curves of Three Skin Bacteria Supplemented with Hydrolactin Liquid PE. The experiment was performed as three technical replicates and three biological replicates. The results from the figure above were discussed in Section 3.22.3. *S. aureus* did not grow well with Hydrolactin Liquid PE, and while *S. epidermidis* grew at every active level, the total yield was low. The highest yield with Hydrolactin was with *C. jeikeium*, however growth was only seen with 0.125% and 0.25% active. Each growth curve of *S. aureus* was not statistically different from the previous growth curve, so growth at 2% active level is not statistically different from growth at 1%, which in turn is not significantly different from growth at 0.5% and so on (P>0.05). Again, for *S. epidermidis*, no growth curve was significantly different from the growth curve directly before it (P>0.05). There was no statistical difference between the growth curves of *C. jeikeium* when grown with 0.125% or 0.25% active levels of Hydrolactin Liquid PE (P>0.05).

3.22.4 Growth Curves of Three Skin Bacteria Supplemented with Hydrosativum P

All results from this Section are referring to graphs in Figure 16. *S. aureus* grew well with the addition of various levels of Hydrosativum P, growth was observed even with 2% active level. The log phase of growth started at 5 hours when *S. aureus* was grown with 0.125%, 0.25%, 0.5%, 0.75% and 1% active levels of Hydrosativum P. When grown with 2% active level, *S. aureus* started the log phase of growth at 13 hours. The exponential growth rate of *S. aureus,* when grown with Hydrosativum P, increased as the active levels increased, starting at 0.17 OD/hour with 0.125% active level, 0.19 OD/hour with 0.25%, 0.24 OD/hour with 0.5%, 0.27 OD/hour with 0.75%, 0.31 OD/hour with 1% and 0.33 OD/hour with 2% active level. The total yield also increased as the active levels increased, excepting with 2% where growth had only begun when the experiment ended. With 0.125% active level, *S. aureus* had a total OD yield of 0.074, 0.117 with 0.25% active level, 0.180 with 0.5%, 0.190 with 0.75% and 0.203 with 1% active level. At the end of the experiment, although growth had started, *S. aureus* had only reached an OD of 0.345 with 2% active level Hydrosativum P. There was no significant difference between the growth curves of *S. aureus* with 0.25%, 0.5%. 0.75% and 1% active level Hydrosativum P ($P>0.231$). Growth with both 0.125% and 2% created the outliers and *S. aureus* grew significantly different with both active levels ($P<0.038$) compared to each other and all other active levels.

S. epidermidis grew with the addition of Hydrosativum P, however, growth started very late with 0.75% and 1% active level and no growth was seen with 2% active level. The start log phase of *S. epidermidis* differed, dependent on what active level it was grown with. With 0.125% active level, the log phase of growth started at 7 hours, with 0.25% the log phase started at 9.5 hours, with 0.5% the log started at 14 hours, with 0.75% the log phase started at 16.5 hours, while *S. epidermidis* did not enter the log phase of growth with either 1% or 2% active levels of the product. The growth rate of *S. epidermidis* increased as the active level increased to 0.5% before slowing again. With 0.125% active level, *S. epidermidis* grew exponentially at a rate of 0.25 OD/hour, with 0.25% at 0.32 OD/hour, with 0.5% at 0.41 OD/hour, with 0.75% at 0.31 OD/hour. The total OD yield reached by *S. epidermidis* when grown with 0.125% active level of Hydrosativum P was 0.56, with 0.25% it was 0.056, with 0.5% was 0.37, with 0.75% was 0.019. Although no log phase was entered, *S. epidermidis* OD readings increased over time when grown at both 1% and 2% active levels and achieved a low total yield OD reading of 0.007 and 0.004, respectively. There was no significant difference between the growth of *S. epidermidis* with 0.125% and 0.25%, as well as between 0.75% and 1% active levels ($P>0.05$). The growth of *S. epidermidis* was significantly different between all other active levels ($P<0.05$).

Again, growth of *C. jeikeium* was only observed with 0.25% and 0.125% active levels of Hydrosativum P. When grown with 0.125%, *C. jeikeium* entered a growth phase at 6.5 hours, which slowed before entering another log phase at 14 hours, before beginning to enter a

stationary phase at 21 hours. When grown with 0.125% active level of protein, *C. jeikeium* entered the one and only log phase at 4.5 hours. The growth rate of *C. jeikeium* with 0.125% active level was calculated at 0.17 OD/hour, while with 0.25% 0.33 OD/hour. However, due to the unconventional shape of the growth curve this may be inaccurate. There was a significant difference of growth between *C. jeikeium* with 0.125% and 0.25% active level of Hydrosativum P ($P<0.001$). All growth curves between all species were significantly different ($P<0.05$), except between *S. epidermidis* and *C. jeikeium* when both grown with 0.25% active level of Hydrosativum P ($P=0.998$).

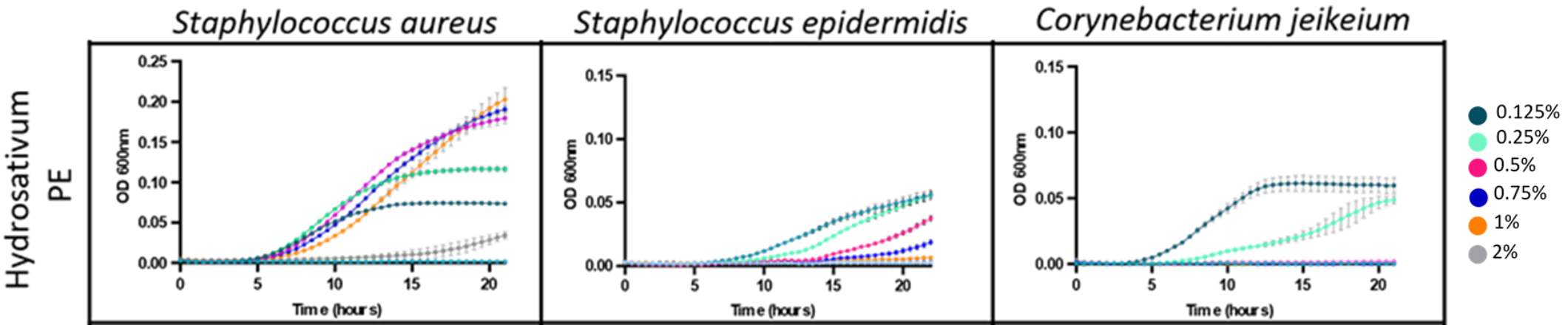


Figure 16. Growth Curves of Three Skin Bacteria Supplemented with Hydrosativum P. The experiment was performed as three technical replicates and three biological replicates. The results from the above figure were discussed in Section 3.22.4. *S. aureus* had growth at various active levels with Hydrosativum P, *S. epidermidis* grew but very little growth seen with the higher active levels, growth of *C. jeikeium* was only seen with 0.125% and 0.25% active. There was no significant difference between the growth curves of *S. aureus* with 0.25%, 0.5%. 0.75% and 1% active level Hydrosativum P ($P>0.231$). There was no significant difference between the growth of *S. epidermidis* with 0.125% and 0.25%, as well as between 0.75% and 1% active levels ($P>0.05$). There was a significant difference of growth between *C. jeikeium* with 0.125% and 0.25% active level of Hydrosativum P ($P<0.001$).

3.22.5 Growth Curves of Three Skin Bacteria Supplemented with Hydrosolanum PE

All results from this Section are referring to graphs in **Error! Reference source not found.**. When *S . aureus* was grown with Hydrosolanum P, there was growth at every active level, except at 2% active level where there was only a slight increase from the starting OD to the end OD. There was, however, a long lag phase of growth and log phase of growth had just started by the time of the last reading. Growth of the *S. aureus* started at 9 hours with 0.125-1% active levels. The growth with 2% active level was so slight it was not possible to calculate a start time. The growth rate of *S. aureus* was calculated at 0.274 OD/hour with 0.125%. 0.279 OD/hour with 0.25%, 0.3202 OD/hour with 0.5%, 0.329 OD/hour with 0.75% and 0.327 OD/hour with 1% active level. Total yield was not reached by *S. aureus* when grown with any active level as the stationary phase was not attained, however, the final OD were similar across active levels 0.125-1% active level. *S. aureus* total OD of 0.0294 with 0.125%, 0.0382 with 0.25%, 0.0425 with 0.5%, 0.397 with 0.75%, 0.0306 with 1%. When grown with 2%, *S. aureus* reached a low OD of 0.006, which was still an 80% increase from the starting OD. There was no statistical difference between the growth of *S. aureus* with the active levels 0.125-1% ($P=0.31$). However, growth with 2% active level was significantly different from growth with all other active levels ($P<0.001$).

A similar pattern was seen when *S. epidermidis* was grown with Hydrosolanum PE. There was a long lag phase, the growth phase of *S. epidermidis* started at 11 hours with all the active levels where clear growth was recorded, 0.125%-1% active level. The growth rate of *S. epidermidis* when grown with 0.125% active level was 0.302 OD/hour, 0.211 OD/ hour with 0.25%, 0.167 OD/ hour with 0.5% and 0.136 OD/hour with 0.75%. *S. epidermidis* grew so gradually with 1% and 2% active level that any exponential growth rate could not be calculated. The final OD reached by *S. epidermidis* when grown with 0.125% active level of Hydrosolanum PE was 0.0192, 0.0151 with 0.25%, 0.009 with 0.5%, 0.007 with 0.75%, 0.008 with 1% and 0.003 with 2% level. There were no significant differences between the growth of *S. epidermidis* when grown with 0.125-0.5% active ($P=0.12$), or between growth with 0.5%-1% active ($P=0.07$). Growth with all active levels 0.125-1%, were significantly different from growth with 2% active level ($P<0.005$).

C. jeikeium only grew with one active level of Hydrosolanum PE, at 0.125%. Exponential growth of *C. jeikeium* started at 15 hours and appeared to just reach a stationary phase at 21 hours. The growth rate of *C. jeikeium* with 0.125% active level was 0.25 OD/hour and the total yield reach an OD reading of 0.021. There was no significant difference between the growth of *S. aureus* at 0.125% and the growth of *C. jeikeium* at 0.125% ($P=0.097$) or between *S. epidermidis* and *C. jeikeium* also with 0.125% active level of Hydrosolanum PE ($P=0.1051$). However, there was a significant difference in growth between *S. aureus* and *S. epidermidis* ($P=0.004$) with 0.25% active level, as well as a significant difference between the three bacteria at all active levels of Hydrosolanum PE ($P>0.05$).

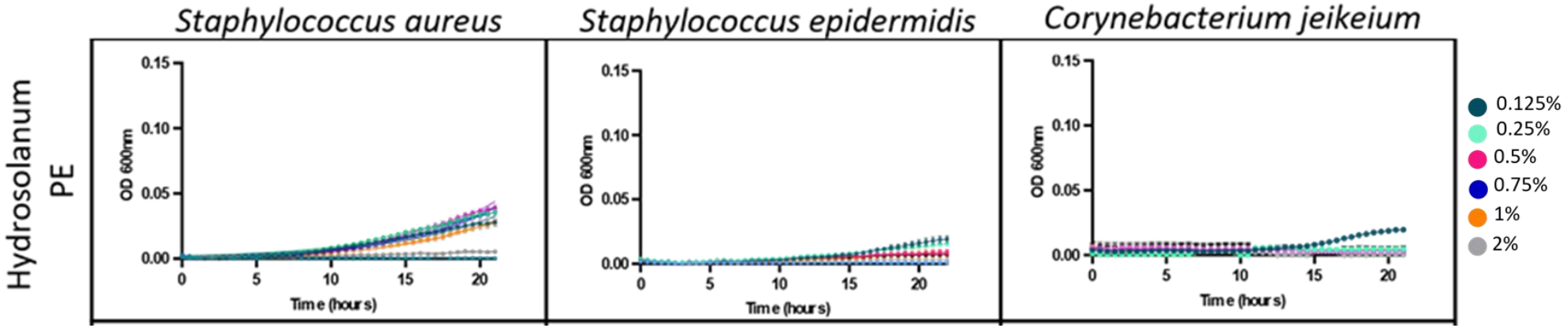


Figure 17. Growth Curves of Three Skin Bacteria Supplemented with Hydrosolanum PE. The experiment was performed as three technical replicates and three biological replicates. The results from the figure above were discussed in 3.22.5. All three achieved very little yield when supplemented with Hydrosolanum PE, *S. aureus* had the higher total yield and *S. epidermidis* had some growth at the higher active levels, but *C. jeikeium* only grew with 0.125% and the yield was low. There was no statistical difference between the growth of *S. aureus* with the active levels 0.125-1% (P=0.31). There were no significant differences between the growth of *S. epidermidis* when grown with 0.125-0.5% active (P=0.12), or between growth with 0.5%-1% active (P=0.07).

3.22.6 Growth Curves of Three Skin Bacteria Supplemented with Prolevium

All results from this Section are referring to graphs in Figure 18. *S. aureus* grew with all active levels of Prolevium, although growth was very stagnant with 2% active level of the cosmetic protein. The log phase of growth started at 5.5 hours for *S. aureus* grown with 0.125-0.75% active level of Prolevium, with 1% active the log phase started at 6.5 hours. As the growth of *S. aureus* with 2% active level was so gradual an exponential growth was never achieved in the 21 hours. The growth rate of *S. aureus* with 0.125% active level of Prolevium was 0.176 OD/hour, 0.154 OD hour with 0.25%, 0.176 OD/hour with 0.5%, 0.247 OD/hour with 0.75% and 0.261 OD/hour with 1% active level. Due to the growth pattern of *S. aureus* with 2%, an exponential growth rate could not be calculated. With 0.125% active level, *S. aureus* had a total OD yield of 0.070, 0.0564 with 0.25% active level, 0.0984 with 0.5%, 0.119 with 0.75%, 0.0977 with 1% active level and 0.012 with 2% active level. There was no significant difference between the growth of *S. aureus* when grown with 0.125-1% ($P=0.071$) but growth with 2% active level was significantly different from growth with the other active levels ($P>0.05$). When grown with Prolevium, *S. epidermidis* grew with 0.125-1% active level of the protein. There was no recorded growth with 2% active level of Prolevium. The log phase of growth started at 5 hours with every active level where growth was recorded. The growth rate of *S. epidermidis* with 0.125% active level of Prolevium was 0.242 OD/hour, 0.221 OD hour with 0.25%, 0.179 OD/hour with 0.5%, 0.170 OD/hour with 0.75% and 0.138 OD/hour with 1% active level. With 0.125% active level, *S. epidermidis* had a total OD yield of 0.037, 0.031 with 0.25% active level, 0.023 with 0.5%, 0. 134 with 0.75% and 0.001 with 1% active level. There was no significant difference of *S. epidermidis* growth when grown with 0.125-0.75% Prolevium ($P=0.307$). There was significant difference in the growth of S*. epidermidis* with 1% active level compared to growth at all other active levels ($P<0.003$). When grown with Prolevium, *C. jeikeium* only grew with 0.125% active level of the protein. The exponential phase started at 10.5 hours and the growth rate was 0.265 OD/hour. The total OD yield of *C. jeikeium* with 0.125% Prolevium was 0.089. Between bacteria, the only non-significant difference in growth was between *S. aureus* and *C. jeikeium* with 0.125% active level of Prolevium ($P=0.43$).

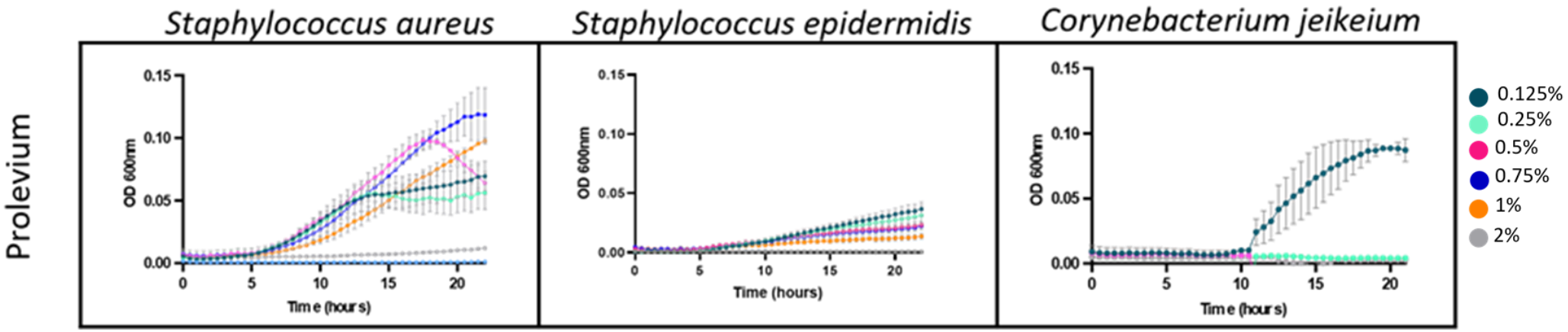


Figure 18. Growth Curves of Three Skin Bacteria Supplemented with Prolevium. The experiment was performed as three technical replicates and three biological replicates. The results from the figure above were discussed in Section 3.22.6. The total yield of *S. aureus* when grown with Prolevium was higher than that of *S. epidermidis*, which grew at nearly all active levels, but yield was low. *C. jeikeium* only grew at 0.125% active, but the yield was better than *S. epidermidis* at any active level. There was no significant difference between the growth curves of *S. aureus* when grown with 0.125-1% (P=0.071) but growth with 2% active level was significantly different from growth with the other active levels (P>0.05). There was no significant difference of *S. epidermidis* growth when grown with 0.125-0.75% Prolevium (P=0.307).

3.23 If the Addition of Cosmetic Proteins Did Not Result in Visible Growth, Are the Cosmetic Proteins Bacteriostatic or Bactericidal Towards the Three Skin Species?

While no growth was recorded when each species was grown in minimal media alone, the colony count revealed that there was no significant ($P>0.05$) gain nor loss in of bacteria after 21 hours of incubation. Where no growth was recorded with a cosmetic protein, the colonies from plates were counted and the final cfu/ml calculated, if this did not significantly differ from the inoculated amount then the preserved cosmetic protein was bacteriostatic. If the final cfu/ml was significantly less than the inoculated amount, then the protein would be bactericidal. The efficacy of the preservatives may differ from previous results (Figure 10, Figure 11, Figure 12) as differences in medium composition can produce different results (Choi et al. 2014; Gomes et al. 2021).

3.23.1 Was the addition of any Active Level of Crosilk Lpc Bacteriostatic or Bactericidal?

When grown with Crosilk, no growth was recorded for both *Staphylococcus* spp. after 0.125% active level, and only a small amount of growth was recorded with *C. jeikeium* at 0.25%. There was a significantly lower ($P<0.05$) final cfu/ml than the inoculated amount when *S. aureus* was grown with 0.75% active level Crosilk and above. Both *S. epidermidis* and *C. jeikeium* had significantly less ($P<0.01$) final cfu/ml when grown with 0.5% active level of Crosilk and above.

3.23.2 Was the addition of any Active Level of Hydroavena HPO Bacteriostatic or Bactericidal?

When grown with Hydroavena HPO, no growth was recorded for *S. aureus* and *S. epidermidis* at 2% active level. Very little growth of *C. jeikeium* was recorded at any active level, but at 0.5% and above there was no growth. While there was, on average, a lower cfu/ml for *S. aureus* at 2% active level, it was not significantly less ($P=0.064$), so the protein was considered as bacteriostatic. *S. epidermidis* had a significantly($P<0.05$) lower cfu/ml than inoculated when grown with 2% active level, so the protein was bactericidal at this active level. Hydroavena HPO was bactericidal for *C. jeikeium* at 0.75% and above.

3.23.3 Was the addition of any Active Level of Hydrolactin Liquid PE Bacteriostatic or Bactericidal?

While differing successes, there was growth with every active level of Hydrolactin Liquid PE for both *Staphylococcus* spp. The only active level in which Hydrolactin Liquid PE was bactericidal for *C. jeikeium* was with the 2% active level ($P<0.01$).

3.23.4 Was the addition of any Active Level of Hydrosativum P Bacteriostatic or Bactericidal?

Staphylococcus aureus grew with every active level of Hydrosativum P, and even though no growth was reported for *S. epidermidis* with 2% active level, the protein was not bactericidal.

Growth of *C. jeikeium* was only recorded with up to 0.25% active level, Hydrosativum P only significantly reduced the starting inoculum cfu/ml at 2% active level ($P<0.05$).

3.23.5 Was the addition of any Active Level of Hydrosolanum PE Bacteriostatic or Bactericidal?

Hydrosolanum PE was not bactericidal to *S. aureus* at any active level, and although reduced the final cfu/ml of *S. epidermidis* at 2% active level, it was not significant ($P>0.05$). However, the final cfu/ml of *C. jeikeium* was significantly lower than the inoculated amount at 0.5% active level and above ($P<0.01$).

3.23.6 Was the addition of any Active Level of Prolevium Bacteriostatic or Bactericidal?

Staphylococcus aureus grew with every active level of Prolevium, even at 2% the final cfu/ml had significantly increased from the inoculated amount ($P<0.01$). However, Prolevium was bactericidal to *S. epidermidi*s at 2% active level, and bactericidal to *C. jeikeium* with 0.75% active level and above.

3.24 Can Cosmetic Proteins Provide Enough Sustenance for the Bacteria to Form Biofilms

While all three species are known to produce biofilms, not all strains and isolates possess the genes capable of creating a biofilm (Zhang et al. 2003; Otto 2008; Martínez-García et al. 2019; Ozdemir et al. 2021). Quantification using the crystal violet method measured the extent of biofilm formation, unattached cells are washed away, and the crystal violet stained the remaining attached cells and extracellular matrix (O'Toole 2011; Welch et al. 2012). Preliminary experiments to determine whether the isolates of the three species could produce a biofilm all generated a positive result. All three species used in the research produced a quantifiable biofilm when grown with both TSB and MH at 37 ^{o}C, pH 7 for 24 hours. All other biofilm formation results are in reference to **Figure 19. Crystal Violet OD of Biofilm Formation of A) S. aureus, B) S. epidermidis and C) C. jeikeium when grown with the six cosmetic proteins.** These experiments were completed with three technical replicates and three biological replicates. The above results were discussed in Section 3.24, including statistical analysis. The yellow column are the control biofilms, formed when the organisms were grown with the minimal medium plus 0.6% w/v casamino acid.Figure 19(A, B and C), which shows the quantity of crystal violet which had stained the adhered cells and biofilm matrix.

When the minimal medium was supplemented with 0.6% v/v casamino acids, the resulting biofilms were recorded. Significantly more cells of *S. epidermidis* adhered and produced a biofilm with these conditions than either *S. aureus* or *C. jeikeium* ($P<0.001$). However, both *S. aureus* and *C. jeikeium* produced biofilms in which the adhered quantity was significantly different to each other ($P=0.72$).

3.24.1 The Effect of Different Active Levels of Crosilk Lpc on Biofilm Formation in the 3 Common Skin Bacteria

When the minimal medium was supplemented with Crosilk Lpc, there were mixed results in biofilm formations. *S. aureu*s (Figure 19, part A) produced a biofilm when grown with 0.125% and 0.25% active levels, there was no significant difference between the biofilms produced (P=0.57). *S. epidermidis* (Figure 19, part B) only produced a weak biofilm when grown with 0.125% active level. *C. jeikeium* (Figure 19, part C) produced a clearly quantifiable biofilm with 0.125%, 0.25% and 0.5% active level of Crosilk, in which there was no significant difference between these (P=0.97). There were also some adhered cells which were stained when *C. jeikeium* grew with 1% and even 2% active levels. There was no statistical difference between the biofilms of *S. aureus* and *C. jeikeium* when grown with both 0.125% and 0.25% active level of Crosilk (P>0.05), but both grew a significantly better biofilm with 0.125% active level than *S. epidermidis* did (P<0.02).

3.24.2 The Effect of Different Active Levels of Hydroavena HPO on Biofilm Formation in the 3 Common Skin Bacteria

When grown with Hydroavena HPO as the protein supplement, *S. aureus* produced a biofilm with 0.125%, 0.25% and 0.5% active levels. *S. aureus*' (Figure 19, part A) biofilm formation with both 0.25% and 0.5% active levels was more significant than with 0.125% (P<0.008). Biofilm formation was most extensive at 0.25% and significantly more than with 0.5% active level (P<0.001). Some adhered cells were stained when grown with 1%, 2% and 4% active levels. *S. epidermidis* (Figure 19, part B) was able to form a biofilm when grown with every active level of Hydroavena HPO (0.125%-4%). There was no significant difference of biofilm production at 0.125% and between 0.5-4% active levels (P<0.06). However, biofilm formation with 0.25% was only slightly significantly greater than with the other active levels (P=0.02). *C. jeikeium* (Figure 19, part C) formed a biofilm with all active levels of Hydroavena HPO, however at 1-4% active levels there were very few adhered cells stained and there was no statistical difference between the biofilm formations (P=0.99). There was no statistical difference between the biofilm formations of C. jeikeium with Hydroavena HPO at 0.125%-0.5%. However, there was a statistical difference between the two groups, *C. jeikeium* with 0.125-0.5% formed significantly more biofilm than with 1-4% active levels of Hydroavena HPO (P<0.04). There was no significant difference between the biofilms formed by *S. aureus* and *S. epidermidis* at 0.25%, 0.5% and 4% active level (P>0.05), however *S. epidermidis* had significantly more cell adherence when grown with 0.125%, 1% and 2% active levels Hydroavena HPO P<0.04). Except with 4% active level, *S. epidermidis* formed significantly more abundant biofilm than *C. jeikeium* with all other active levels (P>0.05). *S. aureus* formed had significantly more adhered cells than *C. jeikeium* at 0.25% and 0.5% (P<0.05), but not with the other active levels.

3.24.3 The Effect of Different Active Levels of Hydrolactin Liquid PE On Biofilm Formation in the 3 Common Skin Bacteria

S. aureus (Figure 19, part A) formed poor biofilms with Hydrolactin Liquid PE, there was no significant differences between the biofilms formed when grown with every active level tested ($P>0.05$). *S. epidermidis* (Figure 19, part B) formed biofilms with every active level of Hydrolactin Liquid PE tested. There was no significant difference between the biofilms with 0.125%, 0.25% and 0.5%, or between 1% and 2% active levels ($P>0.05$). However, the biofilm formed with 4% active level of Hydrolactin Liquid PE was significantly less than at all other active levels ($P<0.04$). *C. jeikeium* (Figure 19, part C) formed biofilms when grown with all active levels of Hydrolactin Liquid PE. There was no significant difference between the biofilms formed with 0.125%, 2% and 4% active levels of Hydrolactin Liquid PE ($P=0.20$). There was no significant difference in biofilm formation with 0.25% and 1% active levels ($P=0.06$) and the biofilm formed with 0.5% active level of Hydrolactin was significantly more than at any other active level ($P<0.03$). *S. aureus* formed significantly less biofilm with every active level of Hydrolactin compared to *S. epidermidis* ($P<0.05$). With 0.25% and 0.5% active level of Hydrolactin Liquid PE, *S. aureus* formed significantly less biofilm formation than *C. jeikeium*, However, at 0.125%, 2% and 4% there was no significant difference between the biofilms formed ($P>0.06$). Again, *S. epidermidis* formed significantly more abundant biofilms with every active level of Hydrolactin Liquid PE than *C. jeikeium* ($P<0.01$).

3.24.4 The Effect of Different Active Levels of Hydrosativum P On Biofilm Formation in the 3 Common Skin Bacteria

S. aureus (Figure 19, part A) was able to form a biofilm when grown with Hydrosativum P, although not abundantly. There was no significant difference between the quantity of adhered *S. aureus* when grown with any active level ($P>0.05$). *S. epidermidis* (Figure 19, part B) also was able to form biofilms when grown with every active level of Hydrosativum P, with many more cells adhering to the well than with *S. aureus* ($P<0.05$). When grown with Hydrosativum P, there was no significant differences between the biofilm formed with 0.125, 0.25. 0.5 and 1% active levels ($P=0.08$), 1 and 2% active levels ($P=0.63$). *S. epidermidis* formed the least abundant biofilm with 4% active level of Hydrosativum P ($P<0.01$). *C. jeikeium* (Figure 19, part C) was also able to form a biofilm when grown with every active level of Hydrosativum P. No significant differences in biofilm formation with 0.125, 0.25 and 0.5% active levels ($P>0.05$), but there was a steady reduction in the numbers of adhered cells from 1-4% active levels, with no significant difference between biofilms with 2 and 4% ($P>0.05$). No significant differences between the abundance of biofilm formed by both *S. aureus* and *C. jeikeium* when grown with 0.125-1% active levels of Hydrosativum P. *S. epidermidis* produced more biofilm with every active level than the other two species ($P<0.014$).

3.24.5 The Effect of Different Active Levels of Hydrosolanum PE on Biofilm Formation in the 3 Common Skin Bacteria

S. aureus (Figure 19, part A) was only able to form a biofilm when grown with 0.125-0.5% active levels of Hydrosolanum PE, with 0.75-4% active level of Hydrosolanum PE there was no staining recorded and therefore no adhered cells. There was no significant difference between the biofilm formed with 0.125% and 0.25% active level of Hydrosolanum PE, but the amount of biofilm was significantly reduced with 0.5% active level ($P<0.02$). *S. epidermidis* (Figure 19, part B) formed a biofilm with 0.125-1% active level of Hydrosativum P, although the adherence was low at 0.125% and significantly less than with any other active level ($P<0.001$). There was no significant difference between the biofilms formed between 0.25-1% active level ($P<0.05$). *C. jeikeium* (Figure 19, part C) also only formed biofilms when grown up to 0.5% active level of Hydrosolanum PE, biofilms formed at 0.5% were significantly less abundant than at 0.125 or 0.25% ($P<0.03$). There were no significant differences between the biofilms formed by *C. jeikeium* at 0.125 and 0.25% ($P=0.54$). There were no significant differences between the biofilms formed by *S. aureus* and *C. jeikeium* when grown with 0.125 and 0.25% active levels of Hydrosolanum PE ($P>0.05$). But both species, with these active levels, formed significantly more abundant biofilms than *S. epidermidis* ($P<0.05$). With 0.5% active level, there was no statistical difference between the biofilms formed by the three species ($P>0.05$).

3.24.6 The Effect of Different Active Levels of Prolevium on Biofilm Formation in the 3 Common Skin Bacteria

When grown with Prolevium, *S. aureus* (Figure 19, part A) was able to form biofilms when grown with 0.125-2% active levels. The significantly most abundant biofilm was formed with 0.125% ($P<0.001$), followed by 0.25% ($P<0.007$), then 0.5% ($P<0.023$). There were no significant differences between the biofilms formed with 1 and 2% Prolevium ($P=0.3$). *S. epidermidis*, (Figure 19, part B) however was able to form biofilms with every active level of Prolevium. There were no significant differences between the abundance of biofilm formed at active levels 0.125-2%, but these were significantly more abundant than the biofilm grown with 4% active level ($P=0.003$). *C. jeikeium* followed a similar pattern to the biofilms formed by *S. aureus*, with the most abundant biofilm formation at 0.125% ($P<0.02$), then 0.25% *et cetera*, with no significant differences between the biofilms formed with 1 and 2% active level ($P=0.3$). Both *S. aureus* and *C. jeikeium* (Figure 19, part C) form non-statistically different biofilm abundance with 0.25, 0.5, 1 and 2% active levels of Prolevium. At 0.125 and 0.25% both species grew significantly more abundant biofilms than *S. epidermidis* with the same active level ($P<0.05$). However, this was reversed at 0.5% and above, when *S. epidermidis* grew more abundant biofilms than the other two species ($P<0.04$).

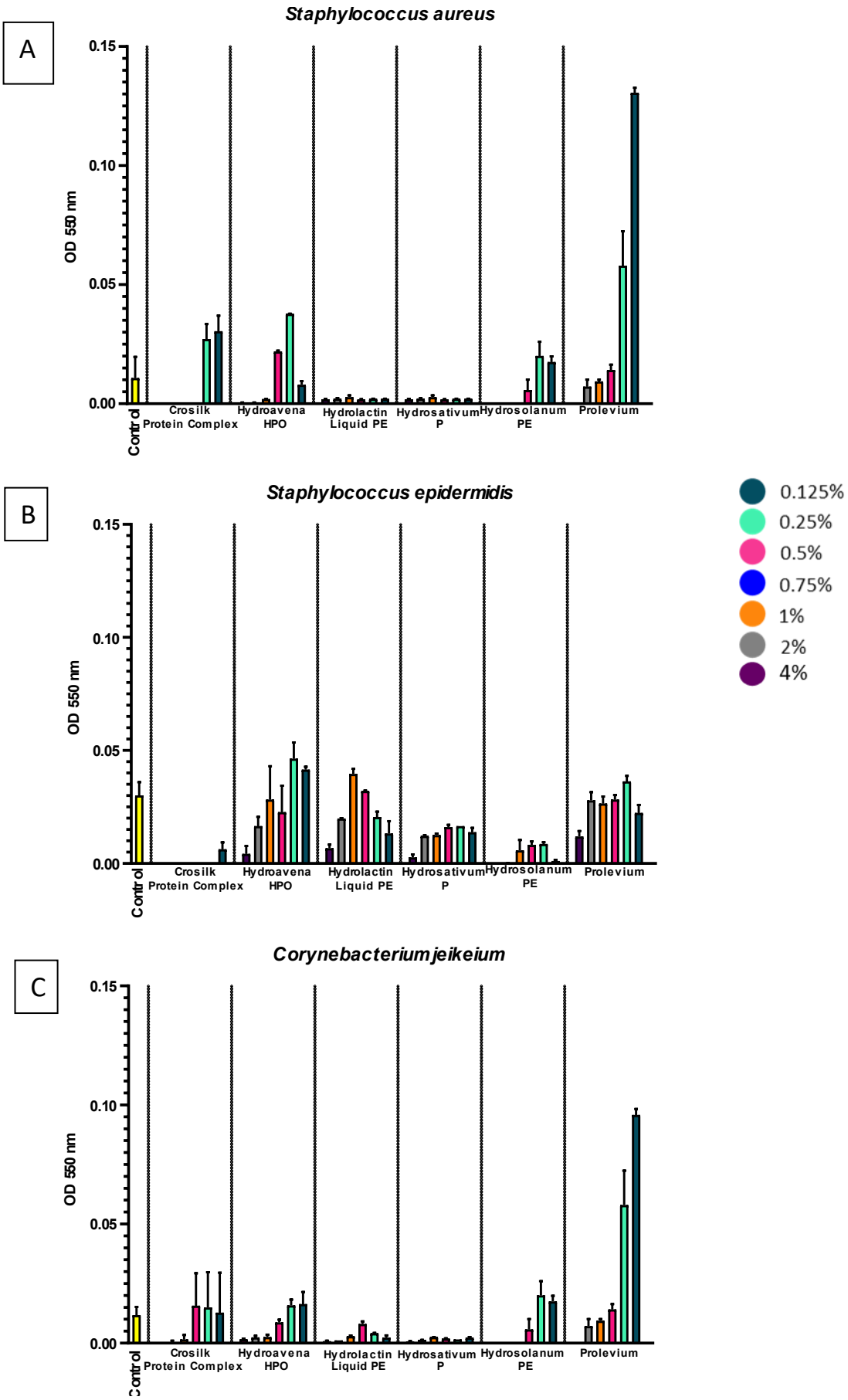


Figure 19. Crystal Violet OD of Biofilm Formation of A) *S. aureus*, B) *S. epidermidis* and C) *C. jeikeium* when grown with the six cosmetic proteins. These experiments were completed with three technical replicates and three biological replicates. The above results were discussed in Section 3.24, including statistical analysis. The yellow column are the control biofilms, formed when the organisms were grown with the minimal medium plus 0.6% w/v casamino acid.

3.25 Discussion

We can reject the null hypothesis that cosmetic proteins do no provide support for any kind of bacterial growth.

3.26 Identifying Bacteria with Extracellular Proteases

Many species of bacteria can release proteases into their surroundings. These extracellular proteases can be part of the organism's virulence portfolio or used to digest large, unusable proteins into smaller peptides and amino acids that are transported into the cell and utilised for cellular processes (Wandersman 1989). Most bacterial species have proteases which are stable and functional between a pH of 5-9 (Rao et al. 1998), while a common staphylococcal commensal has been found to possess resilient proteases which can operate at more extreme acidic and alkaline conditions (Uttatree and Charoenpanich 2018). Therefore, it could be assumed that any proteases secreted by the above bacteria would be likely functionable at most skin sites and in the presence of cosmetic products which have a pH to match skin.

Colonies of *Corynebacterium jeikeium* did not produce zones of clearing on the lactose-free skimmed milk agar. This result corroborates with previous research which found that most *Corynebacterium* spp. do not possess extracellular enzymes which other skin dwellers do, including proteases (Kwaszewska et al. 2014). It's likely that *Corynebacterium* spp. acquire their amino acids from the by-products of protease producing *Staphylococcus* spp. (Bojar and Holland 2002). This survival tactic would make *C. jeikeium* a social cheater, a species which acquires the products of other bacteria for their own metabolic gain (Chen et al. 2019).

Both *Staphylococcus* spp. gave a positive result for extracellular proteases using the LFSM agar method (Figure 9). Both *Staphylococcus* spp. having extracellular proteases corroborates with prior research, in which both *S. aureus* and *S. epidermidis* isolates have produced hydrolysed clear zones on similar media and thus exhibiting their extracellular protease activity (Sudagidan and Aydin 2009; Alkhafaji et al. 2013; Alizadeh-Sani et al. 2018).

While many studies confirm the presence of proteases, few describe the proteolytic activity against different protein sources. For instance, there is little to no research detailing protease activity of *Staphylococcus* spp. against silk proteins, the source of Crosilk Lpc. Silk proteins have been investigated for their antimicrobial potential (Kunz et al. 2016; Xue et al. 2016), and a few studies have examined their potential as a nutrient source (Weide 2003). There have even been studies on the ability of *S. aureus* to form biofilms on clinical silk stitches, which would suggest there is potential for utilisation of silk proteins in biofilms (Hess et al. 2011). To successfully degrade silk protein, either *Staphylococcus* spp. would need to possess the extracellular fibroinase like the Gram-negative soil dwelling species *Variovorax paradoxus* (Forlani et al. 2000).
Casein however, which was the source for Hydrolactin Liquid PE, has been extensively researched

for its utilisation (Taylor 1951; Bryant and Robinson 1962; Reissbrodt et al. 1995). *Staphylococcus aureus* possesses the V8 extracellular protein, while *Staphylococcus epidermidis* possesses the SepA, both of which are caseinolytic (Arvidson 1973; Martínez-García et al. 2018). Therefore, if the proteolysis resulted in large fragments, it would be possible for both *Staphylococcus* spp. to utilise these products. Casein hydrolysates, both acid and enzymatic digests, are ingredients in, or supplemented into media (Moore 2015; Millipore 2018) capable of providing nutrients for a wide range of organisms, *Staphylococcus* spp. and *Corynebacterium* spp. included (Morita et al. 1979; Balci et al. 2002; Moore 2015). If the casein hydrolysis was successful in producing small fragments or free amino acids, then all three species would theoretically be able to grow with Hydrolactin Liquid PE.

Hydrosolanum PE is derived from potato protein and potatoes have long been used to cultivate microorganisms (Zumstein 1936). Potato peptones in bacterial cultivation are becoming more popular and if not equally as successful as meat derived peptones, due to the demand for animal-product free science (Heenan et al. 2002; Solabia 2019). As *Staphylococcus aureus* is a frequent potato contaminant, then it stands to reason that the bacteria can hydrolyse the proteins and utilise the peptones to enable proliferation (Huang 2015). Thus, potato peptones are suitable for a range of Gram-positive organisms and can supply at least some useable peptides. Similar results could be expected from the other vegetable protein source commonly used in cultivation media, Hydrosativum P, which is hydrolysed pea protein (Thermofisher 2018; Merck 2022).

Hydroavena HPO, sourced from oat protein prolamin avenin, has a similar structure to gluten (Balakireva and Zamyatnin 2016; Gilissen et al. 2016). Both *Staphylococcus aureus* and *Staphylococcus epidermidis* have been found to possess extracellular proteases which are effective against prolamins, although the latter was strain dependent (Herrán et al. 2017). Other research has found that even *Staphylococcus* spp. which reside above-ground on cereal species (Legard et al. 1994), do not possess these extracellular proteases (Herranen et al. 2010), highlighting that not all staphylococcal species or strains possess these prolamin degrading extracellular enzymes. However, if both *Staphylococcus* spp. in the experiment do possess these prolamin hydrolysing extracellular proteases then they would be able to easily breakdown and utilise any large peptide fragments.

Bacteria which can utilise cottonseed proteins, the protein source for Prolevium, express extracellular proteases, which also happen to be fibrinolytic, while they utilise the protein as a nitrogen source. The complex structure of cottonseed requires large quantities of protease production to break the proteins down and use the nitrogen source (Bajaj et al. 2013). Staphopains and cysteine proteases, released by *S. aureus* can degrade fibrinogen (Ohbayashi et

al. 2011), *S. epidermidis* also releases enzymes to degrade fibrin (Ma et al. 2017), and therefore could potentially also degrade the complex proteins found in cottonseed.

Due to *S. aureus* being a common contaminant in a range of food sources (Kadariya et al. 2014), it is highly likely that this will be the organism most tolerable at breaking down large fragments and utilising a range of peptide sizes, and as *S. epidermidis* possesses similar extracellular protease activity (Martínez-García et al. 2018), then the bacteria will also likely be able to degrade various protein sources. Though skin is a nutrient desert, free amino acids are easier to utilise, the smaller fragmented cosmetic proteins would provide better nutrients for the growth of both *Staphylococcus* spp. Both *Staphylococcu*s spp. would have an advantage over the proteinase lacking *Corynebacterium jeikeium*, which would only be able to utilise the smallest of fragments and free amino acids.

3.27 Are Preservatives found in Cosmetic Proteins Effective Against the Three Skin Species?

The experiment above was not the standardised testing for the minimum inhibitory concentration as the investigation tested the preservative concentrations as found in the cosmetic protein active levels. Standard MIC tests are carried out on bacteria at 5 x 10^5 cfu/ml, while here the test was carried out at 1 x 10^5 cfu/ml to standardize with the other experiments in the thesis and to lessen the quantity to be more similar to the low biomass found on skin (Wilson 2004). The pH was also dropped to 5 to better represent the likely pH of the final formulations (Lukić et al. 2021; Schulte to Brinke et al. 2021) as well as the skin (Lambers et al. 2006). However, as with previous work, Mueller-Hinton broth was the testing medium (EUCAST 2003) and the minimum inhibitory concentration was met when the preservatives reduced bacterial growth by 80% or above (Rushton et al. 2013). The mechanisms for all preserving substances are found in the Section 1, Table 3.

Only three preservatives reached the 80% and above inhibition of *Staphylococcus aureus*, all at the highest active levels, which are not likely to be used in final formulations. However, even at the lower, widely used active levels, there was still inhibition of growth (Figure 10). Crosilk Lpc at the sold active level of 12.7% is preserved with 0.5% sodium benzoate and 0.01% disodium EDTA. As Crosilk Lpc was sold at the lowest active level of the all the proteins at 12%, therefore, to reach formulation levels, it was diluted the least and thus had the highest preservative concentrations. For example, at 4% active level, the preservative concentrations would have been 0.16% sodium benzoate and 0.00315% disodium EDTA. *Staphylococcus aureus* has shown to be highly sensitive to sodium benzoate at concentrations as low as 0.0032% (Karabay and Sahin 2005). Although ineffective alone (Sangcharoen et al. 2017), EDTA has also been found to enhance antimicrobial

agents against planktonic *S. aureus* through exposure of the inner cell membrane and allowing preservative partners to enter to the cell more easily (Walsh et al. 2003).

The preservatives in Hydroavena HPO at 22.4% active level, also contained 0.5% sodium benzoate, this time with the addition of 0.3% potassium sorbate. However, as Hydroavena HPO is sold at a high active level, it was diluted nearly twice as much as Crosilk Lpc to reach a working formulation active level. For example, at 4% active level, the concentration of sodium benzoate was 0.09%, while potassium sorbate was 0.05%. While the effectiveness of sodium benzoate against *S. aureus* has been noted, potassium sorbate has also shown to be effective at inhibiting *S. aureus*. At pH 5, a low concentration of 0.016% potassium sorbate alone has previously inhibited the growth of *S. aureus* in brain-heart infusion broth (Aminzare et al. 2014), but other work has found a much higher concentration of 0.4% was needed (Wang et al. 2018a). When used in conjunction together, sodium benzoate and potassium sorbate were classed as 'highly effective' against *S. aureus*, causing between 10-20% inhibition (Pinto et al. 2021). While the MIC of both sodium benzoate containing preserving measures were at active levels unlikely to be used in final formulations, *S. aureus* was still somewhat inhibited at the lowest active level concentrations.

The four remaining preservatives were the least effective at inhibiting *S. aureus*. All contained Phenoxyethanol and either disodium EDTA, potassium sorbate or both. Phenoxyethanol has been reported to be less effective against Gram-positive bacteria than Gram-negative bacteria (Wishart et al. 2015) At 18.3% active level, Hydrolactin Liquid PE was preserved with 1% phenoxyethanol and 0.2% disodium EDTA. Once diluted to 4% active level, the concentrations of preservatives were 0.3% phenoxyethanol and 0.06% disodium EDTA. The minimal inhibitory concentration was not reached, but *S. aureus* was inhibited by 70% at 4% active level. Previous work found that to reach the MIC to inhibit *S. aureus*, phenoxyethanol had to be twice as concentrated at 0.64% compared to other bacterial species (Deckner 2015). While the addition of 0.1-0.2% EDTA has shown to improve the efficacy of phenoxyethanol in inhibiting *S. aureus*, the levels found in the used active levels were far too low. If the addition of disodium EDTA had little effect at these low concentrations, then also using phenoxyethanol at pH 5 would also present less staphylococcal inhibition, as phenoxyethanol, alone, works best at pH 7 (Siegart 2013).

Hydrosativum P was sold at 20.3% active and at this concentration the protein was preserved with 1% phenoxyethanol and 0.3% potassium sorbate. At 4% active level this would equate to 0.2% phenoxyethanol and 0.06% potassium sorbate, 0.1% less than phenoxyethanol found in Hydrolactin Liquid PE at 4% active level and less than previous MIC concentrations (Deckner 2015). At the active level they were sold at, both Hydrosolanum PE and Prolevium were preserved with 1% phenoxyethanol, 0.3% potassium sorbate and 0.20% disodium EDTA. Therefore, Hydrosolanum PE when diluted to a 4% active level the protein contained 0.20%

phenoxyethanol, 0.060% potassium sorbate and 0.040% disodium EDTA. At 4% active level of Prolevium contains 0.19% phenoxyethanol, 0.056% potassium sorbate and 0.038% disodium EDTA. Both preservative mixtures faired similarly to each other, and with the preservatives found in Hydrolactin Liquid PE. If disodium EDTA was present in the preservative mixture, those preserving measure were more successful at inhibiting *S. aureus* than preservative mixtures without disodium EDTA, due to EDTA weakening of the cell wall (Miyahara and Arita 2020). The MIC of EDTA when used in conjunction with other antimicrobials has been put at approximately 0.049% (Lambert et al. 2004), which is close, but not quite the level reached at the 4% active level of any of the cosmetic proteins. To inhibit *S. epidermidis*, the MIC of phenoxyethanol alone has previously been calculated at 0.5% or higher (Wang et al. 2019). At 4% active level, the four phenoxyethanol containing cosmetic proteins only contained approximately 0.3% phenoxyethanol, however, three were still able to inhibit *S. epidermidis* by 80% or more. The addition of disodium EDTA to Hydrolactin Liquid PE, Hydrosolanum PE and Prolevium boosted the efficacy of phenoxyethanol, thus allowing greater inhibition at lower concentrations against *Staphylococcus* spp. (Miyahara and Arita 2020). However, the same inhibition 'boost' could not be said for the preserving measures of Hydrosativum P. While prior research found that most double preservative mixtures (both 0.1% concentrated) had completely inhibited the growth of *S. epidermidis*, any preservative paired with potassium sorbate did not repeat such results and growth was recorded (Lalitha and Prasada Rao 2015).

Preservative mixtures containing sodium benzoate were more effective against inhibiting *S. aureus* than other preserving measures. This corroborates with previous work which has highlighted that sodium benzoate is a more effective preservative against *S. aureus* below pH 6 than potassium sorbate. Although phenoxyethanol can be used from pH 3-9 (Williams et al. 2018), it is less effective at the lower the pH ranges (Siegart 2013). The addition of most of these preserving measures, except the preservative combination in Hydrosativum P, could still influence and potentially significantly inhibit *S. aureus* residing on the skin.

The preservatives found at 4% active level in five out of six of the proteins inhibited *S. epidermidis* by 80% or more. The preservatives found at 2% active level of Crosilk Lpc also inhibited *S. epidermidis* by more than 97%. The preservatives found at 2% active level of Hydroavena HPO still inhibited *S. epidermidis* by nearly 63% (Figure 11), corroborating with previous research which has found that like *S. aureus*, *S. epidermidis* is more sensitive to sodium benzoate than most other preservative types, with the MIC of sodium benzoate being as low as 0.0016% (Güven and Kaynak 2014). Figure 11 also shows that the proteins preserved with sodium benzoate were effective at inhibiting *S. epidermidis*, even at the lower concentrations. However, unlike *S. aureus*, *S. epidermidis* was also sensitive to most of the other preservative combinations, all of which

contain phenoxyethanol. While previous research found that phenoxyethanol alone had a MIC of between 0.5- 1% against *S. epidermidis* (Wang et al. 2019; Fournière et al. 2020), the addition of EDTA consistently lowers the MIC to 0.4% (Miyahara and Arita 2020). The preservatives found in Hydrosativum P were the least effective against *S. epidermidis*, the same as *S. aureus*. The lack of EDTA meant that the preserving efficacy of phenoxyethanol was not 'boosted' against *S. epidermidis*. Previous work has found that potassium sorbate is not as effective against *S. epidermidis* than other preserving measures, and the MIC was double that of sodium benzoate (Güven and Kaynak 2014).

As seen in Figure 6, when grown with Mueller-Hinton broth, *C. jeikeium* does not reach stationary phase within the 24-hour incubation. Extension of the incubation period could create inaccuracies as extending could make the MIC appear higher (EUCAST 2003), and the likelihood of products staying in contact with skin greater than this period of time would also be unlikely (Larson 2001; Purnamawati et al. 2017). If total growth is not reached, the MIC will likely appear to be higher and the organism less sensitive than it may be (Mouton et al. 2018).

Much of the previous research regarding *Corynebacterium* spp. and the effect of preservatives have focused on deodorant and antiperspirant preservatives (Halla et al. 2018) as the axilla is the *Corynebacterium* 'problem' area (Lam et al. 2018). These preservatives were chlorhexidine and Trichlorocarbanilide (Halla et al. 2018), which are highly effective at inhibiting skin bacteria (Fahimipour et al. 2018; Kates et al. 2019).

While little data is available about the effectiveness of sodium benzoate, potassium sorbate and disodium EDTA on inhibiting *C. jeikeium*, phenoxyethanol has been found to be highly effective when used in conjunction with other preserving measures. Phenoxyethanol constituted 53% of a preservative mixture which contained naturally found preservatives, the minimal inhibitory concentration of the preservation compound was found to have ranged between 0.025% and 1% dependent of the strain of *C. jeikeium* (Haustein et al. 1993). The strain of *C. jeikeium* used in the above experiments was sensitive to phenoxyethanol. In Prolevium at 0.0625% active level, phenoxyethanol would be found at a concentration of approximately 0.003%, a concentration which had no inhibition against *S. aureus* and *S. epidermidis*, while *C. jeikeium* was inhibited by nearly 6% (Figure 10, Figure 11, Figure 12). The lowest concentrations of phenoxyethanol containing mixtures inhibited *C. jeikeium* by up to 15.3%, while *S. epidermidis* was only inhibited up to 8.3% and *S. aureus* was only inhibited up to 2.6%. While no MIC was reached by the phenoxyethanol containing mixtures, giving the impression that *C. jeikeium* was less sensitive to these, the higher inhibition percentages at the lowest concentrations would suggest the opposite.

While an accurate picture of the sensitivity of *C. jeikeium* to the preservative mixtures is not necessarily possible as stationary phase was not reached, there was clear sensitivity to the

mixtures containing sodium benzoate. However, across the active levels, the sodium benzoate concentration found in Crosilk were nearly double the concentration found in Hydroavena HPO, but the inhibition percentages did not reflect this (Figure 19). The addition of disodium EDTA in Crosilk may have a reduced effect against *C. jeikeium* than other skin species. The cell wall of *Corynebacterium* spp. is considered more complex than other Gram-positive species. *Corynebacterium* spp. possess a layer of mycolic acid outside the peptidoglycan layer, which is comparable to the outer membrane of Gram-negative bacteria (Burkovski 2013,2018). This extra layer has been shown to reduce sensitivity to lytic substances which attack the cell membrane (Burkovski 2018; Theresia et al. 2018). The extra cell membrane layer may well be why the addition of the membrane targeting disodium EDTA (Virto et al. 2005) does not increase the effectiveness of sodium benzoate against *C. jeikeium* compared to the two *Staphylococcus* spp.

All three species showed sensitivity at some level to the preservative combinations found in cosmetic proteins. The addition of preservatives to cosmetic proteins may influence the natural balance of bacteria on the skin due to the varying sensitivities from species to species. This may even affect other microbes too, while the use of potassium sorbate may not widely inhibit most bacteria, fungi are quite sensitive to the preservative (Marshall et al. 2016; Alsudani 2017). *Staphylococcus aureus* has been shown to be the least sensitive to the preservative combinations, a worrying sign as it is the most pathogenic (Bush and Vazquez-Pertejo 2021) of the three species tested. However, this is not a certainty in how the preservatives will react with species when applied to the skin, there will always be strain-to-strain variation (Lambert and Pearson 2000) and previous work has found that the *in vitro* preservative experiment results have not always been repeated *in vivo* (Murphy et al. 2021).

3.28 What do the Protease assay, MIC tests and Growth Experiments reveal?

As seen in Figure 9, both *Staphylococcus* spp. produced extracellular proteases, while *C. jeikeium* did not. These extracellular proteases are predominantly used for virulence, as bacteria favour utilisation of free amino acids as nutritional sources. However, in nutrient poor conditions such as the skin, proteases can be released to hydrolyse proteins into small peptides and amino acids (Kolar et al. 2013). Many of the cosmetic proteins which are used in formulations are derived from proteins, which both *Staphylococcus* spp. can hydrolyse, and contain hydrolysates used to promote bacterial growth under laboratory conditions (Arvidson 1973; Slifkin and Pouchet 1975; Swiatecka et al. 2010). Therefore, with thorough protein analysis of the fragment sizes or free amino acids available, then it would be highlight whether using a cosmetic protein could potentially favour bacteria with extracellular proteases. However, if the hydrolysis produces large fragments, using these proteins in formulations could favour certain species.

Another obstacle which may cause microbial perturbations is the addition of preservatives in the cosmetic proteins. Necessary in liquid products to avoid contamination, but many of these targeted contaminants also happen to be skin microbiota. Figure 10, Figure 11 and Figure 12 show that all three species have some degree of susceptibility to the preservatives found at the highest active levels of proteins tested. However, in nutrient depleted conditions, the efficacy of the preservatives can improve as there is less sustenance available to overcome the antimicrobial activity (Gomes et al. 2021). Therefore, choice of preservatives is important and species with less susceptibility to antimicrobials could be promoted over more sensitive species, in this experiment the most pathogenic species was the least susceptible. Many of these preservative combinations may not be as effective against bacteria, however, they could cause dysbiosis for the other microbiota that reside of skin (Leyva Salas et al. 2017). The cosmetic industry on whole is moving towards using less water in formulations and using dry ingredients (Embleton 2019; Turner 2021). Research into dry cosmetic proteins, in which preserving measures are not required, could be the next step (Halla et al. 2018). However, putting unprotected hydrolysates on skin could cause over-proliferation of species, throwing up new problems (Murphy et al. 2021).

A key question was, could these proteolysates provide amino acids/nitrogen source to sustain growth of the three skin species. Growth of all three species in the minimal medium was bacteriostatic, meaning there was no significant loss or rise in the number of cells inoculated. However, the addition of many of the cosmetic proteins, especially at the lower active levels when preservative concentration was low, provided the sustenance necessary for growth. Growth was not equal across the different bacteria and the differing amino acid content of the proteins provided various levels of nourishment for the three species. For example, *S. aureus* grew better when supplemented with cosmetic proteins which had higher levels of cysteine (Alreshidi et al. 2020). More proteomic analysis would also highlight if any of the hydrolysed proteins fragmented into peptides which could harm skin bacteria (Théolier et al. 2013). While providing sustenance, peptides can also have antibacterial qualities (Ouertani et al. 2018).

While the proteins could provide sustenance for planktonic growth, preservatives hindered growth with higher active levels of proteins. Biofilm formation shielded the skin species from these preserving measures and sessile cells were quantified even at the highest active levels (Dincer et al. 2020). The pH helped downregulate biofilm forming genes in *S. aureus* (Efthimiou et al. 2019), but many of the proteins still provided nutrition needed for adherence of the cells. *S. epidermidis* was able to form consistent biofilms, even with cosmetic proteins in which planktonic growth was poor. The slow growing *C. jeikeium*, also formed biofilms when supplemented with many of the cosmetic proteins, again including with active levels where no planktonic growth was recorded. The least formed biofilms were with the one cosmetic protein,

Crosilk Lpc, where D-amino acids could have been present. Research into using some of these D-amino acids in formulations could curb any potential biofilm formations without harming the bacteria themselves (Kolodkin-Gal et al. 2010; Ampornaramveth et al. 2018).

Introduction of cosmetic proteins to the skin may favour the growth of organisms with extracellular proteins which could utilise large peptide fragments. Without mass spectrometry to analyse the peptide length and peptides produced, it is difficult to say whether they would be useful or even potentially antimicrobial. The use of preservatives could reduce growth of susceptible species and promote growth of less sensitive species. Overuse of low preservative concentrations could also increase resistance amongst skin bacteria (Capita et al. 2019). In amino acid depleted environments, the cosmetic proteins can provide sustenance to aid in growth of bacteria. If adherence of the bacteria begin, cosmetic proteins can also provide nutrients which aid in biofilm production, which can also reduce the efficacy of the preservatives. Promotion of one species over another, even when described as 'beneficial bacteria' could upset the overall balance, but actively promoting pathogens on skin is harmful. In a healthy individual, the most desirable outcome would be using an active level of a cosmetic protein which is bacteriostatic towards all species.

4 Planktonic Growth and Biofilm Formation of Co-Cultured *Staphylococcus* spp. with Cosmetic Proteins

4.1 Introduction

Staphylococcus aureus and *Staphylococcus epidermidis* are often described as the pathogenic and commensal cousin. While *S. epidermidis* is also a major cause of nosocomial infections, the associated virulence factors are not as severe as the ones used by *S. aureus* (Otto 2009,2012). Both species colonise similar areas of the skin and therefore interact with each other (Otto 2010; Gosbell and van Hal 2013). *S. epidermidis,* for example, can modulate *S. aureus* to limit the latter's ability to infect and form biofilms (Byrd et al. 2018). For these reasons, many publications investigate interactions between the two species (Iwase et al. 2010; Fredheim et al. 2015; Lee et al. 2019a; Hardy et al. 2020)

On healthy skin, both species can co-exist without causing any serious health issues (Grice and Segre 2011). However, the introduction of a substance which could manipulate the staphylococcal balance could promote inflammation leading to disease (Park et al. 2019).

4.2 Aims

- Investigate if cosmetic proteins can promote the growth of one staphylococcal species when grown together in a planktonic state
- Investigate whether cosmetic proteins promote the formation of biofilms by one staphylococcal species over another

4.3 Null Hypothesis

Addition of cometic proteins would not cause an imbalance of planktonic growth or biofilm production in a co-culture of *S. epidermidis* and *S. aureus.*

4.4 Methods

4.5 Bacteria

Both the *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates used were the same as in Section 2.6.

4.6 Media

TSB and TSA were used to grow the species separately as found in Section 2.10. Minimal medium was used to co-culture the species (Section 2.8). Mannitol salt agar was used to differentiate and count the species. *S. aureus* can utilise mannitol and colonies are surrounded by yellow halo (**Error! Reference source not found.**), while *S. epidermidis* cannot utilise mannitol and therefore t he colonies are either colourless or red and the surrounding media stays red (Chapman 1945).

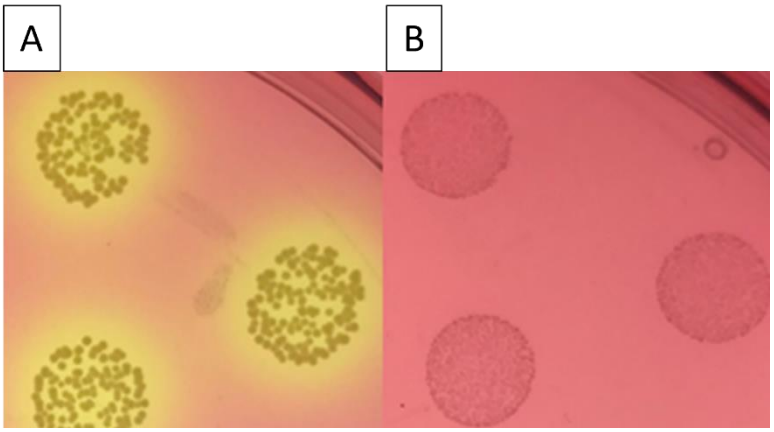


Figure 20. *Staphylococcus* species grown on Mannitol Salt Agar. *Staphylococcus aureus* (A) can utilise mannitol and therefore a yellow halo is formed around the colonies. *Staphylococcus epidermidis* (B) does not utilise mannitol, and therefore the colonies remain grey on a red background.

4.7 Preparation for Planktonic Growth

The minimal medium from Section 2.8, was used as a way of producing conditions which could reflect the skin environment better than traditional growth media. However, when testing the cosmetic proteins, the casamino acid growth supplement was removed from the recipe. Instead, the media was supplemented with the cosmetic protein at active levels in which they may be used in the final formulation 0, 0.125, 0.25, 0.5, 0.75, 1 and 2%.

Bacteria were grown as single colonies on TSA. A single colony was removed and place into 4 ml of TSB, which was grown for 18 hours at 37 ˚C on an orbital shaker at 150rpm. The optical density (OD) was measured at 600 nm and the cell suspensions were diluted $1x10^5$ cfu/ml in minimal media containing the cosmetic protein. To produce the co-culture, 100 µl of each species were added and mixed in a well of a MicroWell™ 96-Well Microplate well plate (Nunc, Denmark) and placed into a shaking incubator at 37 °C for 24 hours.

4.8 Preparation for Sessile Growth

Bacteria were grown overnight, as stated above, and diluted to $1x10^5$ cfu/ml in minimal media. The media was supplemented with the cosmetic protein at active levels in which they may be used in the final formulation 0, 0.125, 0.25, 0.5, 1, 2% and 4%. An 200 µl aliquot of a 1:1 ratio of *S. epidermidis* and *S. aureus* and supplemented minimal media were placed into a flat bottom MicroWell™ 96-Well Microplate well plate (Nunc, Denmark). The layout of the plate was simultaneously duplicated so that they could be treated differently later. Flat bottom plates were chosen due to the non-motile nature of the organisms (O'Toole 2011). These plates were placed into a 37 ˚C incubator for 24 hours without shaking. After incubation, one of the plates was stained with crystal violet as per the protocol set by O'Toole (O'Toole 2011). The other plate was

washed gently three times with 1 x PBS to removed unattached cells and the biofilm removed manually using a sterile pipette tip as suggested by Lemos and colleagues (Lemos et al. 2010). Once scraped, 100 µl of 1x PBS was added to the well and the contents were serially diluted before being plated onto Mannitol salt agar for counting using the Miles and Misra method (Miles et al. 1938). The resulting cfu/ml were converted into percentages. A 100% domination by one species did not mean that the other species was not at all present, but at the dilution counted there were not any of the reduced species to count.

4.9 Statistics

The final cfu/ml of both species for both planktonic growth and biofilm formations were analysed using GraphPad Software (Version 9.1.2). The mean of each were compared using t-tests and significant differences noted.

4.10 Results

4.11 Does Incubation with Cosmetic Proteins Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

There was never any significant difference (P>0.05) between the inoculated cfu/ml of the *Staphylococcus* spp. or after incubation with the minimal medium without supplements, see yellow and blue bars from Figure 21. All results below are in reference to the data shown on Figure 21.

4.11.1 Does Incubation with Crosilk Lpc Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

The addition of Crosilk Lpc to the minimal medium resulted in the planktonic growth of *S. aureus* being favoured over the growth of *S. epidermidis*. When grown with Crosilk Lpc at active levels 0.125-0.75%, both the cfu/ml of *S. aureus* and *S. epidermidis* increased significantly from the starting inoculum. At 1% and 2% however, the total cfu/ml had significantly decreased from the starting inoculum (P<0.001).

4.11.2 Does Incubation with Hydroavena HPO Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

The addition of Hydroavena HPO also showed no significant promotion of one species over another (P>0.05). There was significantly more growth with every active level of Hydroavena HPO than with Crosilk Lpc (P<0.0003). The final cfu/ml of both species were also significantly increased when grown with every active level of Hydroavena HPO (P<0.03), the final cfu/ml was never less than the inoculated amount.

4.11.3 Does Incubation with Hydrolactin Liquid PE Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

When the co-culture was grown with supplemented Hydrolactin Liquid PE, there was no promotion of either *Staphylococcus* spp. with 0.125% active. However, with 0.25% Hydrolactin

Liquid PE and above, *S. epidermidis* was significantly promoted over *S. aureus* (P<0.0079). The final cfu/ml of *S. epidermidis* increased significantly compared to the initial inoculated amount with every active level (P<0.009) and continued to rise as the active levels increased. While the final cfu/ml of *S. aureus* was significantly higher than the inoculated amount with active levels 0.125-1% (P<0.008), the total cfu/ml of *S. aureus* peaked with 0.5% active and significantly reduced with active levels 0.75-2%.

4.11.4 Does Incubation with Hydrosativum P Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

When grown with 0.125% Hydrosativum P, neither *S. aureus* nor *S. epidermidis* were significantly promoted over each other (P>0.05). However, with 0.25% upward, *S. aureus* was promoted significantly more than *S. epidermidis* (P<0.045). The total cfu/ml of *S. aureus* increased significantly until grown with 2% active, where the cfu/ml was significantly decreased compared to growth with 1% (P<0.001). However, even growth with 2% active resulted in a higher end cfu/ml than the initial inoculated amount (P<0.001). While *S. aureus* grew more successfully, the total cfu/ml of *S. epidermidis* never dropped significantly below the initial inoculated amount (P=0.09), but the final cfu/ml did significantly drop when grown with 0.5-2% compared to growth with both 0.125% and 0.25% (P<0.003).

4.11.5 Does Incubation with Hydrosolanum PE Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

When grown with Hydrosolanum PE, *S. aureus* was significantly promoted over *S. epidermidis* when grown with 0.125% active, but only slightly (P=0.0475). When grown with 0.25% active Hydrosolanum PE, this is reversed and *S. epidermidis* is significantly promoted over S. aureus (P=0.0012). However, when grown with 0.5% active and above, *S. aureus* is significantly promoted over *S. epidermidis* (P<0.001). The final cfu/ml of *S. aureus* increased until 0.5%, when it decreased significantly from 0.75% active onwards. However, the final cfu/ml never significantly decreased below the initial inoculated amount, even with 2% active (P=0.44).

4.11.6 Does Incubation with Prolevium Promote One *Staphylococcus* spp. Over Another in a Planktonic Growth Co-Culture?

Growth with 0.125% Prolevium neither promoted the growth of *S. aureus* nor *S. epidermidis*. However, from 0.25% onward, *S. aureus* was significantly promoted over *S. epidermidis* (P<0.0012). The total cfu/ml of *S. aureus* significantly increased when grown with 0.25% compared with 0.125% active (P=0.0008). However, there was no significant difference between the final cfu/ml of *S. aureus* when grown with 0.25-1% active (P=0.149), with 2% active the final cfu/ml was significantly reduced (P=0.0002) but still significantly more than the starting inoculum (P<0.0001). The final cfu/ml of *S. epidermidis* decreased significantly when grown with 0.5-2%

active Prolevium (P<0.05). However, the final cfu/ml only significantly decreased compared to the inoculated amount when grown with 2% active (P=0.0002).

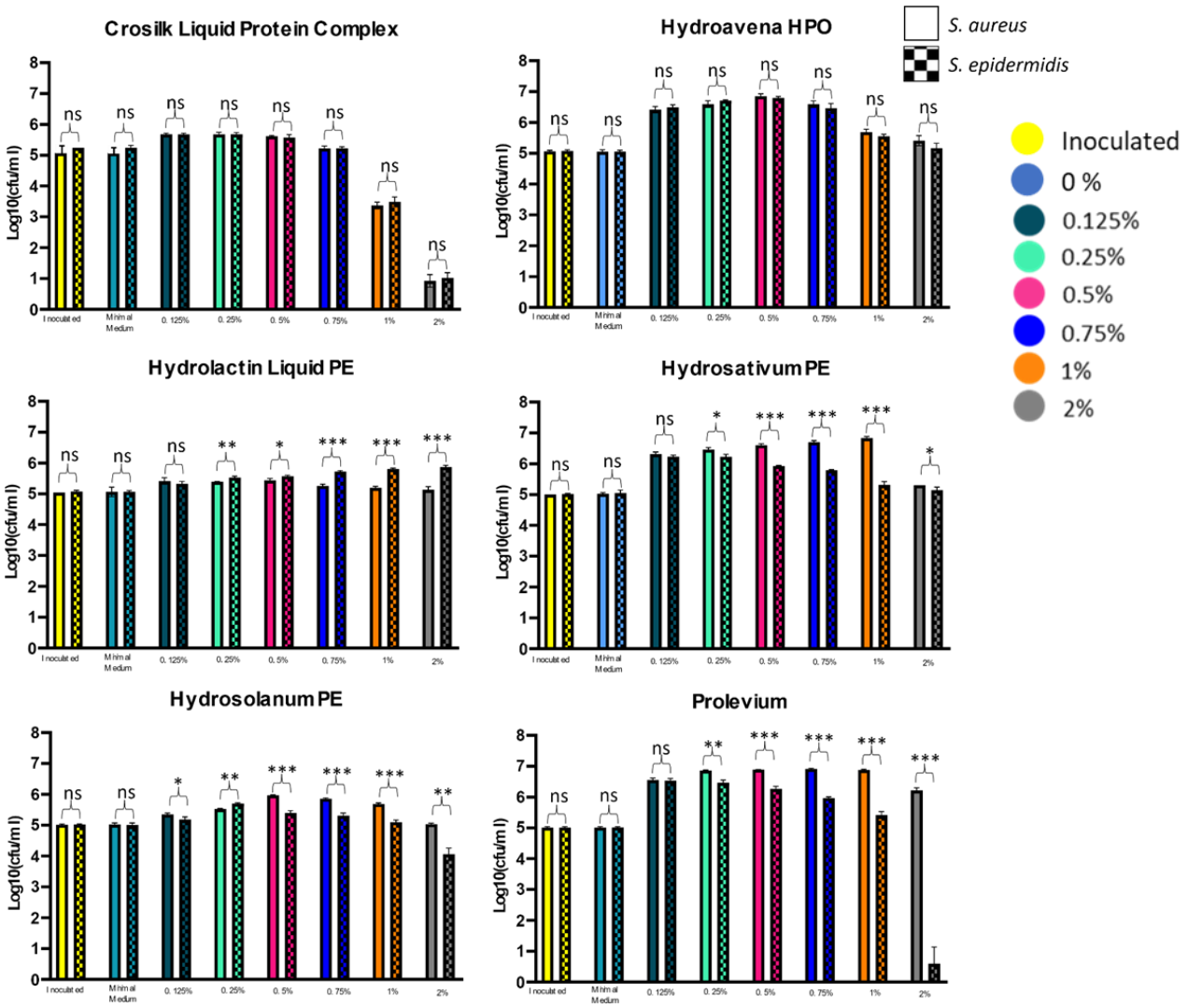


Figure 21. Final Cfu/ml of the Mixed *Staphylococcus* spp. Culture; Planktonic Growth. These experiments were completed with three technical replicates and three biological replicates. *S. aureus* is represented by the non-patterned bars while *S. epidermidis* is referenced by the chequered bars. The yellow bars are the inoculated amount, the blue bars are the species when grown with minimal medium without supplement. There was never any significant difference between the inoculated numbers of each bacterium or the final cfu/ml after incubation with the minimal medium alone.

4.12 Does Incubation with Cosmetic Proteins Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

When grown in the minimal medium supplemented with 0.6% casamino acids, a biofilm was formed which comprised of 59% *S. epidermidis* and 41% *S. aureus*, as seen on the green bar from Figure 22. Results below are referencing data shown in Figure 22.

4.12.1 Does Incubation with Crosilk Lpc Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

When grown with Crosilk Lpc, a quantifiable biofilm was only formed with 0.125 and 0.25% active. There was no significant difference between the measurable biofilm formed at both active levels ($P=0.23$), however there were differences in the quantity of each species. While both biofilms were heavily *S. aureus* dominated, at 0.125%, 28% of the biofilm was formed by *S. epidermidis*. At 0.25% active level, only *S. aureus* was able to be measured using the Miles and Misra method.

4.12.2 Does Incubation with Hydroavena HPO Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

Biofilms were formed with every active level of Hydroavena HPO, with varying dominating species. With 0.125% active, the biofilm formation was split 24.1% *S. epidermidis* compared to 75.9% of *S. aureus*, there was significantly less quantifiable biofilm formed than at 0.5% and 1% active ($P<0.03$). There was no significant difference between the quantifiable biofilm formed with 0.25% and 0.5% active Hydroavena HPO ($P>0.05$), *S. aureus* was also the only detectable species in the biofilm with both active levels. With 1-4% active Hydroavena HPO, there was a significant drop in measurable biofilm compared to the lower active levels ($P<0.0002$). With active levels 1-4% there was also a shift in the dominating *Staphylococcus* spp., with 1% active the biofilm was dominated by 55% *S. epidermidis*, with 2% *S. epidermidis* made up 65% of the biofilm and 63.4% of the biofilm formed with 4%.

4.12.3 Does Incubation with Hydrolactin Liquid PE Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

Biofilms were formed with every active level of Hydrolactin Liquid PE, with the most significant biofilm structures were formed with 0.5 and 1% active ($P<0.05$). There was no significant difference between the biofilms formed with 1 and 2% ($P>0.05$), while the biofilm formed at 0.25% had significantly more established cells than with 0.125% active ($P=0.007$). *S. epidermidis* dominated the biofilm formations at every active level making up 69% with 0.125 and 0.25% active and 79.2% with 0.5% active. With 1%, 2% and 4% active the only quantifiable bacterium was *S. epidermidis*.

4.12.4 Does Incubation with Hydrosativum P Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

The bacteria were again able to form quantifiable biofilms with every active level of Hydrosativum P. There was no significant difference between the biofilms formed with 0.25 and 0.125% active

(P>0.05) which had significantly more established biofilm than with any other active level (P<0.0009). There was also no significance between biofilms formed with 0.5 and 1% active, 1% and 2% active and 2% and 4% active (P>0.05). Again, the dominant species of biofilms formed with every active level was *S. epidermidis*. With 0.125% active Hydrosativum P, *S. epidermidis* formed 66% of the biofilm, with 0.25% *S. epidermidis* made up 80.4%, followed by 97.5% with 0.5, 92.9% with 1%, 89.1% with 2% and formed 81.2% of the biofilm with 4% active Hydrosativum P.

4.12.5 Does Incubation with Hydrosolanum PE Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

Quantifiable biofilm was only detected with up to 1% Hydrosolanum PE, there was no significant difference between the biofilms formed with 0.125, 0.25 and 0.5% active (P>0.05). *S. epidermidis* narrowly dominated the biofilms formed with 0.125, 0.25 and 0.5% active, making up 54%, 53% and 58% respectively. *S. epidermidis* was the only species quantifiable from the biofilm formed with 1% active Hydrosolanum PE.

4.12.6 Does Incubation with Prolevium Promote One *Staphylococcus* spp. Over Another in a Sessile Growth Co-Culture?

A quantifiable biofilm was formed with every active level of Prolevium, with varying results as to which species dominated. The biofilm formed with the most significant number of adhered cells was with 0.125% (P<0.007), followed by 0.25%, 0.5%. 1% and 2% active. The biofilms formed with 0.125 and 0.25% active Prolevium were dominated with 79% and 55.4% *S. aureus,* respectively. From 0.5-4% active *S. epidermidis* was the dominant species in the biofilm, making up 65.2% of the biofilm with 0.5%, 74% of the biofilm with 1%, 79% of the biofilm with 2% and 90% of the biofilms formed with 4% active Prolevium.

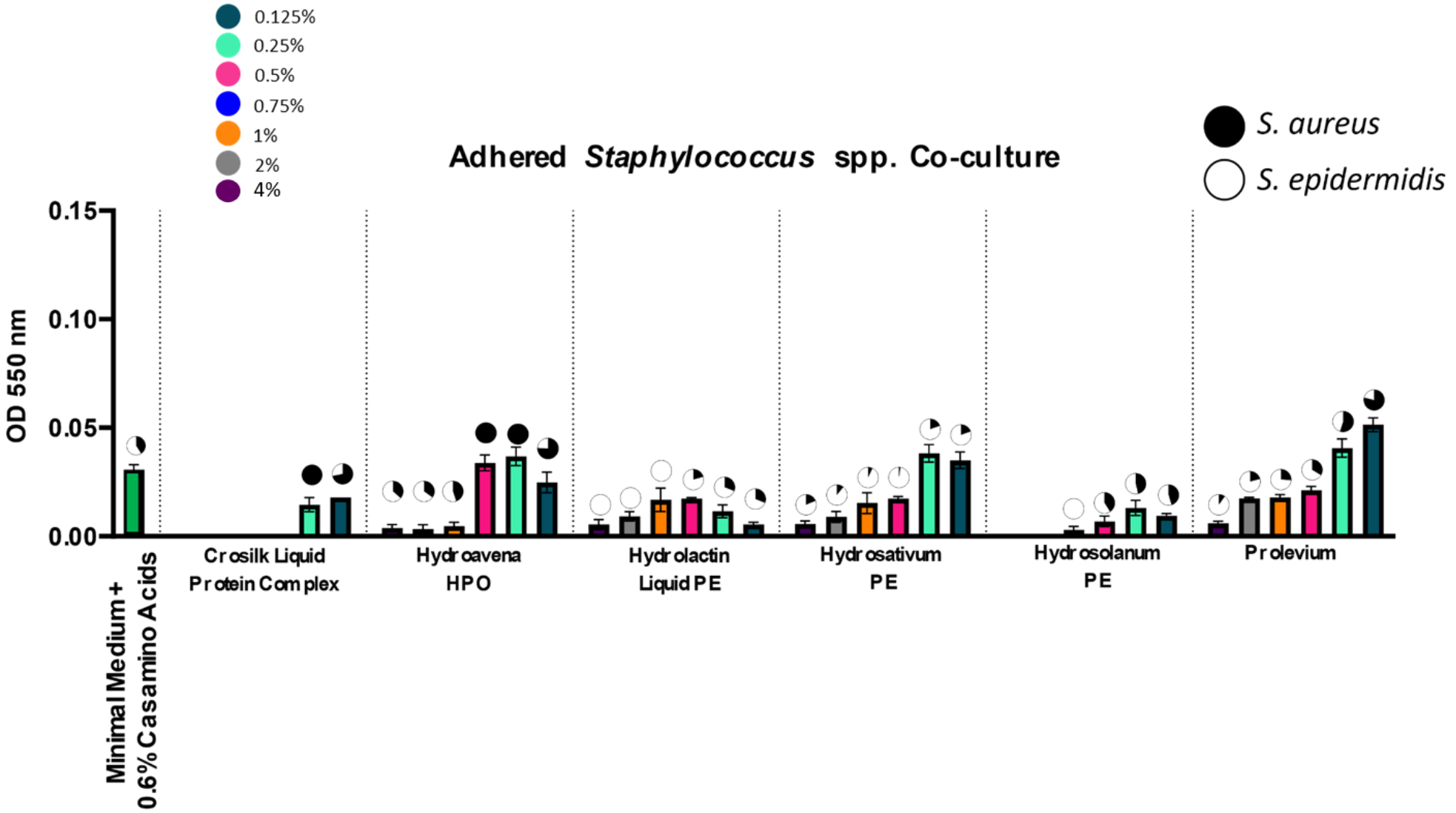


Figure 22. OD readings Of Crystal Violet which Stained Adhered Cells and the Ratio of *Staphylococcus* spp. in the Biofilm. These experiments were completed with three technical replicates and three biological replicates. The first bar is the control of both species grown with minimal medium plus 0.6% w/v casamino acids. The ratios are represented by pie charts above the bars, black is *S. aureus* and white is *S. epidermidis*.

4.13 Discussion

4.14 Planktonic Mixed Species Growth Reflects Individual Species Growth

The planktonic growth of each species in co-culture were reflective of their individual planktonic growth from Section 3.22. If a species grew better than the other with a cosmetic protein, then that species was more likely to be the dominant species in the co-culture. For example, *S. epidermidis* achieved a higher yield and faster growth rates when grown with every active level of Hydrolactin Liquid PE compared to *S. aureus*. This result was reflected in the co-culture where the final cfu/ml of *S. epidermidis* was significantly higher than *S. aureus* when grown with 0.25% active level and up of Hydrolactin Liquid PE. This pattern is again presented when grown with Crosilk Lpc and Hydroavena HPO, both individual species grew equally poorly with Crosilk and well with Hydroavena HPO.

Previous work has shown that *S. aureus'* faster growth rate would make it dominate in a mixed culture with *S. epidermidis*, which was apparent when both were grown with proteins which *S. epidermidis* did not utilise well, Hydrosolanum PE and Prolevium (Figure 21). The slowed growth of *S. aureus* at pH 5 (Stewart et al. 2017), aided *S. epidermidis* to dominate growth with cosmetic proteins in which it grew well. The low pH of this experiment favoured growth of *S. epidermidis*, when a neutral pH may promote the growth of *S. aureus* and previous work at this pH has showed that *S. aureus* can grow with other bacterial species without interrupting the other species' health (Frapwell et al. 2018). This could explain why in cosmetic proteins, which both species either grew equally well or equally poorly, both S*taphylococcus* spp. could cohabit together and grow.

4.15 *Staphylococcus* species May Tolerate the Presence of Each Other

When co-cultured with both Crosilk and Hydroavena HPO, there were no significant differences between the species of their final cfu/ml. Most research has focused on *S. aureus* being an invasive pathogen, but little has been written about its commensal status (Cogen et al. 2008). This organism can be found on many peoples' skins as part of the natural microbiota in and around the nose, therefore interactions with *S. epidermidis* wouldn't always be antagonistic (Sakr et al. 2018; Boxberger et al. 2021). These *Staphylococcus* spp. have possibly previously benefitted from being in close contact, methicillin resistance gene cassettes are thought to have been passed on to *S. aureus* from *S. epidermidis*, through horizontal gene transfer (Otto 2009). As the results from Figure 21 show, the lack of domination by one species would suggest, perhaps not a cooperative relationship, but one of cohabitation.

4.16 The Lower pH favours *S. epidermidis* in Biofilm Domination

S. aureus and *S. epidermidis*, have both been found in multispecies biofilms of skin ulcers (Johani et al. 2017). Proving that, while reported for reducing *S. aureus* colonisation, *S. epidermidis* can co-exist with its close relative in pathogenic circumstances. Both species can also dominate a

mixed staphylococcal biofilm, when equally inoculated and under alkali and neutral conditions, *S. aureus* dominates over *S. epidermidis*. However, at pH 5, this is reversed as in acidic conditions *the* genes which enable *S. aureus* cell adherence are downregulated (Efthimiou et al. 2019) and *S. epidermidis* has the ability to become the dominant species within the biofilm (Stewart et al. 2017; Efthimiou et al. 2019). The dominance of *S. epidermidis* is reflected in many of the results from Figure 22. The only instances in which *S. aureus* dominated the biofilms were with all levels of Crosilk Lpc and with the lower active levels of Hydroavena HPO and Prolevium. If the genes for *S. aureus* are downregulated, this would leave *S. aureus* at risk from the higher concentrations of preservatives, which *S. epidermidis* could tolerate. The dominance of *S. epidermidis* could also be down to the species' auto-inducing peptides which inhibits the quorum-sensing gene regulators (*agr*) of *S. aureus* (Boles and Horswill 2008), which are involved in biofilm formation (Yarwood et al. 2004).

4.17 Planktonic Growth and Sessile Growths are Dominated by Different Bacteria

We can reject the null hypothesis for planktonic growth of the co-culture, when grown with most active levels of Hydrolactin Liquid PE, Hydrosativum P, Hydrosolanum PE and Prolevium. However, planktonic growth of the co-cultured species with either Crosilk Lpc or Hydroavena HPO did not promote one species over the other, therefore we can accept the null hypothesis for these cosmetic proteins. For biofilm production, we can reject the null hypothesis for all co-cultured growth with all the cosmetic proteins as one species was always promoted over another. Planktonic growth versus sessile growth, of all bacteria, differ in their nutritional requirements and susceptibility to antimicrobials (Donlan 2002). This is reflected in the difference in the species which dominated dependent on type of growth. *S. aureus* for example dominated in planktonic growth with most active levels of Hydrosativum P, Hydrosolanum PE and Prolevium. However, *S. epidermidis* made up more than 50% of the biofilm when grown with most active levels of these cosmetic proteins. Planktonic growth of *S. aureus* is not significantly affected by acidic pHs (Korting et al. 1992), that would explain why within the planktonic co-culture *S. aureus* could dominate. However, sensitivity of *S. aureus* to acidic stresses (Iyer et al. 2021) can cause down regulation of biofilm related genes (Efthimiou et al. 2019). *S. aureus'* sensitivity can also cause a reduced colony size (Mirza et al. 1985), which affects the quantity of biomass within a biofilm (Yoshioka and Newell 2016). Therefore, the lack of sensitivity of *S. epidermidis* to lower pHs meant that this species was more capable of biofilm domination with nutrient appropriate cosmetic proteins. Throughout experiments from Sections 4, 3.22.1 and 3.24.1, *S. epidermidis* has never proliferated when grown in the minimal medium supplemented with Crosilk Lpc. The amino acid content of Crosilk had a high percentage of serine (Table 7), if racemization of serine had occurred, while both *S. aureus* and *S. epidermidis* have difficulty growing in the presence of D-serine, *S. epidermidis* is far more sensitive (Sakinç et al. 2009). However, the poor growth of *S.*

epidermidis could also have been due to the high concentration of disodium EDTA found in Crosilk Lpc. Disodium EDTA has been shown to have a bacteriostatic effect against planktonic cells and have the ability to inhibit growth and promote dispersal of *S. epidermidis* biofilms (Juda et al. 2008).

While promoting any type of biofilm formation is unadvisable, promotion of biofilm formations dominated by *S. aureus* over *S. epidermidis* could cause severe illness. Therefore, ensuring products do not alter the natural pH of the skin by keeping them at lower pHs would favour *S. epidermidis* health over *S. aureus*. To ensure that *S. epidermidis* can aid in downregulating *S. aureus* colonisation on the skin, formulations should avoid preservatives in which *S. epidermidis* is highly sensitive too, especially EDTA and EDTA derivatives.

5 *In Vitro* Stimulation of Immune Response Biomarkers of an Immortal Keratinocyte Cell-line by Addition of *Staphylococcus* spp. and Cosmetic Proteins

5.1 Immune Response to Commensal Microbes

The importance of the human microbial community does not simply start and end with the microbes that reside there, but the interactions between them and the host cells. Commensal microbes are recognised by the immune system as little or no threat and induce homeostasis (Belkaid and Harrison 2017). The sheer presence, however, of skin microbes is enough to trigger and reinforce the immune response (Abdallah et al. 2017). This readies the immune system for any microbial dysbiosis, pathogenic invasions and quick wound repair (Linehan et al. 2018; Timm et al. 2020).

Exposing chemicals to the skin may cause its own immune response which manifest as contact dermatitis (Martin et al. 2011; Martin 2017; Nicolai et al. 2020). The addition of these substances may alter the natural composition of the bacterial environment, depleting or promoting species growth (Bouslimani et al. 2019). The change in the microbial arrangement could spark an immune response (Mezouar et al. 2018; Catinean et al. 2019; Faas et al. 2020; Swaney et al.).

5.2 HaCaT Cells in Skin Studies

The cell-line HaCaT are immortal keratinocytes cells, more resilient and longer lasting than primary keratinocyte cell lines (Colombo et al. 2017; Griffoni et al. 2021). As the epidermis is >90% Keratinocytes, HaCaTs are used *in vitro* for numerous experiments regarding skin. This includes experiments which aim to measure the skin's innate immune response to pathogens and disease (Olaru and Jensen 2010; Henrot et al. 2020).

5.3 Immune Response Biomarkers

The pathways of the immune markers can be found in Table 8.

IL-8

High levels of the chemokine Interleukin-8 (IL-8, CXCL8) are associated with numerous diseases (Shahzad et al. 2010). Many types of tissue release IL-8 as part of their immune response and therefore IL-8 is used as a biomarker for clinical diagnosis and *in vitro* experiments (Shahzad et al. 2010; Quist et al. 2016; Ke et al. 2019; Griffoni et al. 2021). Keratinocytes are one of the tissue types which release IL-8 in response to inflammatory stimuli and while this is mostly from pathogenic invasion, interleukins have been produced by HaCaT cells, when stimulated with known chemical skin irritants (Mohamadzadeh et al. 1994).

TNF-α

Tumour necrosis factor- α, as the name suggests, is part of the innate immune system which targets and causes cancerous cells to undergo apoptosis (Wang and Lin 2008). However, production of TNF-α also increases in the presence of a bacterial infection (Ma et al. 2010). Different species or strains of bacteria could vary in the TNF-α levels expressed (Kragsbjerg et al.

1998). Keratinocytes express TNF-α through a multitude of stimulants including autoimmune diseases (Li et al. 2019c), bacterial infections (Johnston and Conly 2006) and UVB stimulation (Bashir et al. 2009).

IFN-ɣ

Interferon-ɣ is a glycosylated proteins involved with the innate and adaptive immune system (Vilček et al. 1998). The specialised cytokine is synthesized mostly within immunity cells (Payne 2017), including Natural Killer cells and T regulatory cells (Griffin et al. 2008). IFN-ɣ receptors are found throughout cells in the body. IFN-ɣ has been used as a biomarker for an immune response against bacterial antigens delivered in vaccines (Weir et al. 2008). It is also used as a biomarker for skin conditions, increased IFN-ɣ levels and IFN-ɣ induced processes are found in individuals with atopic dermatitis (Rebane et al. 2012).

5.4 Null Hypothesis

The addition of bacteria nor the exposure to cosmetic proteins would not cause any significant changes the concentration of immune markers found in the supernatant of HaCaT cells.

5.5 Aims

- Investigate the effect of *Staphylococcus* spp. have on the concentration of immune marker concentration found in the supernatant of HaCaT keratinocytes
- Investigate how the exposure of keratinocytes and bacteria to cosmetic proteins effects the concentration of immune marker concentration found in the supernatant of HaCaT keratinocytes

Table 8. Pathways of Immune Biomarkers of the Skin

Biomarker	Pathway
Interleukin-8 (IL-8)	When pathogen-associated molecular patterns are recognised, Toll-like receptors ligate which prompts the production of many pro-inflammatory proteins, including IL-8 (Christmas 2010). IL-8 then draws in neutrophils to the infection site to neutralise the microbial threat (Kobayashi et al. 2018) IL-8 also upregulates complement receptors on the surface of neutrophils, which boosts the adherence of the leukocytes (Zwahlen et al. 1993). Stimulates respiratory burst which releases reactive oxygen species when neutrophils are infected by a pathogen, which degrades the bacteria (Bréchard et al. 2005).
Tumour Necrosis Factor - α (TNF-α)	Mostly produced by macrophages (Parameswaran and Patial 2010), TNF-α are proteins which are involved in both the innate and acquired immune responses. It is predominantly involved in tissue degradation, repair and signalling. These signalling pathways are dependent on which of the transmemebrane TNF- receptors are activated. TNFR1 starts a pathway in which promotes inflammatory cytokine concentration and survival of the cells. TNFR2 signals a different pathway in which cell proliferation and migration. Both receptors are more focused on cell survival and can inhibit targeted cell apoptosis (Yang et al. 2018). However, TNF-α can also trigger hyper-inflammation which can lead to elongated wound healing times(Ashcroft et al. 2012).
Interferon- ɣ (IFN-ɣ)	When macrophages recognise a pathogenic invasion, IFN-ɣ releasing interleukins are activated (Schroder et al. 2004). IFN-ɣ itself binds to IFNG receptors, which are present on a range of cell types. This starts the activation of two Janus kinases JAK1 and JAK2 (Shtrichman and Samuel 2001). JAK1 and JAK2 release phosphate which signal transducer and activator of transcription (STATs) form a dimer with. The large dimer then moves into the cell and binds to DNA. This, in turn, triggers the activation of genes to further the immune response (Kisseleva et al. 2002).

5.6 Materials

5.7 Proteins

Hydrosolanum PE and Hydrosativum P were chosen for this experiment as both had previously shown either positive or very little negative effects on the growth of the commensal *S. epidermidis* or did not overly promote *S. aureus* over the growth of *S. epidermidis.* The proteins were also in-keeping with cosmetic trends of not having been derived from animal products. Hydroavena HPO fit both profiles, however this cosmetic protein differs from the others tested as a large percentage of the active level contains oligosaccharides rather than peptides. The proteins were diluted in sterile dH2O pH 5 to active levels of 0.125%, 0.25%, 0.5%, 0.75%, 1% and 2%. The controls were fresh media and 0% (sterile dH2O, pH 5).

5.8 Bacteria

Both *Staphylococcus aureus* and *Staphylococcus epidermidis* used were as described previously in Section 2.6. *S .aureus* was stained with the fluorescent dye of CellTrace Red, and *S. epidermidis* was stained with flourescent CellTrace CFSE. Both *Staphylococcus* spp. were grown, as in Section 2.10, then spun at 6000 x g, washed twice with 1x PBS and incubated with the fluorescent dye. The bacteria were diluted down to 0.5 x 10^6 cfu/ml ready to be seeded, this number was chosen because in the preliminary experiments it was the most consistent inoculation number to be able to produce quantifiable adhered cells. Fluorescence for CellTrace red was measured at 635 nm excitation, and at 488 nm excitation for CellTrace CFSE. Fluorescence staining was originally used so that the bacteria could be counted by flow cytometry, but was then used to ensure the bacteria were still present throughout the week long experiment.

5.9 HaCaT Cells

HaCaT cells were kindly donated by the School of Dentistry at Cardiff University. Mycoplasma detection was carried out using polymerase chain reaction analysis involving mycoplasma specific primers (Hopert et al. 1993), the results were negative. The cells were cultivated as per suggestions from the dental school. HaCaTs were grown in high glucose DMEM plus pyruvate, 10% v/v fetal bovine serum, 1% v/v antibiotic-antimyotic (Streptomycin, Amphotericin B, Penicillin), 1% v/v L-Glutamine and in an incubator set at 5% CO_2, 37 ˚C. For the experiments, where bacteria were involved, there was no antibiotic present in the media. Cells were split appropriately once approximately acheived 60-70% confluence. Although the ideal pH for this experiment would have been pH 5, due to the time required to adjust the cells to a new pH, the media was used at the pH supplied. Changing the medium to a lower pH has been shown to reduce viability and the migration rates of cultured keratinocytes (Lönnqvis et al. 2015).

5.10 Methods

5.11 Tissue Experiment

HaCaT cells were grown to approximately 60-70% confluent and diluted to 0.5 x 10^5 cells/ml. These were seeded into 96 well plates for a final volume of 200 µl and incubated overnight. When a confluence of 40% across the plates was reached, this would be taken as time point 1 and the supernatent would be removed and stored for further work. The supernatent would be replaced with either fresh media, fresh media and *S. aureus*, fresh media and *S. epidermidis* or fresh media and a 1:1 *Staphylococcus* mix. Fluorescence was measured on a microplate reader to confirm presence of bacteria. After 48 hours to allow for sufficient bacterial adherence, the supernatent was removed and labelled as time point 2. The adhered cells were washed once with 1xPBS before being incubated with 100 µl of verying levels of proteins or controls for 15 minutes. The proteins were replaced with fresh media before fluorescence was measured to confirm bacterial presence and incubated. After 24 hours, supernatent for time point 3 was saved and the bacteria quantified.

5.12 Microscope Images

After bacteria were seeded with the established HaCaT cells, they were left to incubate for 48 hours. The wells were examined using the GXM-XDS Inverted Biological Phase Contrast Microscope (GT-Vision Ltd, Sudbury) at 40x magnification and images captured using a GXM-camera extension (GT-Vision Ltd, Sudbury). The images taken were of the control of HaCaT cells alone, HaCaT cells that were incubated with *S. aureus*, HaCaT cells which were incubated with *S. epidermidis* and HaCaT cells which were incubated with a 1:1 ratio *Staphylococcus* spp. mix.

5.13 Biomarker Assays

Sandwich ELISA assays for IL-8, IFN-ɣ and TNF-α were purchased from Peprotech. Assays were carried out per the manufacturer's protocols. Biomarker levels were measured in the supernatent that was removed at each time point, at neat, 1/25 and 1/50 dilutions.

5.14 Measurement of Adhered Bacteria

The quantity of each *Staphylococcus* species which had adhered to the plate or HaCaT cells were due to be measured on a flow cytometer. However, due to pandemic restraints on the University equipment, the flow cytometer was unavailable for use. Instead, the adhered bacteria were twice washed with 1xPBS and scratched off with a pipette tip. To quanitify, the bacteria were diluted in 1xPBS, plated, incubated and counted using the Miles and Misra method. Indivudal species were plated onto TSA, while co-culutred species were plated onto MSA.

5.15 Statistics

The mean concentrations of immune markers from the ELISA results and mean cfu/ml from the Miles and Misra method were analysed using t-tests using GraphPad Prism (Version 9.1.2). Graphs below were also made using this software.

5.16 Results

5.17 24 Hours After Seeding HaCaT Cells

The following results are in reference to Figure 23. The other Immune markers were expressed 24 hours after seeding into the 96 well plate (Figure 23). IL-8 had a mean concentration of 144.0 pg/ml, with a range of 216.2 pg/ml and a standard deviation of 83.19 pg/ml. TNF-α concentration was on average 1.178 pg/ml with a range of 4.571 pg/ml and a standard deviation of 1.848 pg/ml. IFN-ɣ had the largest range of concentration with 1233 pg/ml and an average concentration of 846.7 pg/ml, due to this large discrepancy in concentration the standard deviation is 469.6 pg/ml.

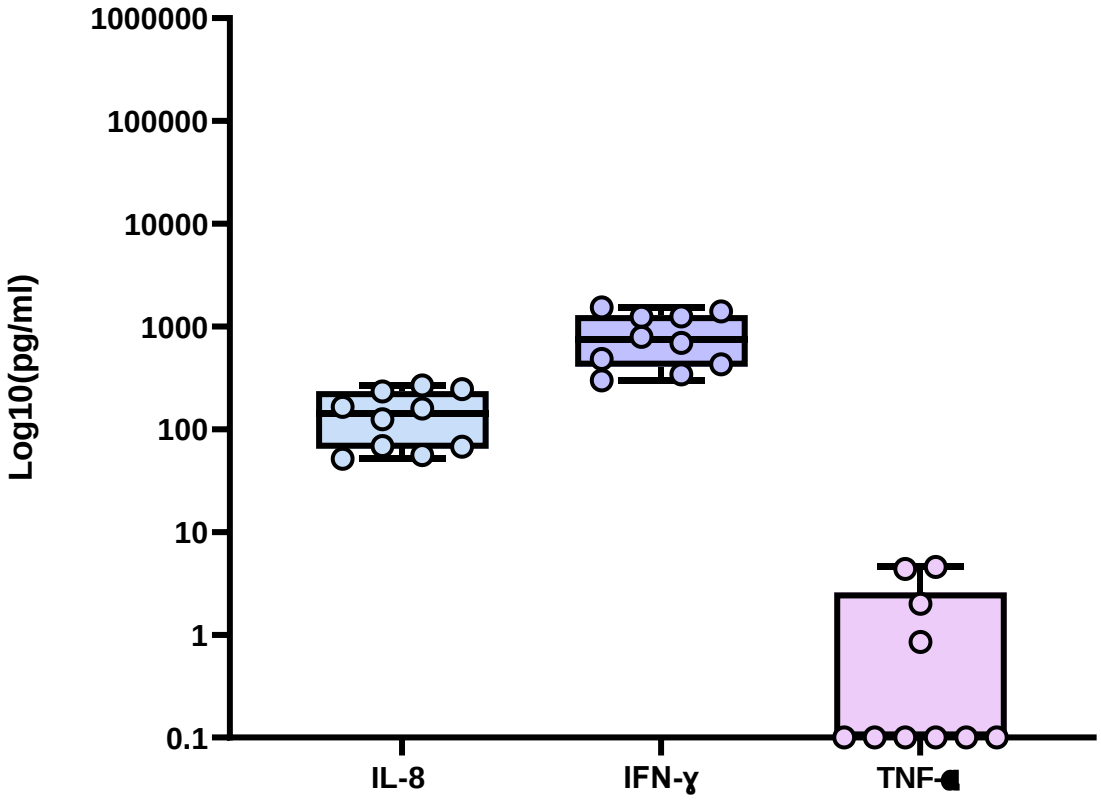


Figure 23. Concentration of Immune Markers by HaCaT cells 24 hours after Seeding. The experiment was carried out with three technical replicates and two biological replicates. IFN- Y was the most expressed marker, with a large range of different concentrations expressed, between 500-5000 pg/ml. IL-8 was the next most expressed marker with expression between 50-500 pg/ml. TNF-α was the least expressed of the quantifiable markers, with some results showing negligible expression, no more than 10 pg/ml of the immune marker was expressed after 24 hours.

5.18 48 Hours After Incubation with Bacteria

The following results are in reference Figure 24 A, B and C. The addition of *S. epidermidis* to HaCaT cells did not increase the immune markers expressed by HaCaT cells. Both IL-8 (Figure 24A) and TNF-α (Figure 24C) concentration were not significantly changed by the addition of *S. epidermidis* ($P>0.7$), while IFN-ɣ (Figure 24B) concentration was significantly reduced ($P=0.006$). Incubation with *S. aureus* or the *Staphylococcus* spp. co-culture elevated the concentration of all immune markers. Increased concentration of IL-8 and TNF-α were the most prominent ($P<0.0001$), while IFN-ɣ still significantly increased ($P=0.0044$). Despite double the number of bacteria inoculated to the HaCaT cells, compared to *S. aureus* alone, the co-culture of bacteria did not significantly change the concentration of IL-8 and IFN-ɣ ($P>0.07$) and had significantly decreased the concentration of TNF-α ($P=0.013$).

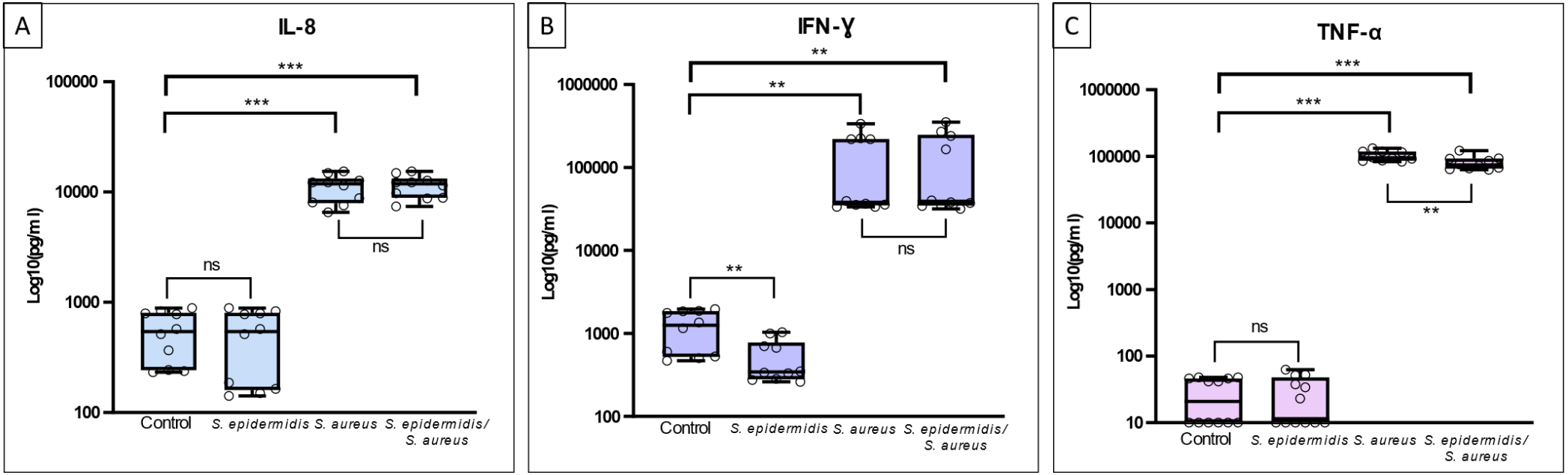


Figure 24. Concentration of Immune Markers measured in the HaCaT Supernatant after 48 hours Incubation with Bacteria. The experiment was carried out with three technical replicates and two biological replicates. A) IL-8 B) IFN-Y and C) TNF-a. There was no significant difference between the concentration of IL-8 in HaCaT supernatant between the control and HaCaT cells incubated with *S. epidermidis* ($P>0.05$). However, incubation with *S. aureus* increased the concentration of IL-8 by at least 10-fold compared to both the control and HaCaT with *S. epidermidis* ($P<0.001$). A similar result is seen when HaCaT cells were incubated with both *Staphylococcus* spp., however the concentration of IL-8 was not significantly different from when HaCaT cells were incubated with *S. aureus* alone ($P>0.05$). A similar result to IL-8 was seen with IFN-Y, however incubation with *S. epidermidis* significantly decreased the concentration of IFN-Y in the supernatant ($P<0.01$). Again, incubation with S. aureus and the Staphylococcus spp. mix increased the concentration of TNF-a in the supernatant, however this was nearly 10,000-fold ($P<0.001$), as TNF-a was barely negligible when HaCaT cells were alone or incubated with *S. epidermidis*.

5.19 Microscope Images of HaCaT Cells After 48 hours, Incubation with and without Staphylococcus spp.

Images (Figure 25) from the microscope show the physiological health and attachment of the HaCaT cells 72 hours after initial seeding and 48 hours after introduction of *Staphylococcus* spp. The results in Section 5.18 are a qualitative and descriptive assessment of the images.

5.19.1 How did the HaCaT Cells Look without Bacteria?

Without the addition of bacteria, the HaCaT cells had a continuous unbroken mosaic formation around the perimeter of the well and proliferation of the cells were moving towards the centre of the well. There was evidence of cell death, due to some shrivelled cells that had remained attached to the monolayer (Figure 25A).

5.19.2 How did the HaCaT cells look when Grown with *Staphylococcus epidermidis*?

The HaCaT cells still were in a mosaic pattern, but there were more gaps between clumps of cells. Cells were still proliferating towards the centre of the well. There were more signs of dead cells and some cells had become swollen, and some cells had detached (Figure 25B).

5.19.3 How did the HaCaT cells look when Grown with *Staphylococcus aureus*?

HaCaT cells were still mostly confined to the perimeter but were separated into clumps. The cells were extremely swollen, and many were shrivelled and dead. Any proliferation into the centre of the well had now stopped and there were many cells that had removed from attachment (Figure 25C).

5.19.4 How did the HaCaT cells look when Grown with a Co-culture of *Staphylococcus* spp.?

HaCaT cells were still surrounding the perimeter, but again, separated into lots of clumps of cells. The cells were again swollen, and many appeared shrivelled and detached, but not to the extent of *S. aureus* alone. There was no evidence that the cells were proliferating into the centre of the well. Again, many cells had detached from the bottom of the well (Figure 25D).

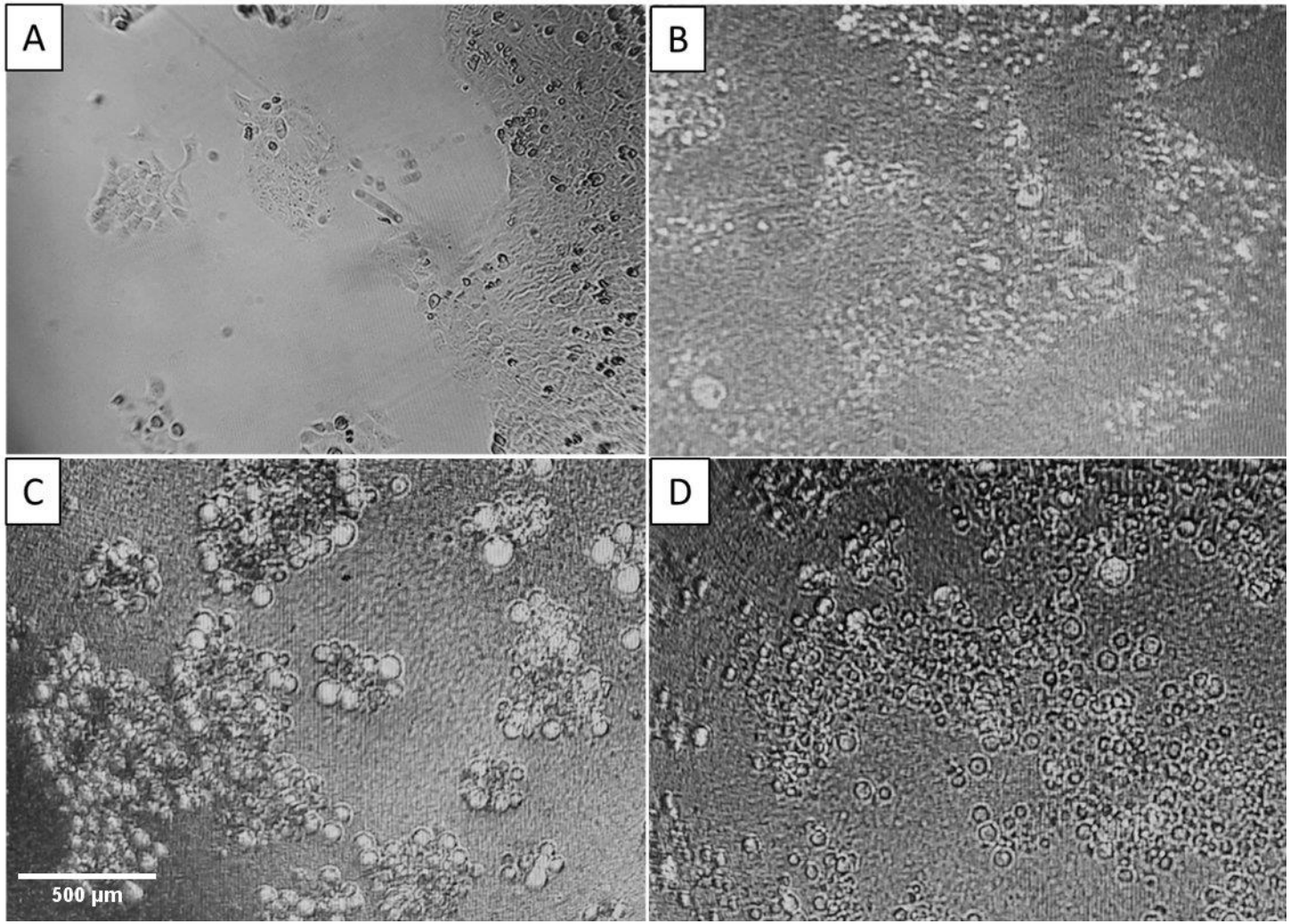


Figure 25. Microscope images of HaCaT cells Grown with and without *Staphylococcus* spp. A) HaCaT alone B) HaCaT with *S. epidermidis* C) HaCaT with *S. aureus* D) HaCaT with *S. epidermidis* and *S. aureus* Mix. After 48 hours of incubation, there are naturally dead cells present without the addition of bacteria. However, clear stress signs can be seen with HaCaT cells incubated with the bacteria, especially with *S. aureus* alone or with the co-culture. Reduced adhesion and cell swelling are clear indications of the keratanocytes are under immune stresses.

5.20 Does Exposure to Hydrosativum P Affect Interactions Between the *Staphylococcus* spp. and HaCaT Immune Marker Concentration?

5.20.1 How did exposure to Hydrosativum P alone Affect the Concentration of IL-8 from HaCaT cells?

All results below are in respect to Figure 26. Exposure of HaCaT cells to sterilised water (0%), 0.5% and 0.75% active levels of Hydrosativum P had no significant effect on the concentration of IL-8 ($P>0.06$). Exposure to 0.125, 0.25, 1 and 2% active levels of Hydrosativum P significantly reduced the amount of IL-8 expressed by the HaCaT cells ($P<0.05$). There were no significant differences between the amount of IL-8 expressed when exposed to 0.5 and 0.75% active levels compared to 2% active level of Hydrosativum P. Therefore, the concentration of IL-8 was on trend to be less when exposed to Hydrosativum P, compared to the control, reducing expressed IL-8 from an average of 509.6 pg/ml to as low as 362.6 pg/ml.

5.20.2 How did exposure to Hydrosativum P Affect the Concentration of IL-8 from HaCaT cells incubated with *S. epidermidis*?

The addition of *S. epidermidis* alone to HaCaT cells reduced the concentration of IL-8 significantly ($P<0.001$), from an average concentration of 551.7 pg/ml to 134.1 pg/ml. Exposure to water and the 0.125% active level of Hydrosativum P did not significantly change ($P>0.10$) the expressed IL-8 compared to *S. epidermidis* alone. Exposure to 0.25-0.75% active level significantly increased ($P<0.001$) the expressed IL-8 compared to *S. epidermidis* alone. With 0.25% active level concentration levels were still significantly less ($P=0.005$) than HaCaT cells without *S. epidermidis*, 509.6.7 pg/ml compared to 280.4 pg/ml. With 0.5 and 0.75% active level it was not significantly more than HaCaT cells without *S. epidermidis* ($P>0.42$), 509.6 pg/ml compared to 555.3 pg/ml and 522.2 pg/ml, respectively. Exposure of HaCaT cells with *S. epidermidis* to 1% active level of Hydrosativum P significantly increased the concentration of IL-8 compared to HaCaT cells alone ($P<0.003$). HaCaT cells alone expressed 509.6 pg/ml, while when grown with *S. epidermidis* and exposed with 1% active level this increased to 825.7 pg/ml. Exposure to 2% active levels of Hydrosativum P overall significantly reduced the concentration of IL-8 to 489.3 pg/ml, however the range of expressed IL-8 with this active level is large between 380-676.56 pg/ml. While *S. epidermidis* reduced the concentration of IL-8, exposing HaCaT grown with *S. epidermidis* to increased levels of Hydrosativum P increased the IL-8 expressed.

5.20.3 How did exposure to Hydrosativum P Affect the Concentration of IL-8 from HaCaT cells incubated with *S. aureus*?

Incubation of HaCaT cells with *S. aureus* significantly increases the average concentration of IL-8 compared to HaCaT cells alone or with *S. epidermidis* ($P<0.001$), concentration of IL-8 was raised up to 3039 pg/ml. Exposure to Hydrosativum P only slightly significantly raised the concentration level compared to HaCaT with *S. aureus* alone with active levels 0.125 and 0.25% ($P<0.05$). With 0, 0.5, 0.75, 1 and 2% active level of Hydrosativum P did not significantly change the concentration of IL-8

compared to HaCaT cells with *S. aureus* alone (P>0.05). As a trend, addition of Hydrosativum to HaCaT cells grown with *S. aureus* did not increase IL-8 concentration of the cells, and if there was an increase it was only just significant.

5.20.4 How did exposure to Hydrosativum P Affect the Concentration of IL-8 from HaCaT cells incubated with *Staphylococcus* spp. Co-culture?

Again, when HaCaT cells were incubated with the *Staphylococcus* spp. mix, the IL-8 levels were significantly higher than when HaCaT cells were grown alone (P<0.001) and with *S. epidermidis* (P<0.001). The concentration of IL-8 was also slightly significantly increased compared to HaCaT cells with *S. aureus* alone (P=0.04). Exposure to Hydrosativum P active levels 0.5 and 0.75% significantly increased the IL-8 levels expressed by the HaCaT cells grown with the *Staphylococcus* co-culture, however it was only a slight significant increase (P=0.02). When HaCaT cells grown with *Staphylococcus* co-culture was grown with 0, 0.125, 25, 1 and 2% active levels of Hydrosativum P there was no significant change in concentration levels (P>0.06). Again, the addition of Hydrosativum P, generally, did not significantly affect the concentration of IL-8 by HaCaT cells grown with the *Staphylococcus* spp. co-culture, and if it was increased it was only slightly significant.

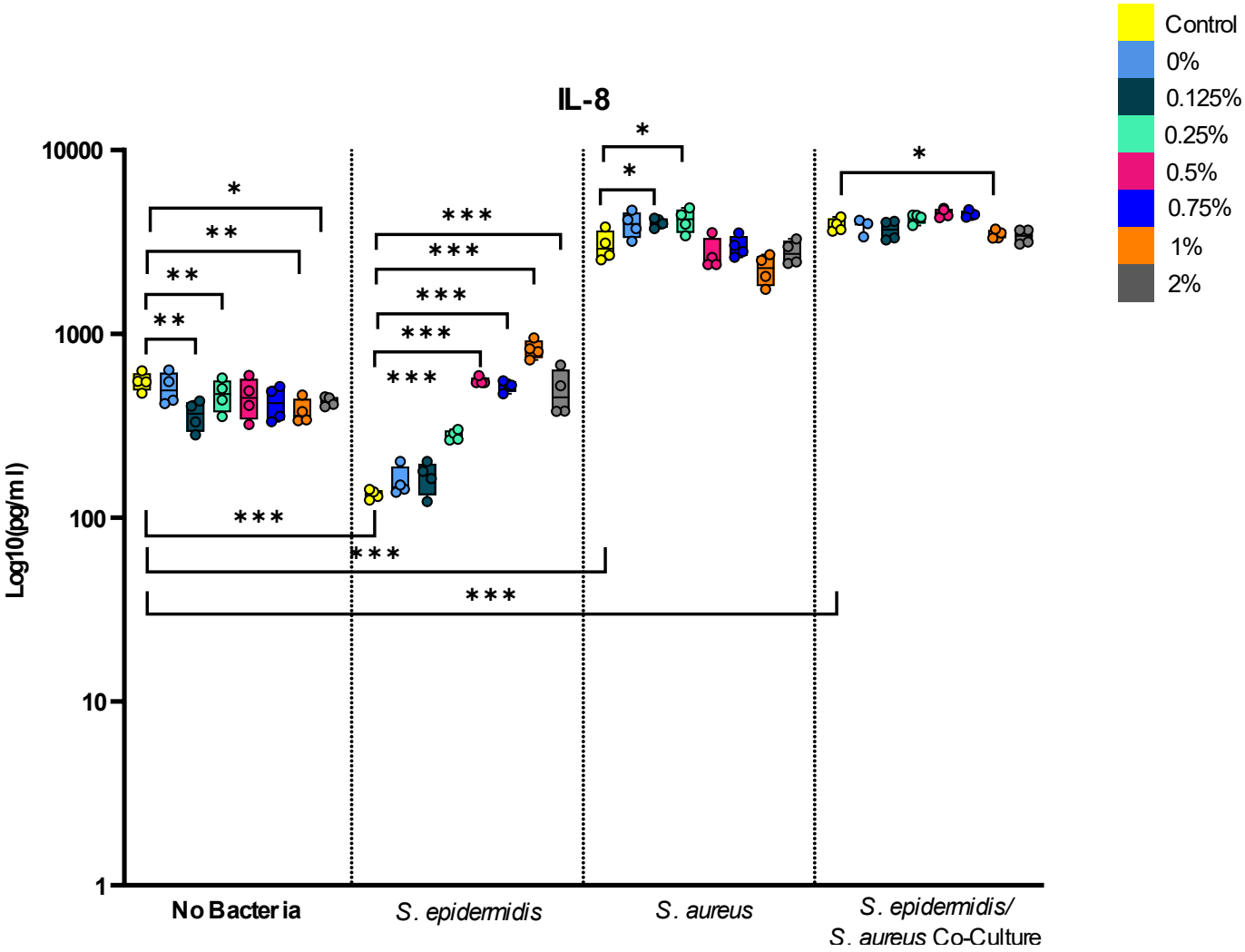


Figure 26. Concentration of IL-8 from HaCaT cells With or Without Bacteria after Being Exposed to Various Active Levels of Hydrosativum P. Yellow bars represent HaCaT cells (with or without bacteria) in which they have not been exposed to any outside source, only the media was changed out. The blue bar represents the HaCaT cells being exposed to 0% of the cosmetic protein, otherwise known as sterile water. The other colours are exposure to active levels 0.125-2% of the cosmetic proteins. Non-significant values were not added, nor values between species. *<0.05, **<0.01, ***<0.0001. For all statistics, please see above Section 5.19.1-5.19.4.

5.20.5 How did exposure to Hydrosativum P alone Affect the Concentration of IFN-Y from HaCaT cells?

The following results are in relation to Figure 27. HaCaT cells grown alone expressed a varied level of IFN-ɣ, but the average value was 443.8 pg/ml. When exposed to 0, 0.125, 0.25, 0.5, 0.5, 0.75 and 2% Hydrosativum P, there was no significant change in the IFN-ɣ expressed by the HaCaT cells. There was a slightly significant increase (P=0.045) in IFN-ɣ when HaCaT cells were incubated with 1% active level of Hydrosativum P, up to 818.4 pg/ml, but exposure to all active levels resulted in a large range of concentrations.

5.20.6 How did exposure to Hydrosativum P Affect the Concentration of IFN-ɣ from HaCaT cells incubated with *S. epidermidis*?

While the addition of *S. epidermidis* did not significantly change the concentration of IFN-ɣ from HaCaT cells alone, the mean concentration at 206.6 pg/ml and only slightly significant result would suggest that it does have some reducing affect (P=0.054). The addition of 0.125-2% active levels of Hydrosativum also significantly increased (P<0.003) the concentration of IFN-ɣ compared to HaCaT cells grown with *S. epidermidis* alone, up to 1504 pg/ml. Even the exposure to 0%, (sterile water), increased the concentration of IFN-ɣ significantly to 994 pg/ml (P=0.0023). While the addition of *S. epidermidis* slightly decreased the concentration of IFN-ɣ, although not significantly, addition of water and Hydrosativum P increased the concentration significantly.

5.20.7 How did exposure to Hydrosativum P Affect the Concentration of IFN-ɣ from HaCaT cells incubated with *S. aureus*?

Addition of *S. aureus* to HaCaT cells significantly increased (P<0.001) to 35,616 pg/ml the production of IFN-ɣ compared to HaCaT cells grown alone or with *S. epidermidis*. Exposure to 0-0.5% Hydrosativum P significantly increased the concentration (P<0.031) of IFN-ɣ by HaCaT cells grown with *S. aureus* alone, up to 53,024 pg/ml with 0.5%. Exposure to 0.75-2% active level of Hydrosativum did not significantly change the concentration of IFN-ɣ by HaCaT cells grown with *S. aureus* (P>0.15). Exposure of HaCaT cells grown with *S. aureus* to Hydrosativum P did increase the concentration of IFN-ɣ, but not with 0.5% active level and above.

5.20.8 How did exposure to Hydrosativum P Affect the Concentration of IFN-ɣ from HaCaT cells incubated with *Staphylococcus* spp. Co-culture?

Incubation of HaCaT cells with the *Staphylococcus* spp. co-culture did increase the concentration of IFN-ɣ compared to HaCaT cells grown alone, or with *S. epidermidis* (P<0.001). However, compared to HaCaT cells grown with *S. aureus* alone, there was no significant change in the IFN-ɣ expressed (P=0.24). Exposure of HaCaT cells grown with *Staphylococcus* spp. mix to water, 0.125 or 2% active levels of Hydrosativum P did not significantly affect the concentration of IFN-ɣ

(P>0.2). However, exposure to 0.25, 0.5, 0.75 and 1% active did significantly increase the concentration of IFN-ɣ (P<0.026).

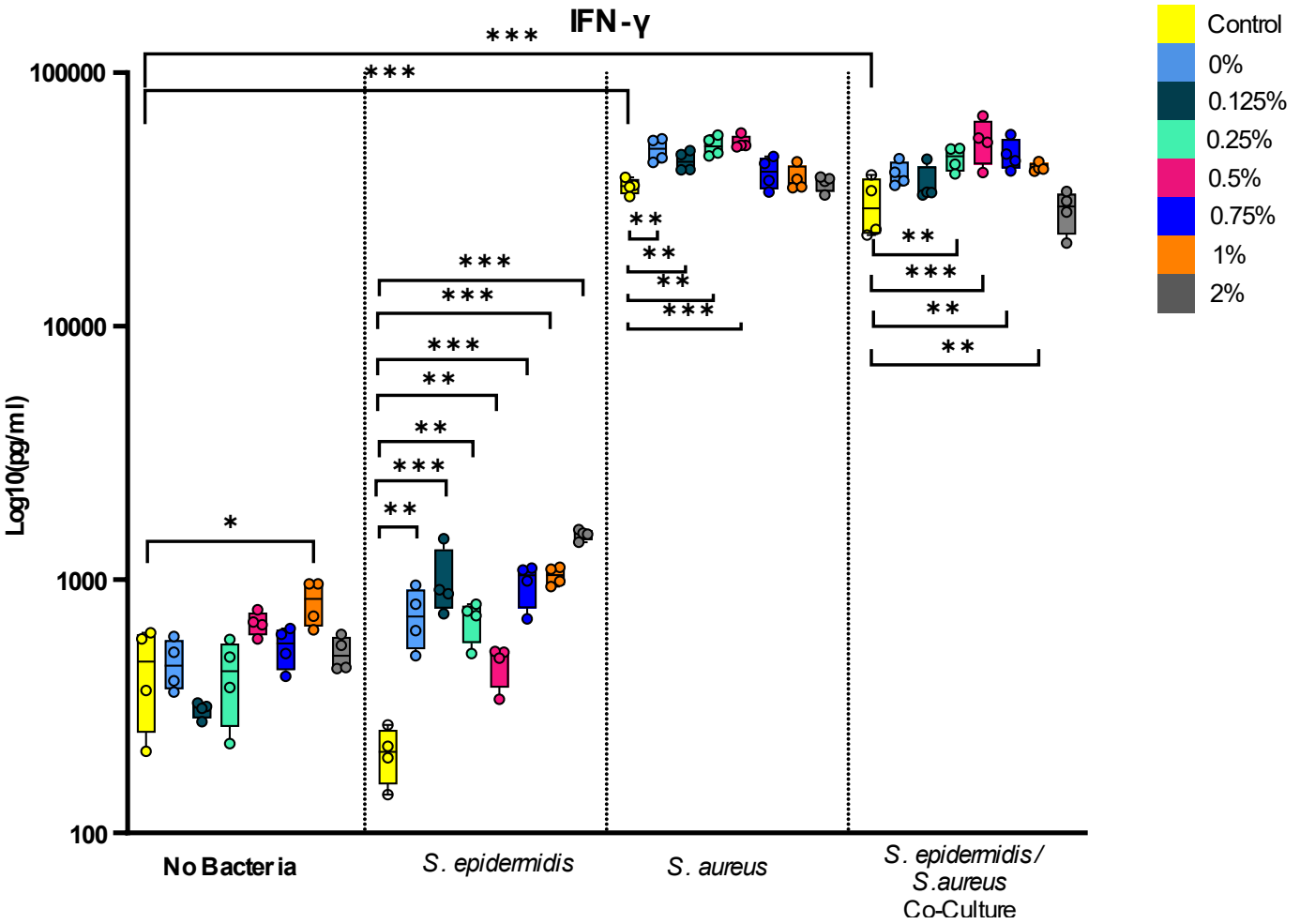


Figure 27. Concentration of IFN-γ from HaCaT cells With or Without Bacteria after Being Exposed to Various Active Levels of Hydrosativum P. Yellow bars represent HaCaT cells (with or without bacteria) in which they have not been exposed to any outside source, only the media was changed out. The blue bar represents the HaCaT cells being exposed to 0% of the cosmetic protein, otherwise known as sterile water. The other colours are exposure to active levels 0.125-2% of the cosmetic proteins. Non-significant values were not added, nor values between species. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.19.5-5.19.8.

5.20.9 How did exposure to Hydrosativum P alone Affect the Concentration of TNF-α from HaCaT cells?

All results below are in reference to Figure 28. HaCaT cells alone expressed 50.77 pg/ml of TNF-α. Exposure of Hydrosativum P to HaCaT cells alone increased concentration of TNF-α when exposed to 0, 0.125 and 0.25%, in which concentration increased up to 97.05 pg/ml ($P<0.02$). The biggest significant increase in TNF-α concentration was when HaCaT cells were exposed to Hydrosativum P at active level 2%, in which concentration shot up to 234.4 pg/ml (<0.001). However, exposure to 0.5, 0.75 and 1% active did not significantly increase concentration of TNF-α by HaCaT cells alone ($P>0.05$).

5.20.10 How did exposure to Hydrosativum P Affect the Concentration of TNF-α from HaCaT cells incubated with *S. epidermidis*?

Growing HaCaT cells with *S. epidermidis* significantly reduced the concentration of TNF-α compared to HaCaT cells alone, no TNF-α was detected in the supernatant ($P<0.001$). Again, TNF-α was undetectable when HaCaT cells grown with *S. epidermidis* were exposed to sterile water (0%). However, exposure to 0.125% active level of Hydrosativum P and above increased the TNF-α expressed significantly compared to HaCaT cells with *S. epidermidis* alone ($P<0.0006$). However, exposure to 0.125, 0.25 and 0.5% Hydrosativum P still significantly reduced the concentration of TNF-α of HaCaT cells with *S. epidermis* compared to than HaCaT cells alone expressed ($P<0.03$). Even exposure of HaCaT cells with *S. epidermidis* to Hydrosativum P at 0.75-2% active levels did not result in TNF-α concentration which was significantly more than HaCaT cells alone ($P>0.2$).

5.20.11 How did exposure to Hydrosativum P Affect the Concentration of TNF-α from HaCaT cells incubated with *S. aureus*?

S. aureus being incubated with HaCaT cells made their concentration increase to 20, 409 pg/ml, significantly more than HaCaT cells alone or with *S. epidermidis* ($P<0.0001$). Exposure of HaCaT cells incubated with S*. aureus*, to any level of Hydrosativum P did not significantly change the concentration levels of TNF-α ($P>0.05$).

5.20.12 How did exposure to Hydrosativum P Affect the Concentration of TNF-α from HaCaT cells incubated with *Staphylococcus* spp. Co-culture?

The *Staphylococcus* spp*.* co-culture being incubated with HaCaT cells made their concentration increase to 15, 597 pg/ml, significantly more than HaCaT cells alone or with *S. epidermidis* ($P<0.001$). However, this was significantly less concentration of TNF-α compared to HaCaT cells grown with *S. aureus* alone ($P=0.045$). Again, exposure of any active level of Hydrosativum P did not significantly change the concentration of TNF-α from HaCaT cells with *Staphylococcus* spp. mix ($P>0.057$).

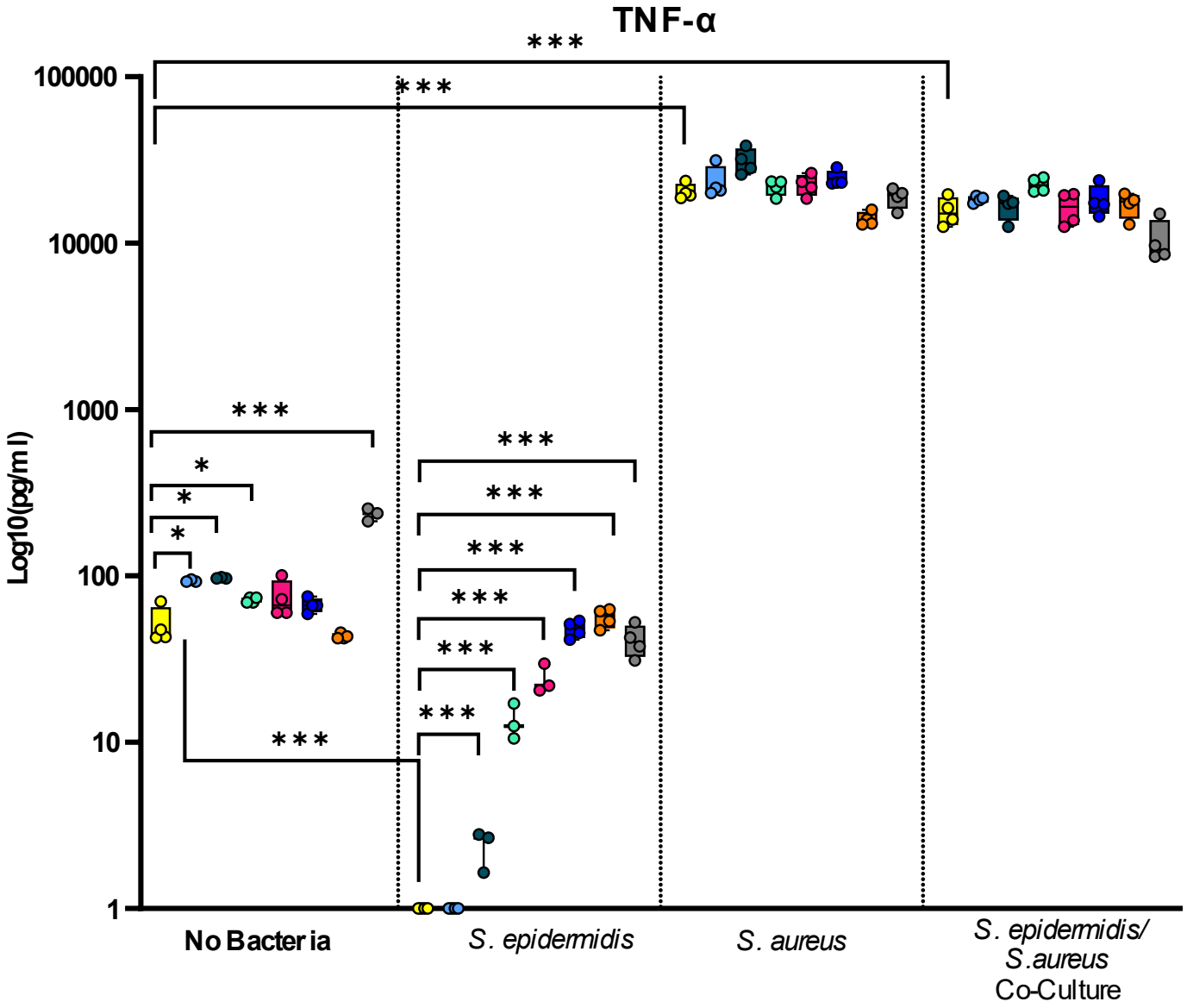


Figure 28. Concentration of TNF-α from HaCaT cells With or Without Bacteria after Being Exposed to Various Active Levels of Hydrosativum P. Yellow bars represent HaCaT cells (with or without bacteria) in which they have not been exposed to any outside source, only the media was changed out. The blue bar represents the HaCaT cells being exposed to 0% of the cosmetic protein, otherwise known as sterile water. The other colours are exposure to active levels 0.125-2% of the cosmetic proteins. Non-significant values were not added, nor values between species. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.19.9-5.19.12.

5.20.13 How did exposure to Hydrosativum P Affect the Final CFU/ml of *S. epidermidis* grown with HaCaT cells?

All results below are in reference to Figure 29. For the control, the final number of attached *S. epidermidis* grown with HaCaT cells was, on average, $2x10^9$ cfu/ml. When exposed to the 0% control, i.e., sterile water, this final cfu/ml was not significantly reduced (p=0.55). However, when exposed to Hydrosativum P, the final cfu/ml of *S. epidermidis* was significantly reduced when exposed to active levels 0.125-2%, compared to both controls (for first control, P<0.0002, for 0% control P<0.004). The final cfu/ml of *S. epidermidis* was significantly reduced when grown with 0.25% compared to 0.125%, with 0.5% compared to 0.25% and with 2% compared to 1% (P<0.03). However, there was no statistical difference between the final cfu/ml of *S. epidermidis* when exposed to 0.5 and 0.75% active levels or 0.75% and 1% active levels of Hydrosativum P (P>0.05). The higher the active level of Hydrosativum P exposed to *S. epidermidis*; the fewer bacteria were able to remain attached. However, even at the highest active level tested, the final cfu/ml was still significantly higher than the original inoculated amount.

5.20.14 How did exposure to Hydrosativum P Affect the Final CFU/ml of *S. aureus* grown with HaCaT cells?

For the control, the final number of attached *S. aureus* grown with HaCaT cells was, on average, $2.2\ x10^9$ cfu/ml, not significantly different to the final number of adhered of *S. epidermidis* (P=0.44). Exposure to the 0% Hydrosativum P, sterile water, significantly reduced the final cfu/ml of adhered *S. aureus* (P=0.014), however after exposure to 0.125% active level of Hydrosativum P the final cfu/ml of adhered cells increased compared to 0% but was not significantly different to the original control (P=0.22). Exposure of 0.125-2% active levels of Hydrosativum P did not significantly change the number of adhered cells of *S. aureus* compared to the original control (P>0.2) and the final cfu/ml of *S. aureus* was not significantly different when all active levels were compared against each other (P=0.10). Although looking at the data from Figure 29 would suggest that increasing the active level of Hydrosativum P increased the final cfu/ml of adhered *S. aureus*, this was only a trend and was not significant.

5.20.15 How did exposure to Hydrosativum P Affect the Final CFU/ml of *Staphylococcus* spp. Co-culture grown with HaCaT cells?

For the control, the final number of attached *S. epidermidis* cells was on average $2.4x10^8$ cfu/ml, while the number of attached *S. aureus* cells was on average $2.3x10^8$ cfu/ml, no significant difference between the two (P=0.61). The final cfu/ml for both species did not significantly change when exposed to the 0% control (P>0.11). Exposure to 0.125-1% active levels of Hydrosativum P did not significantly alter the final cfu/ml of *S. epidermidis* compared to the control, with 2%

active level the final cfu/ml was significantly reduced to $1.3x10^8$ (P=0.0009). Exposure of *S. aureus* to 0.125 and 0.25% Hydrosativum P significantly increased the final cfu/ml to $2.8x10^8$ with both active levels (P<0.02). However, exposure to 0.5-1% Hydrosativum P did not significantly alter the final cfu/ml of adhered cells compared to the control (P>0.05). Exposure of *S. aureus* to 2% active level of Hydrosativum P significantly reduced the final cfu/ml to $1.5x10^8$ (P=0.001). The only significant difference between adhered *Staphylococcus* spp. cells was when the two species were exposed to 0.25, 0.5 and 0.75% Hydrosativum P (P<0.04), *S. aureus* was the dominant species. When exposed to 0.125, 1 and 2% active level of Hydrosativum P there was no significant difference between the quantity of adhered cells (P>0.05).

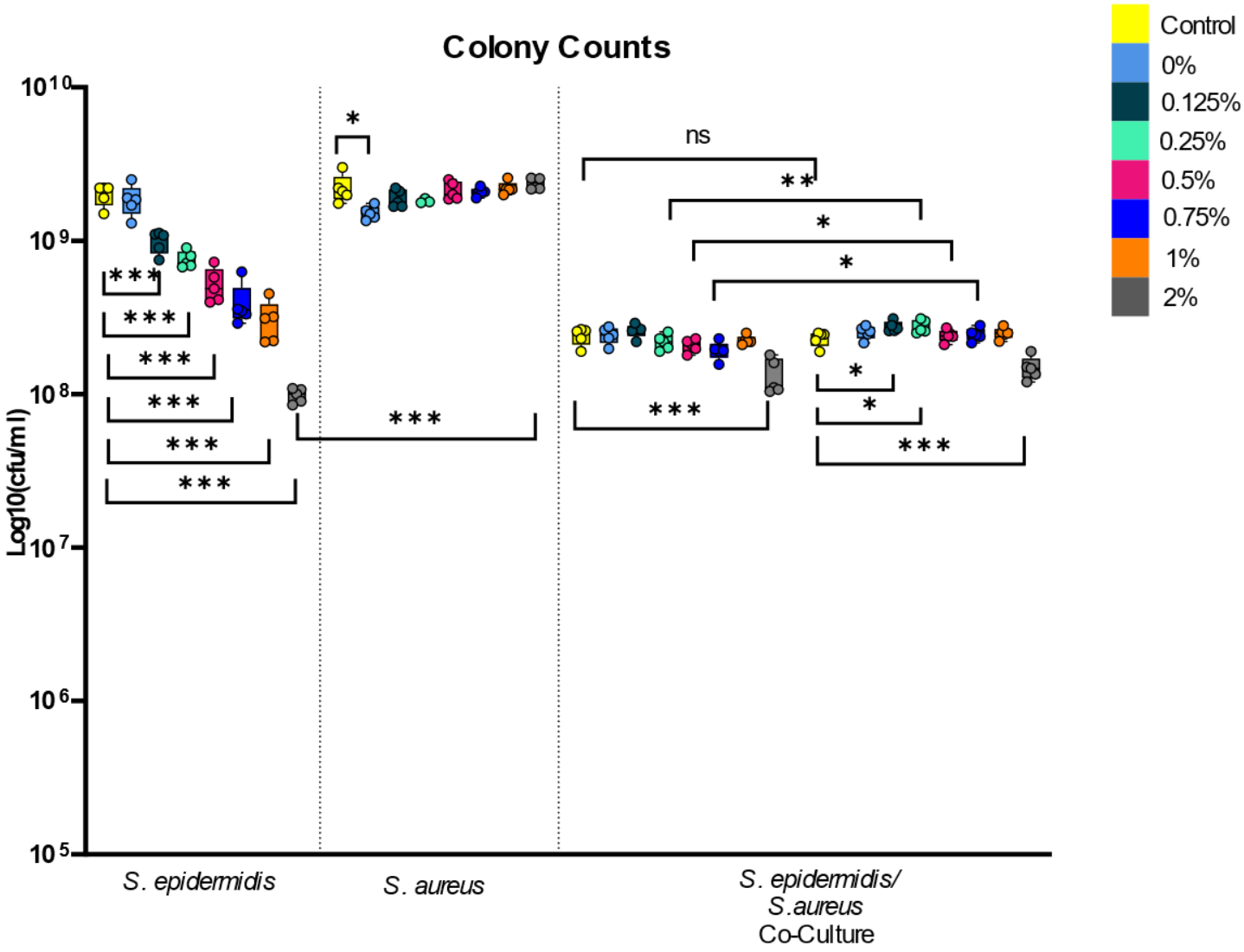

Colony Counts
Log10(cfu/ml)
10^10
10^9
10^8
10^7
10^6
10^5
Control
0%
0.125%
0.25%
0.5%
0.75%
1%
2%
ns
S. epidermidis
S. aureus
S. epidermidis/
S.aureus
Co-Culture

Figure 29. Final CFU/ml of *S. epidermidis*, alone, *S. aureus* alone and the *Staphylococcus* spp. Co-culture after being exposed to Various Active levels of Hydrosativum P. Statistical values between individual species were not all added, when exposed to active levels 0.125-2%, individual *S. aureus* had a significantly higher cfu/ml than *S. epidermidis*. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.19.13-5.19.15.

5.21 Does Exposure to Hydrosolanum PE Affect Interactions Between the *Staphylococcus* spp. and HaCaT Immune Marker Concentration?

5.21.1 How did exposure to Hydrosolanum PE alone Affect the Concentration of IL-8 from HaCaT cells?

All results below are in reference to Figure 30. HaCaT cells grown alone expressed 1092 pg/ml of IL-8, when exposed to 0-0.75% active levels of Hydrosolanum PE the concentration of IL-8 was significantly reduced ($P<0.0007$) to between 360.1-565.9 pg/ml. When exposed to 1 and 2% active levels of Hydrosolanum PE, the expressed IL-8 was increased compared to the lower active levels ($P<0.05$), but not significantly more than the control ($P>0.06$).

5.21.2 How did exposure to Hydrosolanum PE Affect the Concentration of IL-8 from HaCaT cells incubated with *S. epidermidis*?

Addition of *S. epidermidis* to HaCaT cells significantly reduced the concentration of IL-8 compared to HaCaT cells alone, from 1092 pg/ml to negligible quantities ($P<0.0001$). No quantifiable IL-8 was measured by HaCaT cells with *S. epidermidis* when exposed to 0-0.5% Hydrosolanum PE. After being exposed to 0.75-2% active levels of Hydrosolanum PE, the concentration of IL-8 increased significantly ($P<0.0001$) to between 23-26 pg/ml but was still significantly less IL-8 than HaCaT cells expressed when grown alone ($P<0.0001$). There was no significant difference in the concentration of IL-8 from HaCaT cells grown with *S. epidermidis* exposed to 0.75 and 1%, or 1 and 2% Hydrosolanum PE ($P<0.05$).

5.21.3 How did exposure to Hydrosolanum PE Affect the Concentration of IL-8 from HaCaT cells incubated with *S. aureus*?

When incubated with *S. aureus*, HaCaT expressed 8229 pg/ml of IL-8, a significant increase compared to HaCaT cells alone or with *S. epidermidis* ($P<0.0001$). Exposure of HaCaT cells grown with *S. aureus* to between 0-0.75% Hydrosolanum PE significantly reduced the concentration of IL-8 to as low as 5065 pg/ml, compared to the *S. aureus* control ($P<0.02$). Exposure of HaCaT plus *S. aureus* to 1 and 2% active levels of Hydrosolanum significantly increased concentration of IL-8 compared to the lower active levels ($P<0.009$) but not significantly more than the *S. aureus* control ($P>0.05$). Addition of the lower active levels of Hydrosolanum PE reduced IL-8 concentration, but even at the higher active levels, the IL-8 concentration was not increased.

5.21.4 How did exposure to Hydrosolanum PE Affect the Concentration of IL-8 from HaCaT cells incubated with *Staphylococcus* spp. Co-culture?

Incubation of HaCaT cells with the *Staphylococcus* spp. co-culture significantly increased the concentration of IL-8 compared to HaCaT alone, with *S. epidermidis* and with *S. aureus* ($P<0.0005$). Exposure of the HaCaT cells with the *Staphylococcus* spp. mix to 0-0.25% active levels of Hydrosolanum PE did not significantly change the concentration of IL-8 ($P>0.2$). However, exposure to 0.5-2% Hydrosolanum PE significantly increased the concentration compared to the control ($P<0.03$). There was no significant difference between the concentration of HaCaT cells plus *Staphylococcus* spp. mix when exposed to 0.5-2% active levels of Hydrosolanum PE ($P>0.6$).

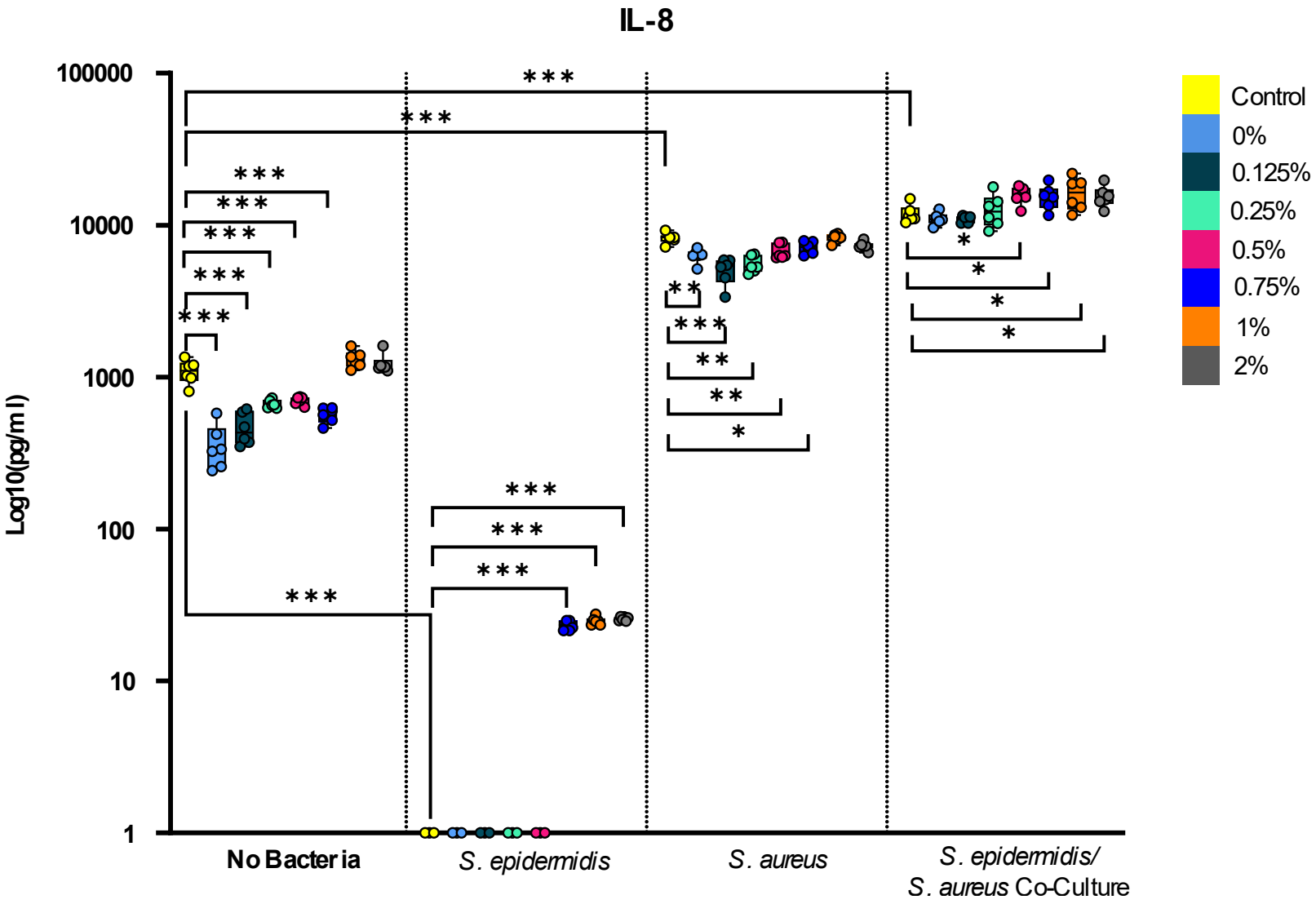


Figure 30. Concentration of IL-8 from HaCaT cells With or Without Bacteria after Being Exposed to Various Active Levels of Hydrosolanum PE. Yellow bars represent HaCaT cells (with or without bacteria) in which they have not been exposed to any outside source, only the media was changed out. The blue bar represents the HaCaT cells being exposed to 0% of the cosmetic protein, otherwise known as sterile water. The other colours are exposure to active levels 0.125-2% of the cosmetic proteins. Non-significant values were not added, nor values between species. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.20.1-5.20.4.

5.21.5 How did exposure to Hydrosolanum PE alone Affect the Concentration of IFN-ɣ from HaCaT cells?

All results below are in reference to Figure 31. HaCaT cells grown alone expressed 2265 pg/ml of IFN-ɣ. Exposure of these cells to every active level of Hydrosolanum PE, including 0% control, increased the concentration of IFN-ɣ significantly to as high as 7825 pg/ml with an active level of 2% ($P<0.0001$). There was no significant difference with any active level in the concentration of IFN-ɣ from HaCaT cells when exposed to 0.125-2% Hydrosolanum PE ($P>0.05$), however concentration with active levels was significantly higher than concentration with the 0% control ($P<0.02$).

5.21.6 How did exposure to Hydrosolanum PE Affect the Concentration of IFN-ɣ from HaCaT cells incubated with *S. epidermidis*?

HaCaT cells grown with *S. epidermidis* significantly reduced the concentration of IFN-ɣ to 261.5 pg/ml, compared to HaCaT cells grown alone ($P<0.0001$). Exposure to 0, 0.125 and 0.75% Hydrosolanum PE did not significantly change the concentration of IFN-ɣ from HaCaT cells with *S. epidermidis* ($P>0.85$). However, exposure to 0.25, 0.5, 1 and 2% Hydrosolanum PE significantly increased ($P<0.05$) the concentration of IFN-ɣ to as high as 495 pg/ml, however this was still significantly less than HaCaT cells alone ($P=0.0002$).

5.21.7 How did exposure to Hydrosolanum PE Affect the Concentration of IFN-ɣ from HaCaT cells incubated with *S. aureus*?

Incubation of HaCaT cells with *S. aureus* increased the concentration of IFN-ɣ from 2265 pg/ml to 18105 pg/ml which is a significant increase ($P<0.0001$). Exposure to 0 and 0.125% Hydrosolanum PE significantly reduced the concentration of IFN-ɣ from HaCaT cells with *S. aureus* compared to the control ($P<0.04$). Exposure to 0.25-1% Hydrosolanum PE did not significantly alter the concentration of IFN-ɣ from HaCaT Cells with *S. aureus* ($P>0.40$), however exposure to 2% active level significantly increased the concentration of IFN-ɣ ($P=0.024$).

5.21.8 How did exposure to Hydrosolanum PE Affect the Concentration of IFN-ɣ from HaCaT cells incubated with *Staphylococcus* spp. Co-culture?

Addition of the *Staphylococcus* spp. to the HaCaT cells significantly increased the concentration of IFN-ɣ compared to HaCaT alone and with *S. epidermidis* ($P<0.0001$), up to 13,713 pg/ml. However, it did not significantly increase the concentration of IFN-Y compared to incubation with *S. aureus* alone (0.04). Exposure to 0-2% significantly increased the concentration of IFN-ɣ compared to the control ($P<0.001$).

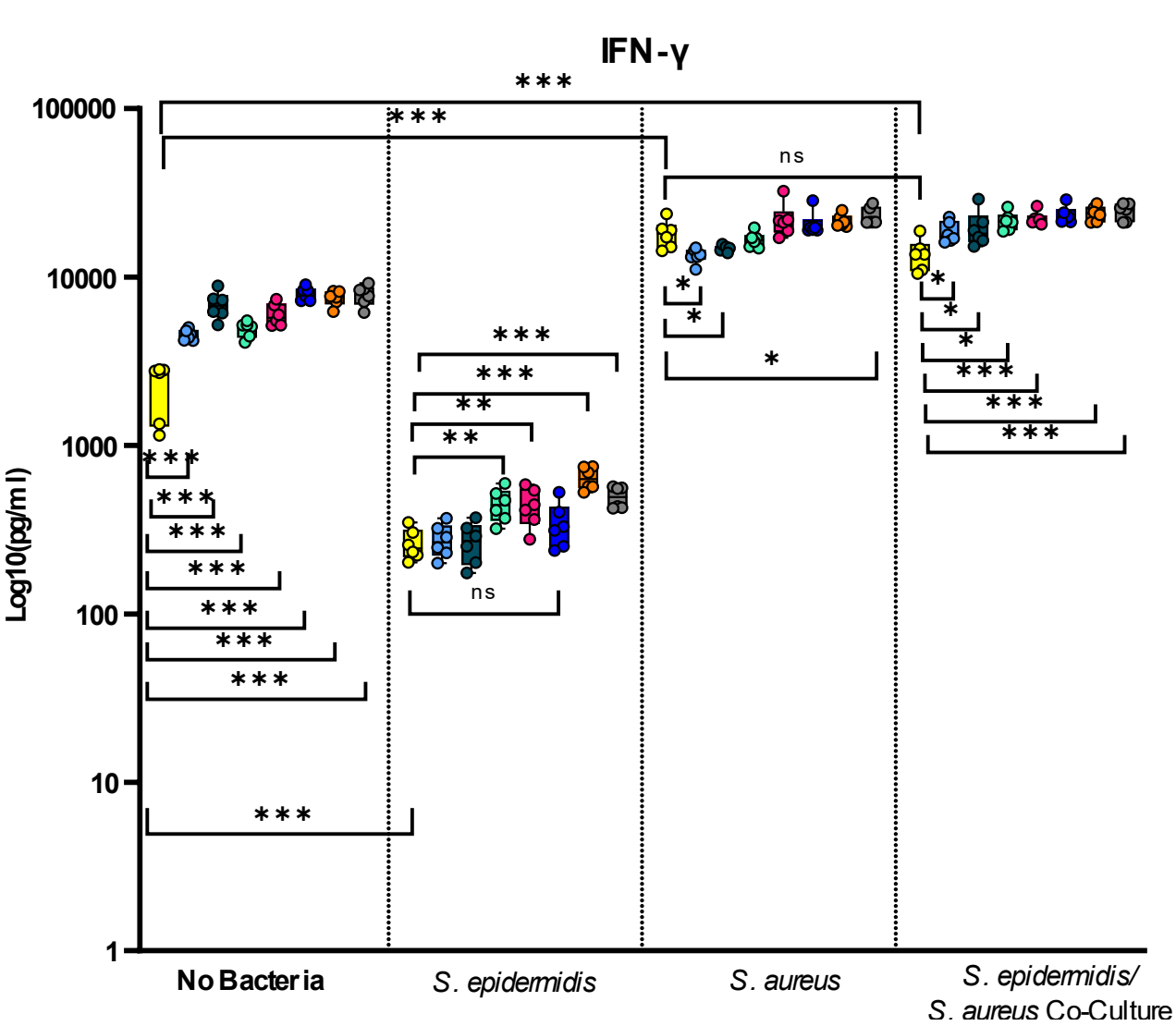


Figure 31. Concentration of IFN-ɣ from HaCaT cells With or Without Bacteria after Being Exposed to Various Active Levels of Hydrosolanum PE. Yellow bars represent HaCaT cells (with or without bacteria) in which they have not been exposed to any outside source, only the media was changed out. The blue bar represents the HaCaT cells being exposed to 0% of the cosmetic protein, otherwise known as sterile water. The other colours are exposure to active levels 0.125-2% of the cosmetic proteins. Non-significant values were only added if important and not obvious from the figure, nor values between species. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.20.5-5.20.8.

5.21.9 How did exposure to Hydrosolanum PE alone Affect the Concentration of TNF-α from HaCaT cells?

All results below are in reference to Figure 32. HaCaT cells alone expressed an average 10.67 pg/ml of TNF-α and when exposed to the active levels 0 and 0.125% this did not significantly change ($P>0.50$). Exposure to active levels of 0.25-1% Hydrosolanum PE significantly increased ($P<0.0002$) the concentration of TNF-α from 10.67 pg/ml to between 41-35 pg/ml but these were not significantly different to each other ($P=0.44$). Exposure to 2% Hydrosolanum PE significantly increased the concentration of TNF-α compared to the other active levels ($P<0.05$), up to 66.99 pg/ml.

5.21.10 How did exposure to Hydrosolanum PE Affect the Concentration of TNF-α from HaCaT cells incubated with *S. epidermidis*?

Incubation of HaCaT cells with *S. epidermidis* significantly reduced the concentration of TNF-α ($P<0.0001$) to a negligible amount. Exposure to 0-2% active levels of Hydrosolanum PE did not significantly change this as there was no TNF-α detected ($P>0.05$).

5.21.11 How did exposure to Hydrosolanum PE Affect the Concentration of TNF-α from HaCaT cells incubated with *S. aureus*?

Incubation with *S. aureus* significantly increased the concentration of TNF-α from HaCaT cells alone from 10.67 to 31,931 pg/ml ($P<0.0001$). Exposure to active levels 0, 0.125, 0.25 and 0.5% did not significantly change the concentration of TNF-α ($P<0.2$), although the exposure to 0.125% and 0.25% did reduce the concentration and was only just not significant ($P>0.05$). Exposure to 0.75 and 1% active levels of Hydrosolanum PE significantly increased ($P<0.03$) the concentration of TNF-α to 41,476 and 46,931 pg/ml respectively, but these Figures were not significant from each other ($P=0.4$). Exposure to 2% active level Hydrosolanum PE increased the concentration of TNF-α from the control ($P<0.0001$) and compared to the other active levels ($P<0.0005$).

5.21.12 How did exposure to Hydrosolanum PE Affect the Concentration of TNF-α from HaCaT cells incubated with *Staphylococcus* spp. Co-culture?

Incubation of HaCaT cells with the *Staphylococcus* spp. co-culture significantly increased the concentration of TNF-α to 23,351 pg/ml compared to HaCaT cells alone or with *S. epidermidis* ($P<0.0001$). However, the concentration of TNF-α was significantly less than when HaCaT cells were grown with *S. aureus* alone ($P=0.002$). Exposure to 0% control did not significantly alter ($P=0.25$) the concentration of TNF-α from the HaCaT cells, however exposure to 0.125-2% significantly increased the TNF-α concentration ($P<0.02$) to between 33,868-60,715 pg.ml. There was no significant difference in concentration of TNF-α when exposed to 0.125-0.75% active levels of Hydrosolanum PE ($P>0.05$). Exposure to 1 and 2% active levels significantly increased the

concentration compared to the lower active levels ($P<0.002$), but the concentration after incubation to 1 and 2% was not significantly different ($P=0.30$).

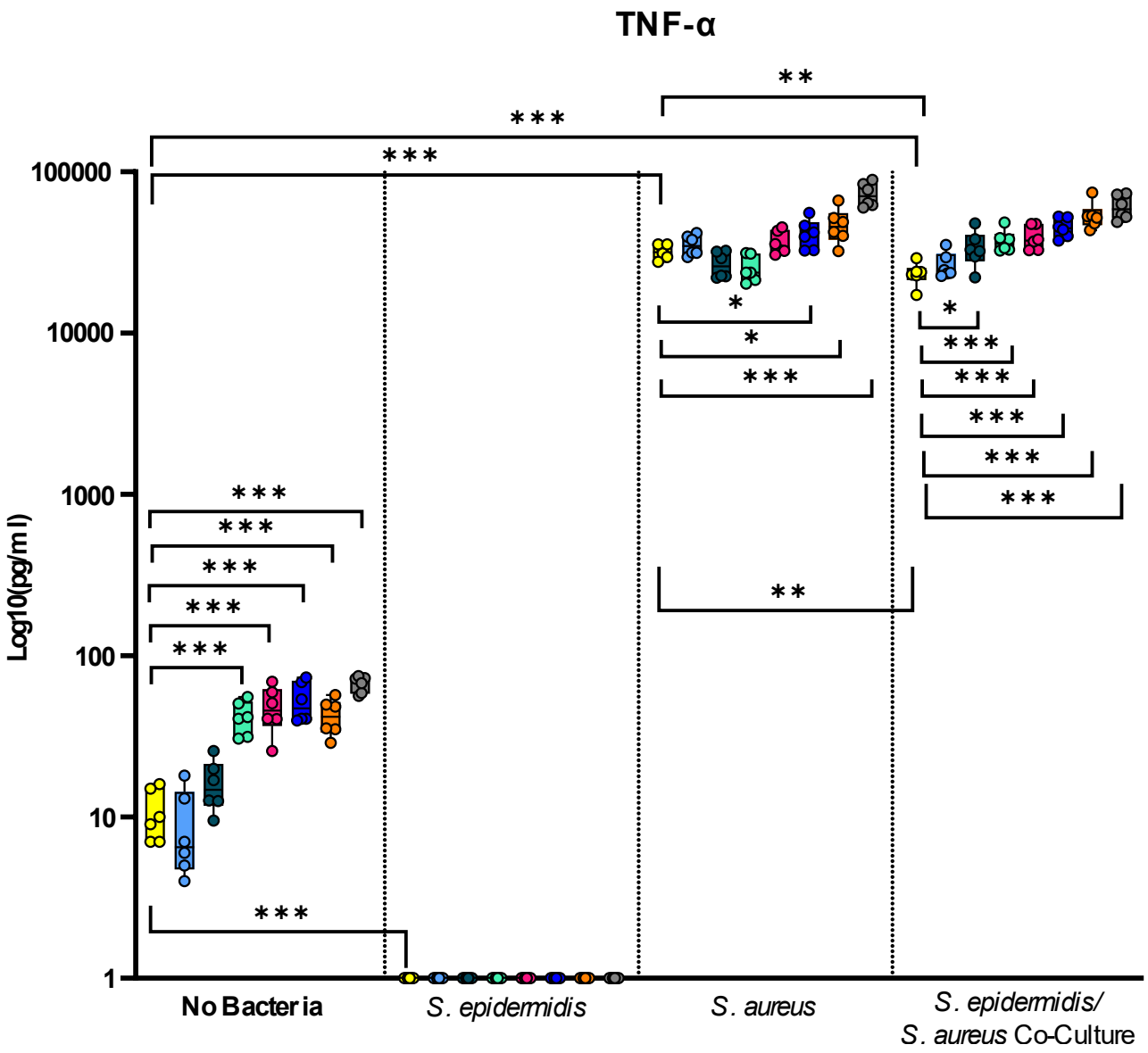


Figure 32. Concentration of TNF-α from HaCaT cells With or Without Bacteria after Being Exposed to Various Active Levels of Hydrosolanum PE. Yellow bars represent HaCaT cells (with or without bacteria) in which they have not been exposed to any outside source, only the media was changed out. The blue bar represents the HaCaT cells being exposed to 0% of the cosmetic protein, otherwise known as sterile water. The other colours are exposure to active levels 0.125-2% of the cosmetic proteins. Non-significant values were not added, nor values between species. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.20.9-5.20.12.

5.21.13 How did exposure to Hydrosolanum PE Affect the Final CFU/ml of *S. epidermidis* grown with HaCaT cells?

All results below are in reference to Figure 33. The final cfu/ml of adhered *S. epidermidis* cells when grown with HaCaT cells was 2.46 x 10^9, when exposed to 0% control or 0.125% Hydrosolanum PE, this did not significantly change (P>0.053). Exposure to 0.25-2% active levels of Hydrosolanum PE did however significantly reduce the final cfu/ml to between 6.9 x 10^8 to 1.19 x 10^8 (P<0.003). Exposure to increasing active levels significantly decreased the number of attached *S. epidermidis* cells (P<0.05), except for 0.75 and 1% active (P=0.45).

5.21.14 How did exposure to Hydrosolanum PE Affect the Final CFU/ml of *S. aureus* grown with HaCaT cells?

The final cfu/ml of adhered *S. aureus* cells when grown with HaCaT cells was 2.3 x 10^9 cfu/ml which was not significantly different to the adhered cells of *S. epidermidis* alone (P=0.74). Exposure to any active level of Hydrosolanum PE did not significantly alter the final cfu/ml of attached *S. aureus* cells (P=0.22).

5.21.15 How did exposure to Hydrosolanum PE Affect the Final CFU/ml of *Staphylococcus* spp. Co-culture grown with HaCaT cells?

The final cfu/ml of adhered *S. epidermidis* in the *Staphylococcus* spp. co-culture grown with HaCaT cells alone was 2.5 x 10^8 and the final cfu/ml of *S. aureus* was 2.8 x 10^8, which were not significantly different from each other (P=0.61). Exposure to 0-1% Hydrosolanum PE, showed no significant reduction in adhered *S. epidermidis* cells (P>0.13), however the general trend is a reduction in adhered cells, from 2.5 x 10^8 cfu/ml down to 1.9 x 10^8cfu/ml. Exposure to 2% active level Hydrosolanum PE significantly reduced the number of adhered *S. epidermidis* cells down to 1.36 x 10^8 cfu/ml, compared to the lower active levels (P<0.001) and the control (P=0.0008). Again, exposure to 0-1% active level of Hydrosolanum PE did not significantly alter (P>0.13) the final cfu/ml of attached *S. aureus* cells, however the trend again was a reduction down to 1.9 x 10^8 cfu/ml. Exposure to 2% active level of Hydrosolanum PE did significantly reduce the final cfu/ml of *S. aureus*, down to 1.36 x 10^8 cfu/ml (P<0.0008). Although both species final cfu/ml of adhered cells followed a similar pattern, *S. aureus* significantly dominated (P<0.04) the adhered cells when exposed to 0.25, 0.5 and 0.75% active levels of Hydrosolanum PE.

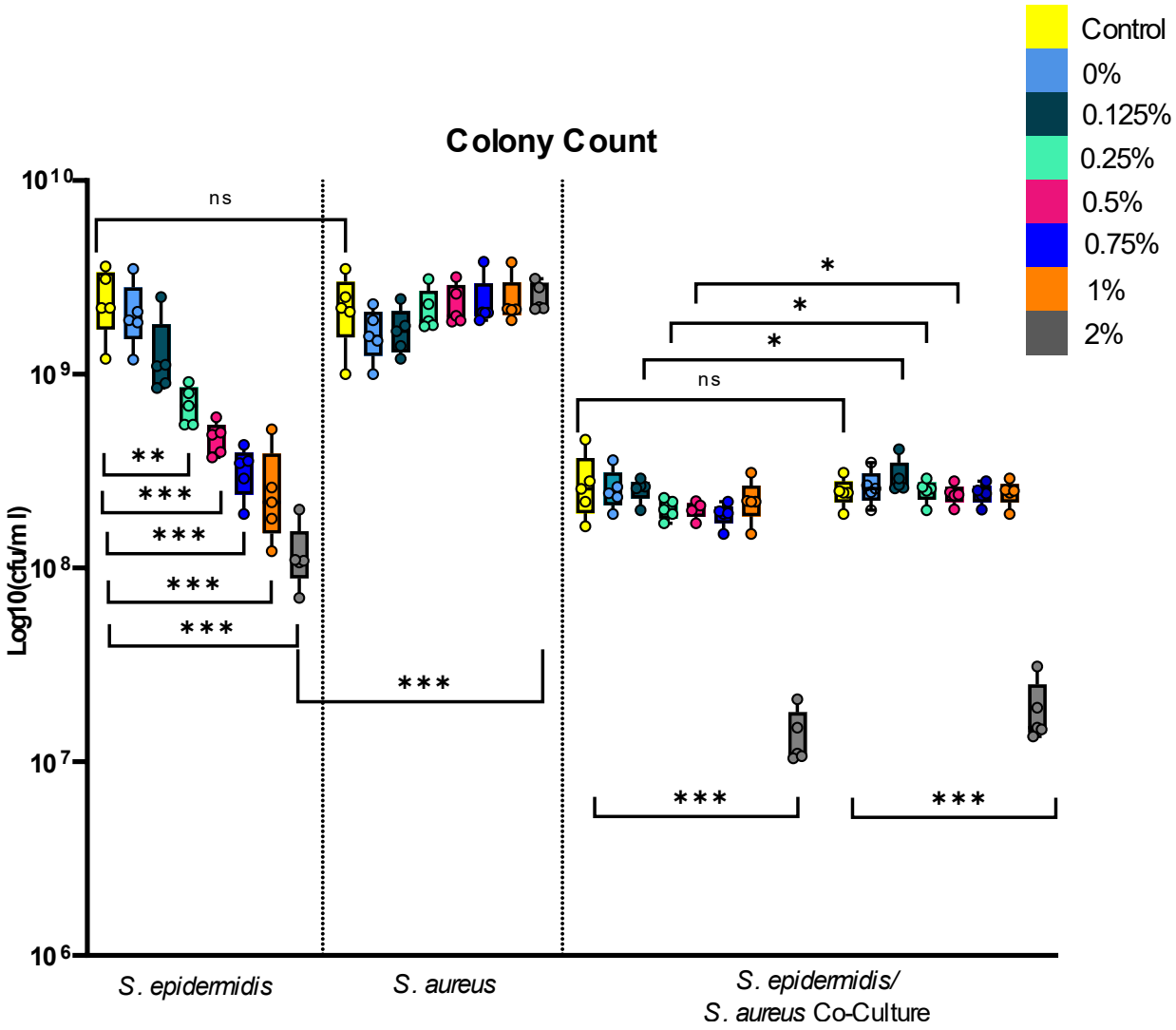


Figure 33. Final CFU/ml of *S. epidermidis* alone, *S. aureus* alone and the *Staphylococcus* spp. Co-culture after being exposed to Various Active levels of Hydrosolanum PE. Statistical values between individual species were not all added, when exposed to active levels 0.25-2%, individual *S. aureus* had a significantly higher cfu/ml than *S. epidermidis*. *<0.05, **<0.01, ***<0.0001. For all statistics, please see Section 5.20.13-5.20.15.

5.22 Discussion

5.23 Microscopic Observation of HaCaT cells shows addition of Bacteria Stresses the Cellular Monolayer

The images shown in Figure 25 were microscope images of the HaCaT cells after 48 hours of incubation with the bacteria. Without bacteria, there was clear adherence and continuous mosaic pattern around the periphery of the well, reaching in towards the centre of the well. Some of the cells had become condensed with uneven shrivelled edges which is a sign of normal developmental cell death (Ziegler and Groscurth 2004), the majority were forming a healthy merged structure. When grown with both *Staphylococcus* spp., separately and together, cell swelling was observed at varying degrees. Some swelling was noted when grown with *S. epidermidis,* but a larger proportion of swollen cells were found when incubated with *S. aureus* or the co-culture of both species.

Keratinocyte swelling is a morphological process which is attributed to several potential causes, commonly microbial infections which trigger oncosis (Rock and Kono 2008; Lauffer et al. 2018). Oncotic cell swelling is due to increased membrane permeability (Wei et al. 2020) causing an inflow of sodium and chloride ions and outflow of potassium ions. The resulting osmotic gradient swells the cell with water, eventually leading to death (Loh et al. 2019). The lack of membrane integrity during oncosis is linked to pore-forming toxins (Lin et al. 2010). *Staphylococcus aureus* releases a pore-forming cytotoxic agent called alpha toxin (α-toxin) which is known to lyse human epithelial cells (Essmann et al. 2003). The α-toxins are released and bind to monomers on the membrane of cells, converting them into a multicellular complex which forces a β-barrel through the lipid bilayer. This opens a new channel which leads to the cell swelling (Seilie and Bubeck Wardenburg 2017). In future, to explore whether the swelling was initiated through the release of Staphylococcal α-toxin, there are highly sensitive enzyme-linked immunosorbent assays available. These assays can detect α-toxins in highly diluted samples (Surujballi and Fackrell 2021). *Staphylococcus epidermidis* does not exhibit as many virulent factors as its more pathogenic cousin, however it does secrete δ-toxin (Otto 2012). In other species, δ-toxin shares similarities with α-toxin, in that it forms pores in the cell membrane with β-strands (Manich et al. 2008). Some swelling could be attributed to this toxin and ELISAs have been used to confirm the presence of δ-toxins (Scheifele et al. 1987). However, some of the bloated cells could be attributed by introduction of bacteria into the well, causing changes to the pH and utilisation of nutrients (Segeritz and Vallier 2017).

The HaCaT cells were also observed to have reduced adhesion when grown with all *Staphylococcus* spp. combinations, however the most pronounced was with *S. aureus* alone. HaCaT cells have been known to have reduced adherence when under different stress conditions

(Luangpraditkun et al. 2021), the presence of other bacteria species has also shown the ability to reduce the HaCaT adhesion (Siemens et al. 2011).

Due to the nature of this experiment, the focus was on the effect of cosmetic proteins on the final cfu/ml of the bacteria. Without performing an extra time-consuming control group, the exact viability of the HaCaT cells could not be quantified. This would be useful for future experiments to investigate the overall health of the keratinocytes.

5.24 Detection of Immune Biomarkers

5.24.1 Immune Markers are Expressed without Stimulation

Even before the incubation for several days or incubation with bacteria, HaCaT cells expressed low levels of bioactive markers Figure 23. This was normal, as previous studies on immune marker concentration reported low levels of these markers from the control results, the added stimulant usually increases the concentration compared to the control level (Colombo et al. 2017; El Darzi et al. 2017; Lee et al. 2019b). As keratinocytes are the first line of defence, having low levels of immune responses allows for quick response when invading pathogens are detected (Nestle et al. 2009; Piipponen et al. 2020).

5.24.2 Addition of either Cosmetic Protein did not Increase Concentration of IL-8 but did increase Concentration of other Immune Markers

The exposure of either cosmetic protein to HaCaT cells alone did not significantly increase the concentration of IL-8 compared to HaCaT cells alone and some active levels even reduced the concentration compared to HaCaT cells alone. Cosmetic proteins are key ingredients in moisturising products which can be used in conjunction to help treat inflammatory diseases (Lynde 2008). While there was no significant change in IL-8 concentration with the pea protein derivative, Hydrosativum P, the trend was a reduction in concentration. Pea proteins have been shown to reduce excessive concentration of pro-inflammatory interleukins in atopic dermatitis damaged skin cells (Campolo et al. 2020). The lower active levels of the potato protein derived cosmetic protein, Hydrosolanum PE, significantly reduced HaCaT cell concentration of IL-8. When exposed to the higher active levels of Hydrosolanum PE, the concentration started to increase but still not more than HaCaT cells alone. While there's little research into anti-inflammatory effects of potato proteins, proteins from similar species have been found to promote wound healing in damaged skin (Suzuki et al. 1996). Unlike IL-8 concentration, both IFN-ɣ and TNF-α were either significantly increased or the trend was increased concentration when exposed to both proteins. Both proteins were preserved with 1% phenoxyethanol (Section 3, Table 6), a substance which has been shown to able to cause slight erythema of skin, a rash which is associated with elevated levels of IFN-ɣ and TNF-α (Caproni et al. 2006; Miller et al. 2009; Ma et al. 2021). It should be noted that any increase in immune marker concentration from HaCaT cells exposed to cosmetic proteins, does not compare to the increased concentration of bioactive markers from HaCaT cells

incubated with *S. aureus*. When HaCaT cells were incubated with S. aureus, the concentration of IL-8 in the supernatant increased by at least 10-fold, the concentration of IFN- ɣ increased by at least 100-fold and the concentration of TNF- α increased by at least 1000-fold compared to HaCaT cells grown alone or with *S. epidermidis*. The extent of the immune marker increase by incubation of HaCaT cells with *S. aureus* was discussed in Section 5.25.

5.25 The Presence of *Staphylococcus epidermidis* Alone Reduces the Immune Response

When HaCaT cells were grown with *S. epidermidis*, concentration of immune markers compared to the control group was either significantly reduced, sometimes to negligible levels, or not significantly different (Figure 24ABC, 26-28, 30-32). However, as exposure to higher active levels of the cosmetic proteins decreased the final cfu/ml of *S. epidermidis*, the concentration levels of immune markers often increased, even when the bioactive marker concentration levels increased, they were often less compared to HaCaT cells grown alone (Figure 23, Figure 24). This resonates with previous literature in which *S. epidermidis* is recognised as a commensal by skin cells (Claudel et al. 2019) and has been shown to be able to reduce excessive immune responses (Skabytska and Biedermann 2016).

5.25.1 *S. epidermidis* Possess Many TNF-α Reducing Techniques

Reduction in TNF-α concentration by HaCaT cells incubated with *S. epidermidis* was seen after 48 hours (Figure 24C) and when grown with both proteins (Figure 28, Figure 32). *S. epidermidis* modulates immune responses from skin cells to limit exaggerated reactions and promote wound healing through inhibition of toll-like receptor 3 facilitated inflammatory pathways (Li et al. 2019a). These pathways include the activation of TNF-α (Covacu et al. 2009), which is involved in the early stages of wound healing (Ritsu et al. 2017; Frank et al. 2021) but is also linked to persistent wounds due to hyper-inflammation (Ashcroft et al. 2012). Concentration of TNF-α can trigger an extreme build-up of glycation products at tissue sites, which initiate apoptosis of fibroblasts and integral vascular cells, thus stopping successful wound healing (Tanaka et al. 2000). *S. epidermidis* prevents TNF-α hyper-inflammation by targeting upstream processes. The bacterium produces a Lipopeptide (LP78), which activates β-catenin which is known to supress toll-like receptor 3 (Li et al. 2019a). One way to establish if TNF-α levels were suppressed due to the presence of *S. epidermidis*, previous work has used horseradish peroxidase (hrp) immunohistochemistry to identify elevated concentration of β-catenin in tissue samples. A primary antibody was used to detect the presence of β-catenin, with diaminobenzidine as the chromogen (Bhattacharya et al. 2019). Concentration was scored from multiplying the percentage of positive β-catenin expressed cells by their staining intensity (Mauri et al. 1998). Other research has found that *S. epidermidis* can reduce proinflammatory TNF-α over-expression caused by pathogens. It was deduced that staphylococcal lipoteichoic acid (LTA), a key component in the cell

membrane of *S. epidermidis*, induced certain miRNAs which inhibited pathogen induced cytokines (Skabytska and Biedermann 2016). Abrogation of TNF-α by systems triggered by LTA could be confirmed by sampling the supernatant and confirmation by use of an LTA sandwich ELISA kit (Tadler et al. 1989; LSBio 2022)

5.25.2 Modulation of IL-8 Can be Direct and Indirect

Incubation with *S. epidermidis* either did not significantly change the concentration of IL-8 by HaCaT cells (Figure 24A) or significantly reduced the concentration of IL-8 compared to HaCaT cells alone (Figure 26, Figure 30). However, exposure to the 1% active level Hydrosativum P did increase the concentration of IL-8 from HaCaT cells with *S. epidermidis* compared to HaCaT cells alone. Many of the TNF-α reducing strategies of *S. epidermidis* are also effective against suppression of IL-8. Lipoteichoic acid, effective against reduction in TNF-α over-expression, also limits IL-8 concentration in various tissues by other species (Blease et al. 1999; Im et al. 2015). Keratinocytes treated with a product isolated from *S. epidermidis* culture medium have found it successful in reducing multiple bioactive markers, including IL-8 (Lai et al. 2009). *S. epidermidis* released extracellular vesicles can directly increase IL-8 production, however they can also indirectly reduce the chemokine through modulation of LL37 which induces IL-8 production in psoriasis inflamed skin (Gómez-Chávez et al. 2021).

5.25.3 IFN-ɣ Concentration May have Increased due to Irritation as well as Reduced *S. epidermidis*

IFN-ɣ concentration was reduced in HaCaT cells incubated with *S. epidermidis* (Figure 24B), however exposure to Hydrosativum P significantly increased concentration (Figure 27). Previous work has shown that *S. epidermidis*-produced biofilms interact with the immune system and can manipulate the immune response inhibiting inflammatory cytokines, including IFN-ɣ (Le et al. 2018). While the increased immune markers could be attributed to the decreased *S. epidermidis* levels, it is worth noting that the preservative phenoxyethanol is found in both of these cosmetic proteins (Section 3, Table 6), which has previously been found to cause slight erythema of the skin during patch tests (Ma et al. 2021). Higher levels of IFN-ɣ are associated with erythema and can exacerbate the symptoms (Miller et al. 2009). Therefore, phenoxyethanol could have reduced *S. epidermidis*, while potentially promoting skin irritation.

5.25.4 Adhered *S. epidermidis* Cells were Reduced as the Active Level of Proteins Increased

In Section 3.21.4, *S. epidermidis* was least sensitive to the preservative mix and concentrations found in Hydrosativum P (phenoxyethanol and potassium sorbate). However, in Figure 29, the final cfu/ml of *S. epidermidis* decreased as the active level concentration increased. While the pH of the proteins was pH 5 ± 0.2, the rest of the experiment could only be undertaken at pH 7. Unlike other preservative types, phenoxyethanol is the most efficient at inhibiting microbes when

used at pH 7, including against *Staphylococcus* spp. (Siegart 2013). Phenoxyethanol has also been shown to be able to increase the permeability and reduce integrity of bacterial cell membranes which can increase the efficacy of the inhibition effect (Walsh et al. 2019). A reduction in final adhered cfu/ml was also noted when *S. epidermidis* was exposed to Hydrosolanum PE, the preservative mixture has the heightened preserving measure of disodium EDTA (Section 3, Table 6). Disodium EDTA increases cell membrane permeability which allows entry into the cell more easily and enhances the inhibition of preservatives (Walsh et al. 2003). *In vitro*, EDTA has been shown to be bacteriostatic against planktonic *S. epidermidis*, and also affective at stopping adhesion of the species and subsequent formation of biofilms (Juda et al. 2008). As discussed in 3.25, due to the lack of protein analysis of the hydrolysed cosmetic proteins, there is always a chance that some of the peptides produced could have antimicrobial effects against *Staphylococcus epidermidis*. However, simple size exclusion data, from Croda Europe Ltd., of both Hydrosativum P and Hydrosolanum PE did show that the hydrolysis produced a range of peptides at varying lengths. The size exclusion data is commercially confidential and cannot be added or be discussed in depth in this thesis.

5.25.5 Future Investigation into Whether *S. epidermidis* being a Commensal or a Pathogen?

As *S. epidermidis* can be both a useful commensal and an opportunistic pathogen, the bacteria's immune modulation could also be part of its immune evasion tactics (Otto 2009; Le et al. 2018). Pathogenic *S. epidermidis* has been shown to activate IL-8 (Sachse et al. 2008), while in this study the IL-8 levels were reduced and therefore unlikely to be acting as a pathogen. However, to investigate whether the reduced immune marker concentration across the board was caused by *S. epidermidis* acting as an immune calming commensal or an evasive pathogenic threat, previous research has identified several markers (Ortega-Peña et al. 2019). SesC is a surface protein in which elevated levels are associated with the latter stages of biofilm forming cells compared to their planktonic counterparts (Shahrooei et al. 2009). The SesC producing gene has been shown to be present in all *S. epidermidis* clinical isolates in multiple studies (Bowden et al. 2005; Khodaparast et al. 2016b), including the strain used in the above work (Bahonar et al. 2019). However, the protein has been absent in isolates from healthy skin (Shahrooei et al. 2009). Identification of this protein would involve separation of the adhered and planktonic cells of *S. epidermidis* from the wells. The next step would then be isolation of the proteins through electrophoresis and SesC antibody added before visualizing on a Western blot (Khodaparast et al. 2016a). Comparison of planktonic and adhered cells would show whether SesC levels were elevated and therefore the presence of a later-stage biofilm.

5.26 *Staphylococcus aureus* Increases Concentration of Immune Markers by HaCaT Cells

Incubation of the HaCaT cells with *S. aureus* saw the concentration of the three markers increase compared to the concentration for HaCaT cells alone or grown with *S. epidermidis*. *Staphylococcus aureus* is a pathogen which is targeted by the innate and acquired immune system (Karauzum and Datta 2017). The pH of this experiment would have suited *S. aureus* better than if the bacteria had found itself on the skin, *S. aureus* proliferates best at neutral pHs while growth is slowed in more acidic conditions (Mirza et al. 1985; Stewart et al. 2017). Multiple immune biomarkers are activated when keratinocytes have been incubated with the species *in vitro* (Hong et al. 2014). While there were some differing concentration levels after exposure to Hydrosativum P, the overall trend was that the cosmetic protein was unlikely to influence the concentration, when HaCaT cells were grown with *S. aureus*. When exposed to Hydrosolanum PE, the overall trend was, when exposed to the higher active levels of the protein the concentration of IFN-ɣ and TNF-α increased, while IL-8 was slightly reduced.

5.26.1 *S. aureus* increased IL-8 Concentration at least 10-Fold More Than HaCaT cells Alone

When HaCaT cells were grown with *S. aureus*, the concentration of IL-8 increased significantly compared to HaCaT cells grown alone or with *S. epidermidis*, whether exposed to cosmetic proteins or not (Figure 24A, Figure 26, Figure 30). Increased levels of IL-8 are an indication of a pathogenic infection (Arican et al. 2005), as a pro-inflammatory chemokine they can activate pathways which increase lesions and exacerbate skin disorders (Kemény et al. 1994; Oka et al. 2000; Amarbayasgalan et al. 2013), which is advantageous to bacteria (Bowler et al. 2001; Arican et al. 2005). *S. aureus* is one such species involved in skin diseases that is known for multiple virulence factors which trigger both direct and indirect IL-8 concentration (Sasaki et al. 2003; Bien et al. 2011). These virulence factors known for inducing IL-8 around all different parts of the body include protein A, enterotoxin B and haemolysin (Thakur et al. 1997; Räth et al. 2013; Ledo et al. 2021). While there was a slight increase in IL-8 when grown with Hydrosativum P, the trend was no change in concentration when exposed to the cosmetic protein. When HaCaT grown with *S. aureus* were exposed to Hydrosolanum P, the IL-8 concentration was reduced. Plant based peptides have been shown to be able to reduce inflammation markers, and potato hydrolysates can activate mechanisms in skin cells (Apone et al. 2019). Potato peptides have been found to have anti-inflammatory properties elsewhere in the body, including the gut (Basilicata et al. 2019).

5.26.2 Could EDTA Induce a Stress Response in *S. aureus*?

Again, concentration of both IFN-ɣ and TNF-α were significantly increased with HaCaT cells grown with *S. aureus* than compared to HaCaT cells grown alone or with *S. epidermidis* (Figure 24B&C, Figure 27, Figure 28, Figure 31, Figure 32). Many of the *S. aureus* virulence factors that modulate IL-8 concentration also promote concentration of both IFN-ɣ and TNF-α. Protein A had been shown to stimulate and cause over concentration of both TNF-α, IFN-ɣ and an array of interleukins in soft nasal tissue (Patou et al. 2008) and keratinocytes (de Souza Feitosa Lima et al. 2019; Ledo et al. 2021). Skin cells recognise lipoproteins released from *S. aureus* as antigens and trigger inflammatory responses, including accumulation of CD4+ and CD8+ T-cells, which produce IFN-ɣ (Saito and Quadery 2018). While the concentration of both IFN-ɣ and TNF-α were, as a trend, not affected by being exposed to Hydrosativum P, both immune markers are increased when exposed to Hydrosolanum PE. One of the main differences between the two proteins is the addition of EDTA to the preservative mixture of Hydrosolanum PE. EDTA has been shown to spark stress responses in Gram-negative species, including release of extracellular vesicles (Steenhuis et al. 2020). It is thought that these stress responses might also be found in gram-positive species (Mozaheb and Mingeot-Leclercq 2020). It is already known that *S. aureus* can release extracellular vesicles which can release pore forming toxins (Wang et al. 2021). These toxins, as discussed above, can lead to cell swelling as seen in Figure 25 (Surewaard et al. 2018). So, the increase in some of the inflammatory bioactive markers could be due to exposure of EDTA, even if it did not affect the final cfu/ml. To investigate whether the addition of EDTA can cause these stress responses in *S. aureus*, techniques analysing supernatants could include RNA extraction and confirmatory western blots, size exclusion chromatography or microfluidics (Kang et al. 2017).

Adhered *S. aureus* Cells were not Reduced by Preservatives

Previous work in Section 3.21.5, found that the preservatives found in Hydrosolanum PE and even more so Hydrosativum P, were the least effective at inhibiting *S. aureus* (Figure 10). The minimal inhibitory concentration of phenoxyethanol against *S. aureus*, is higher than other preservatives at around 1%, (Lundov et al. 2011) more than both cosmetic proteins would be found in formulations. Exposure to EDTA can reduce biofilm formation in *S. aureus*, but over 24 hours and at higher concentrations than found in the cosmetic proteins (Liu et al. 2018). These reasons would explain why the increased active levels of each cosmetic protein did not significantly alter the final cfu/ml of adhered cells (Figure 29, Figure 33). Also, one of *S. aureus*' stress responses, sigma B has been shown to aid in overcoming antimicrobial stresses (Ranganathan et al. 2020).

5.27 The Co-culture of *Staphylococcus* spp. Increased the Immune Marker Concentration

The original inoculation amount of the co-culture was twice as much as HaCaT cells with individual species. However, the final cfu/ml of both species was significantly less than individual species, as competition reduced total population due to limited resources (Celiker and Gore 2012; Osmond and de Mazancourt 2013), but concentration levels were not too dissimilar to *S. aureus* alone (Figure 24).

5.27.1 *Staphylococcus aureus* Was More Likely to Dominate a Sample

Although there was not always a dominant species in the co-culture, when there was, the one species which was the most prevalent was *S. aureus*. The neutral pH would have favoured *S. aureus* as it has the faster growth rate at pHs close to neutral (Stewart et al. 2017). The increased levels of IFN-ɣ has also been shown to promote growth of *S. aureus*, but not commensal *Staphylococcus* spp. (Di Domenico et al. 2018). As both species had a reduction in the adhered population when exposed to the active level 2% of both cosmetic proteins, internalisation of *S. aureus* into the keratinocytes was unlikely to have occurred, as this would protect the species from high concentrations of preservatives (Al Kindi et al. 2019).

5.27.2 *Staphylococcus epidermidis* alerts the Immune System to *Staphylococcus aureus*

As IL-8, IFN-ɣ and TNF-α levels were akin to the levels expressed by HaCaT cells with *S. aureus* alone, it could be hypothesized that *S. epidermidis* is now promoting the immune response. Instead of downregulating a hyper immune reaction, as *S. epidermidis* did when grown alone, the commensal may have aided in promoting the bioactives due to the presence of its pathogenic relative.

Previous work has found that *S. epidermidis* can stimulate the innate immune system into targeting invasive *S. aureus* (Laborel-Préneron et al. 2015; Pastar et al. 2020). *S. epidermidis* can activate a response to limit *S. aureus* virulence against the skin. This includes activating keratinocytes to produce the antimicrobial peptide LL-37 (Elias et al. 2021). As well as antimicrobial properties, the peptide can modulate the pro-inflammatory responses including early upregulation of IL-8 and suppression of TNF-α or IFN-ɣ promoted interleukins (Chen et al. 2013b). The high concentration of IFN-ɣ and TNF-α when HaCaT cells were incubated with *S. aureus* and the *Staphylococcus* spp. coculture could be why the HaCaT cells did not form a continuous mosaic pattern, and there were large gaps between adhered cells and less attachment. Previous work has shown that concentration of these bioactive markers reduces HaCaT cell proliferation (El Darzi et al. 2017). Over expression of IL-8, along with TNF-α, IL-6 and IL-1B, has been postulated as a biomarker for staphylococcal sepsis (Sachse et al. 2008).

Other markers could be examined to further investigate interactions between the two *Staphylococcus* spp. The anti-inflammatory IL-10 is often induced by *S. epidermidis*, which can reduce tissue damage and help inhibit bacterial pathogenesis (Laborel-Préneron et al. 2015). However, *S. aureus* has been shown to be able to manipulate IL-10 into hyperexpression, thus supressing T-cell induction of the pathogen targeting proinflammatory bioactives and aiding *S. aureus* into evading the immune response (Leech et al. 2017; Islam et al. 2021). Using ELISAs to investigate IL-10 hyperexpression could explain why *S. aureus* could continue to successfully adhere even when grown with *S. epidermidis*. However, even with the pH advantage, the adhered cells of *S. aureus* did not excessively proliferate compared to *S. epidermidis* (Figure 29, Figure 33). Extracellular proteases released from *S. epidermidis*, discussed in 3.26, also aid in reduction of adherence and biofilm formation of *S. aureus*. The Esp protease targets the cell wall fractions and hydrolyses more than 50 proteins and nearly a dozen involved in biofilm forming, adherence and fibronectin binding proteins (Iwase et al. 2010; Chen et al. 2013a; Sugimoto et al. 2013).

5.27.3 Conclusion

Exposure of Hydrosolanum PE to HaCaT cells alone only increased the concentration of the three immune markers in the supernatant at the higher active levels. The exposure of Hydrosolanum PE increased the concentration of IL-8 at the higher active levels, but the exposure of Hydrosolanum PE increased both IFN-Y and TNF-a at most active levels. However, to highlight that while mostly the addition of the 0% control did not affect the concentration of the immune markers, sometimes the levels could be both increased and decreased. The uncertainty of the expression levels could be down to the inconsistent growth and stress responses of the HaCaT cells, rather than the introduction of the cosmetic proteins. However, the addition of *S. epidermidis* to HaCaT cells decreased the concentration of immune markers found in the supernatant. The higher active levels of both cosmetic proteins could reduce the final cfu/ml of *S. epidermidis* and therefore the concentration of immune markers in the supernatant. Even if the cosmetic proteins were the cause for the increased concentration of immune markers, it was minimal compared to the 10-1000-fold increase of immune marker concentration when HaCaT cells were incubated with *S. aureus*, whether alone or in co-culture. When in co-culture, both *Staphylococcus* spp. can ‘keep check’ of each other and stop wild proliferation of either species.

5.27.4 Future Work to Enhance the Experiment to be More Akin to the Habitat of Skin

As previously mentioned, this experiment was carried out at pH 7 which is not like the average acidic conditions of skin (Ali and Yosipov 2013). Keratinocytes grown in an acidic pH have shown to have reduced migration and viability (Lönnqvis et al. 2015), so conditioning HaCaT cells to grow in a lower pH may take extra time. Lowering the pH would reduce the advantages a neutral pH awards *S. aureus,* and *S. epidermidis* would likely dominate (Stewart and William Costerton 2001). To make the experiment more comprehensive, the total viability of the HaCaT cells would more

quantitively portray the overall keratinocyte health, rather than simply qualitatively as seen in Figure 25 and discussed in 5.18. An extra control experiment focussing on the viability of HaCaT cells rather than the final cfu/ml of the bacterial species would achieve this. Limiting access to highly nutritious media, as discussed in 3.26, would also allow growth of slower growing organisms if other species were to be added to the mixed culture (Davis et al. 2005). This could be achieved using Transwell inserts which can separate media, for instance DMEM in the lower compartment and minimal medium in the upper compartment (Sip et al. 2014). Transwells have also been successful in producing air-liquid interface, which would be more analogous to the skins ecosystem (Li et al. 2009) (Figure 34). The use of Transwells were investigated but was unsuccessful and abandoned due to time constraints.

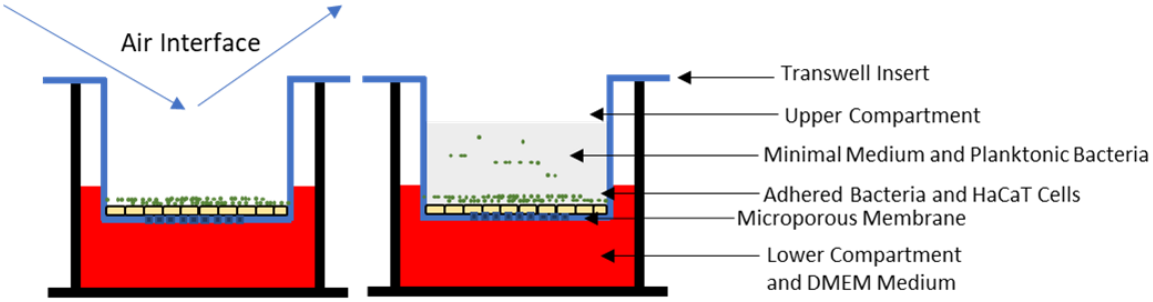


Figure 34. **Using Transwells to Assimilate Conditions More Akin to Skin.** Using a liquid-air interface (left) and using minimal medium (right).

6 Investigating the Effect of Swab Head Type and Buffer Composition on Bacterial Collection using a Porcine Skin Model

6.1 Introduction

6.2 Skin Microbe Research is Lagging

One of the largest organs of the human body and the first line of immunological defence is the skin (Byrd et al. 2018). However, the residing microbial community is often overlooked for areas of the body with greater microbial mass – mainly the gut microbiota (Proctor et al. 2019). The understanding of how the microbes within the gut are connected to nutrition, digestion and immunology is far from complete (Chang and Kao 2019) but the overall research topic is leaps and bounds ahead (Dréno et al. 2016). However with the emergence of modern skin diseases (Cordain et al. 2020) and the increased cases of skin cancers (Ishihara 2006), only recently has the microbial population of the skin been recognised as an equally important area of the human microbiota that requires investigation (Sohn 2018). To find a gold standard of collection of skin microbes could help ensure conformity across the research, bad collection technique could skew and cause inaccurate results (Ogai et al. 2018; Allaband et al. 2019).

6.3 Collecting Skin Microbiota

Understanding how these microbial skin communities could defend against or even influence these diseases and cancers are vital for cure and prevention (Grice and Segre 2011). This requires a robust and consistent method for collecting an accurate as possible representation of these skin communities. For example, a common method to produce a fingerprint of the microbial gut community is the analysis of faecal sample (Wang et al. 2018b).

There are numerous techniques used to collect skin microbiota including tape stripping, skin scraping, biopsies and swabbing (Ogai et al. 2018). Tape stripping is inexpensive but requires the area to be flat and without scarring (Kong et al. 2017) . Skin scraping and biopsies are useful for collecting low microbial mass but are invasive and not suitable for exploring multiple sites (Ogai et al. 2018). Swabbing is by far the easiest, least invasive and most versatile at collecting from all crevices of the skin (Grice et al. 2008). Nevertheless, previous papers have used varied methods in skin microbe collection using swabs, including numerous swab head types, and differing buffer compositions (Keyworth et al. 1990; Huttenhower et al. 2012; Ogai et al. 2018; Prast-Nielsen et al. 2019). Any previous research in to swab head types have either collected samples from surfaces dissimilar to the skin (Park et al. 2015) or have evaluated the release of microbial matter from swabs which have been dipped straight into a cell suspension (Van Horn et al. 2008; Mokomane et al. 2016).

6.4 Skin Models

Animal skin models are commonly used as replacements for human skin in research (Abd et al. 2016). This includes cancer models (van der Weyden et al. 2016), permeation drug studies (Neupane et al. 2020), drug discovery (Avci et al. 2013), and as a practice for surgical techniques (Denadai et al. 2013). Animal skins are readily available in the large quantities needed to

undertake experiments (Schmook et al. 2001) and using them bypasses a lot of ethical and legal issues that would arise by using human skin (Petrini 2012). While a range of species are used, some animal skins are more akin to human skin than others. Murine (mouse and rat) skin have differing dermal thickness (Zomer and Trentin 2018) and higher permeability (van Ravenzwaay and Leibold 2004) than human skin. While porcine skin for example, is structurally similar to human skin, pigs have a similar number of dermal layers as well as comparable skin thickness and elasticity (Keiko et al. 2018). The number of follicles on the pigskin is also akin to our own and while there is often more hair on adult pig skin, this can be rectified by removal (Yu et al. 2015) or using neonatal subjects (Keiko et al. 2018).

6.5 Aims

- Produce an ethically sound experiment in which human skin can be reproduced
- Compare different swab head types and buffer combinations at collecting bacteria from a skin model

6.6 Null Hypothesis

Swab head type or buffer composition have no significance on the quantity of bacteria collected from skin.

6.7 Materials

6.8 Bacteria

Staphylococcus aureus and *Staphylococcus epidermidis* used were the same isolates as previously described in Section 2.6.

6.9 Media

TSB and TSA were used to grow both species and quantify them for the experiment, preparation as seen in Section 2.10. Mannitol salt agar (MSA) from Thermo Scientific was prepared as to specifications on the packaging. MSA was used to quantify the species removed from the swab.

6.10 Porcine Skin

Porcine skin was purchased from a local supermarket butcher. Hair and subcutaneous fat were already removed, and this meant that no animal was killed specifically for the purpose of this investigation.

6.11 Swab and Buffer Types

The swab head types, and buffer compositions were all used in previous skin swabbing papers. For this study, the different buffer solutions were renamed buffer 1, buffer 2 and buffer 3. For swab types and buffer compositions please see

Table ***9***.

Table 9. Swab head type and Buffer combinations used in Previous Swabbing Papers

Paper	Swab Head Type	Buffer Composition	Buffer Name
(Keyworth et al. 1990)	Dacron™ (Deltalab, Barcelona)	0.075 mol litre-1 of phosphate buffer [pH 7.9] containing 0.1% v/v Triton X-100	Buffer 1
(Ogai et al. 2018)	Cotton (Technical Service Consultants, Heywood)	0.9% v/v Saline + 0.1% v/v Tween 20	Buffer 2
(Huttenhower et al. 2012)	Flocked (4N6FLOQSwabs™, Life Technologies, Carlsbad)	50 mM Tris buffer [pH 7.6], 1 mM EDTA [pH 8.0] + 0.5% v/v Tween-20	Buffer 3[9]

6.12 Methods

6.13 Bacterial Suspension Preparation

Both species were cultured in TSB at 37 °C for 18 hours and then diluted to OD 1 as previously described in 2.9. These were then further diluted in 1xPBS to approximately 1 x 10^5 cfu/ml. For mixed cultures, the species were diluted individually to approximately 2 x 10^5 cfu/ml and added together at a 1:1 ratio to produce approximately 1 x 10^5 cfu/ml of each. To verify the quantity of bacteria inoculated, the colonies were plated onto TSA and quantified using the Miles and Misra method (Miles et al. 1938).

6.14 Porcine Skin Preparation

The porcine skin was prepared by washing with 70% ethanol three times, to remove any contaminating microbes residing on the skin. The inoculation areas of 3 x 3 cm^2 were then drawn on using 70% ethanol washed templates and permanent marker. A control area was swabbed and plated onto a mannitol salt agar plate to confirm no residual *Staphylococcus* spp. on the porcine skin.

[9] For this study the buffers were renamed Buffer 1, Buffer 2, and Buffer 3

6.15 Inoculation of Porcine Skin

The porcine skin was inoculated with 50 µl of either individual or mixed, bacterial suspension and, using an L-shaped cell spreader, spread evenly across the designated 3 x 3 cm^2 area. The bacterial suspension was allowed to dry into the skin for one hour. The segments were then swabbed similarly to the report by Ogai and co-workers (Ogai et al. 2018). The swabs were immersed into the buffer for 5 seconds to saturate the tip and swabbed the skin with 10 vertical and 10 horizontal rotational strokes. The swab tips were removed and immersed into sterile phosphate buffer saline (PBS). To shake out the collected biota, the swabs were vortexed for 10 minutes, followed by centrifugation at 6000 x g for 10 minutes and the swab removed. The pellet was resuspended and serially diluted in 1 ml of 1 x PBS, then 20 µl plated onto mannitol salt agar plates. After overnight incubation at 37 °C, the colonies were quantified using the Miles and Misra method (Miles et al. 1938). Figure 35 illustrates the process.

6.16 Statistics

Graphs were made and data analysed using GraphPad Prism (Version 9.1.2). T-tests were used to compare the quantities of bacteria removed from the swabs.

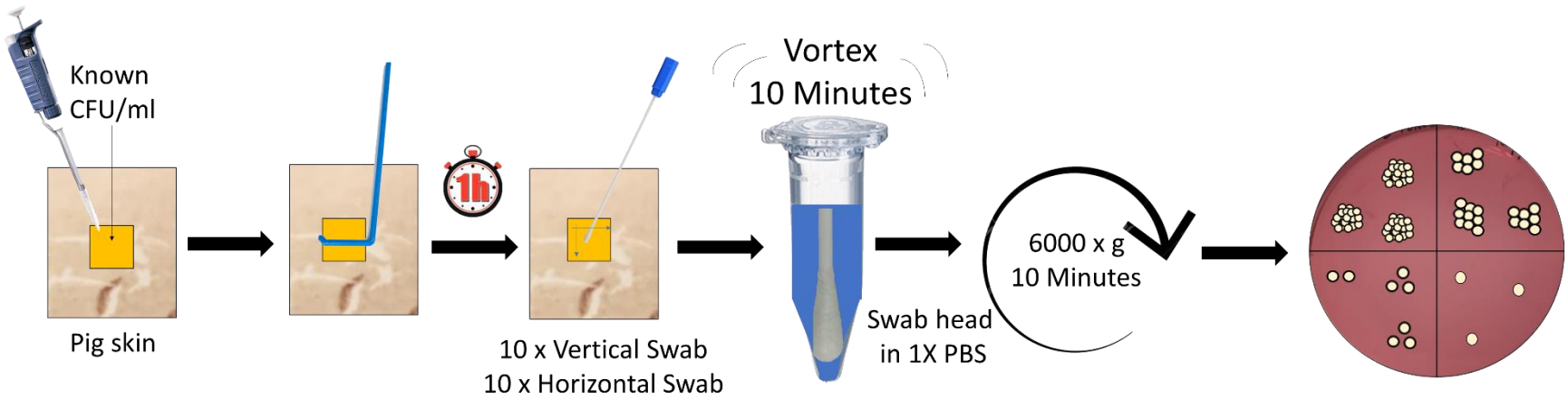


Figure 35. Inoculation, Swabbing and Quantification of *Staphylococcus* spp.. 50 µl of cell suspension was inoculated onto porcine skin and spread around the 3x3 cm^2 area. The bacteria were left to soak into the skin for 1 hour before being swabbed.

6.17 Results

6.18 Collecting Individual Species from Porcine Skin

The results below are in reference to Figure 36 and Figure 37. Both buffer composition and swab head type made a significant impact on the quantity of individual *Staphylococcus* spp. collected from the pig skin. Dry cotton swabs collected and released the lowest percentage of either species, consistently less than 1% of the inoculated amount. Cotton swabs used in conjunction with any buffer type collected and released between 7-12% of *S. epidermidis* and 10-29% of *S. aureus*. There was no significance between using buffer 1 or 2 to collect.

For *S. epidermidis* there was no difference between the buffers (P=0.339), however, buffer 2 was significantly better than buffer 1 when *S. aureus* was collected (P=0.001). Both buffer 1 and 2 were significantly more efficient at collecting and releasing both species than using buffer 3 (P<0.05). Again, dry Dacron swabs collected less than 1% of either species, which is significantly less than using any buffer type (P<0.001). While there was no significant difference between buffer 1 and 2 with Dacron swabs at collecting *S. epidermidis*, buffer 1 is slightly better than buffer 2 with a significance of P=0.022 when *S. aureus* was collected. Again, both buffer 1 and 2 still collected significantly more bacterial cells than buffer 3 when both species were collected (P<0.05). While there is inconsistency with which buffer is better when using cotton or Dacron swabs, the best buffer type is clear when using flocked swabs. Using a dry swab once again, resulted in the least number of bacterial cells being collected, less than 1%. Flocked swabs with any buffer collected and released between 12-50% of the inoculated *S. epidermidis* and 11-32% of *S. aureus*. When both species were collected, buffer 1 collected significantly more than both buffer 2 (P<0.001) and buffer 3 (P<0.001). Buffer 2 still collected significantly more of both species than buffer 3 (P<0.001).

When collecting both *S. epidermidis* and *S. aureus*, there was no clear dry swab which was better than any other. While flocked swabs were significantly better at collecting *S. epidermidis* (P<0.001), Dacron swabs were significantly the best at collecting *S. aureus* (P<0.05). No more than 0.6% of the original inoculum was collected by any swab. When utilising buffer 1, the results were very similar when collecting both species. There was no statistical difference between both cotton and Dacron swabs. When used with buffer 1, cotton swabs collected between 5-16%, Dacron collected between 6-16% and flocked between 41-69% of the inoculated *S. epidermidis*. There was no statistical difference between cotton or Dacron swabs, but flocked swabs collected significantly more (P<0.001). Similar results were seen with *S. aureus*, with buffer 1 cotton swabs collected between 14-25%, Dacron between 16-29% and flocked between 25-32%. Again, flocked swabs were significantly better than the other types, while there was no statistical difference between cotton and Dacron. When using buffer 2, less consistent results were observed. Cotton swabs collected between 6-15%, Dacron between 5-16% while flocked collected upwards of 41-

69% of *S. epidermidis*. Again, there was no significance between both cotton and Dacron swabs ($P>0.05$), but flocked swabs collect significantly more ($P<0.001$). There were much different results when *S. aureus* was collected, Cotton and flocked swabs collected percentages which were not significantly different, between 16-29% for cotton and 21-29% for flocked ($P=0.44$). However, Dacron collected 14-25% of the bacteria, which was significantly less than both cotton and flocked ($P<0.002$). When used with buffer 3, there was not a swab which consistently collected more than any other type. Flocked swabs collected between 9-13% of *S. epidermidis*, which was significantly more than the 4-11% collected by Dacron ($P<0.001$) and 5-15% collected by cotton ($P=0.001$). However, when *S. aureus* was collected there was no overall significant difference between the percentage of bacterial cells collected and released ($P>0.05$). Cotton collected between 10-17%, Dacron 11-21% and flocked between 11-16%.

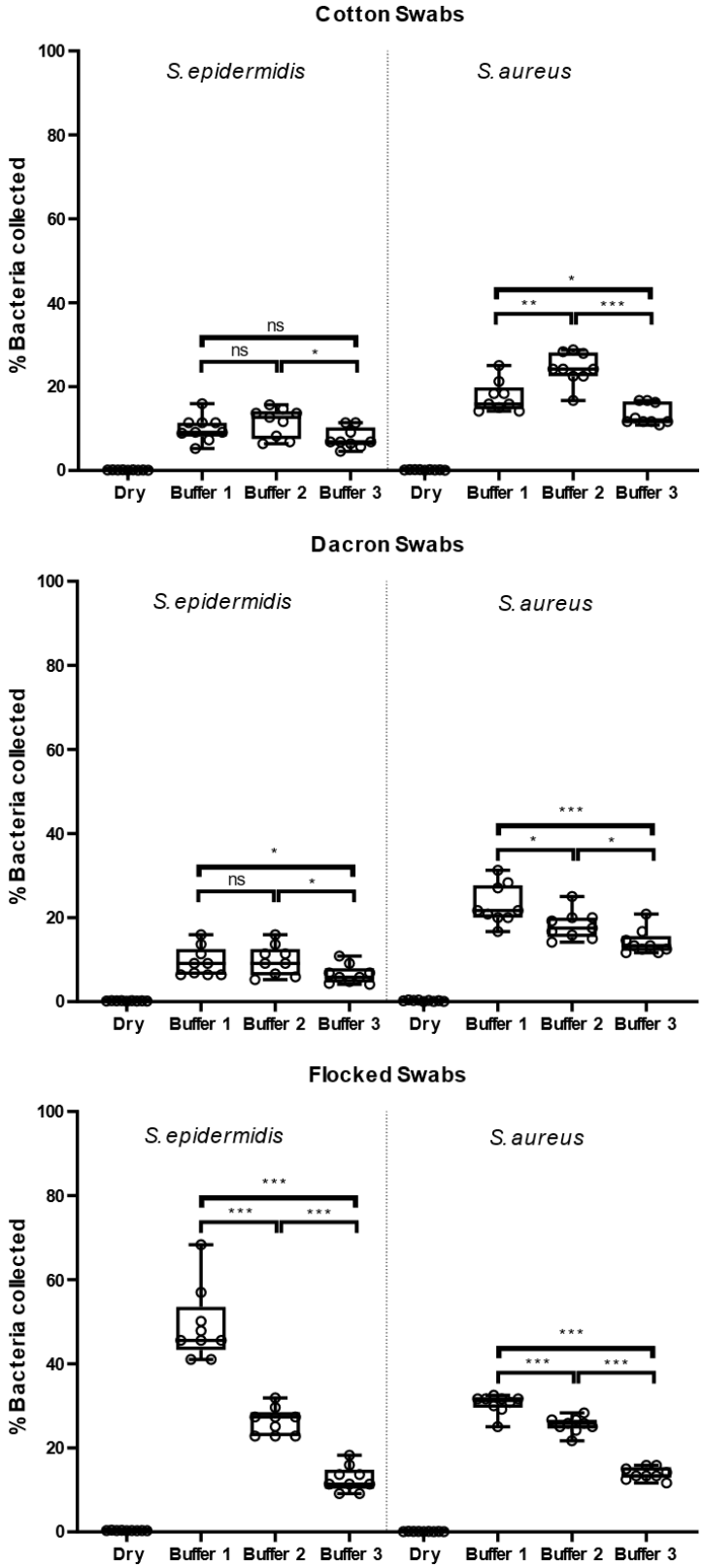


Figure 36. Percentage of Bacteria Collected from Each Swab Type. Using a buffer was always significantly better than using a dry swab. Buffer 2 was either trending or significantly better than buffer 1 and 3 when used with cotton, or Dacron swabs. Buffer 3 consistently collected the least percentage of bacteria, most often significantly worse than the other buffers with any swab head type used. Buffer 1 was significantly more effective when used with flocked swabs. When used with buffer 1, flocked swabs collected mostly between 30-70% of the inoculated bacteria. While the range of collected is large, it is still significantly more than any other swab/buffer combination.

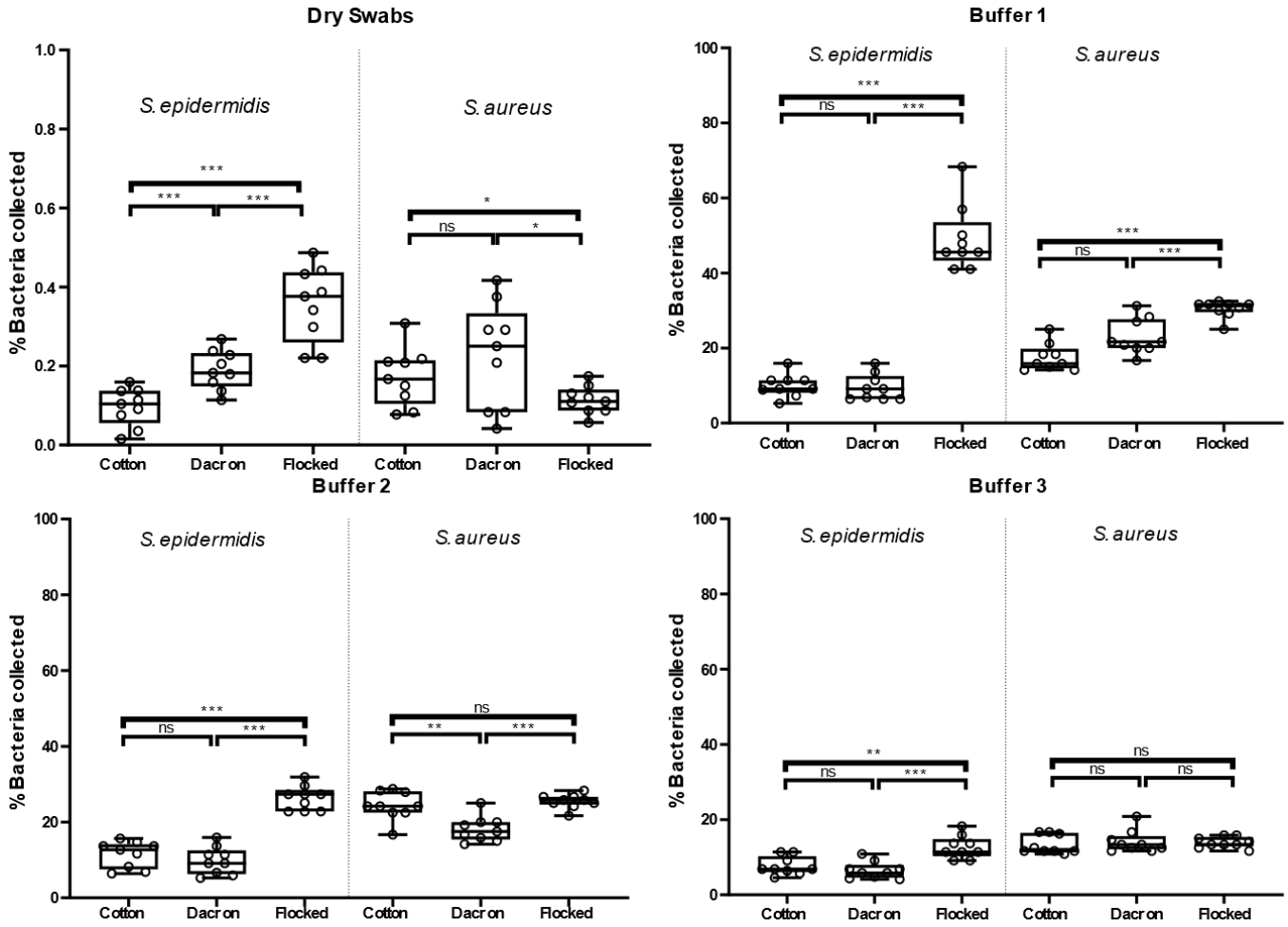


Figure 37. Comparison of Buffer Composition and Swab head Type on Bacterial Collection. Notice how with dry swabs the y-axis had to be changed from 0-100% to 0-1% as the collection percentage was so low. While there are statistical differences between swab head type, there is no pattern or one swab type which is significantly better, biomass collection was below 1%. Again, when used with buffer 1, flocked swabs collected a significantly higher percentage of bacteria than the other two swab head types. This is much more apparent when *S. epidermidis* was collected compared to *S. aureus*, but the pattern is the same. It is not completely clear when using buffer 2, but on trend flocked swabs collected more than cotton or Dacron, again more prevalent when S. epidermidis was collected. When using buffer 3 there was no clear 'winner' in swab head type, flocked swabs collected significantly more *S. epidermidis*, but not significantly more *S. aureus* than the other swab head types.

6.19 Collection of the Mixed *Staphylococcus* spp. from Porcine Skin

The following results are in reference to Figure 38 and Figure 39. Any dry swab head type, once again, reproduced the lowest recovery of bacteria. A dry cotton swab collected between 0.1-0.8% of both species, a dry Dacron between 0.2 and 0.5% and a dry flocked swab bucked the previous trend and collected between 2-8% of the inoculated bacteria, still significantly less than any buffer type with any swab head ($P<0.001$). Cotton swabs when using buffer 1 collected 17-26% of the inoculate, with buffer 2, 23-33% and with buffer 3 between 9-15%. As also seen when collecting *S. aureus* alone, cotton swabs with buffer 2 collected and released significantly more bacterial cells than buffer 1 or 3 ($P<0.001$). Cotton swabs with buffer 1 collected more than buffer 3 with a higher significance than the previous experiment in Section 6.18($P<0.001$). When a Dacron swab was used to collect the mix of staphylococcal species, buffer 2 collected between 15-20% of the inoculate, which was significantly more than buffer 1 which was between 13-16% and buffer 3, 7-16% ($P<0.001$). Such significant differences between buffer efficiency were unlike the individual species collection, where there was either no significance between buffer 1 and 2 for *S. epidermidis*, or buffer 1 was only better by a small significance when *S. aureus* was collected. When a flocked swab was used, the results matched the results from Section 6.18, in which buffer 1 collected between 41-63% which was highly more significant than the 23-48% buffer 2 collected ($P<0.001$) and the 15-22% that buffer 3 did ($P<0.001$).

Dry flocked swabs were statistically better at collecting and releasing the staphylococcal mix than either of the other two swab head types ($P<0.001$). When flocked swabs collected the Staphylococcal mix, that was the only occurrence where a dry swab had achieved over 1% of the inoculant recovery. Flocked swabs again recovered more bacteria with both buffer 2 and 3 in this experiment than the other two swab head types. Therefore, in this experiment, flocked swabs consistently collected and released the highest percentage of the mixed *Staphylococcus* spp.. There was no significant difference between cotton and Dacron swabs when used with buffer 3 or a dry swab ($P>0.05$). Cotton swabs were significantly more efficient at recovery of biota than Dacron swabs when used with buffer 1 and buffer 2 ($P<0.001$).

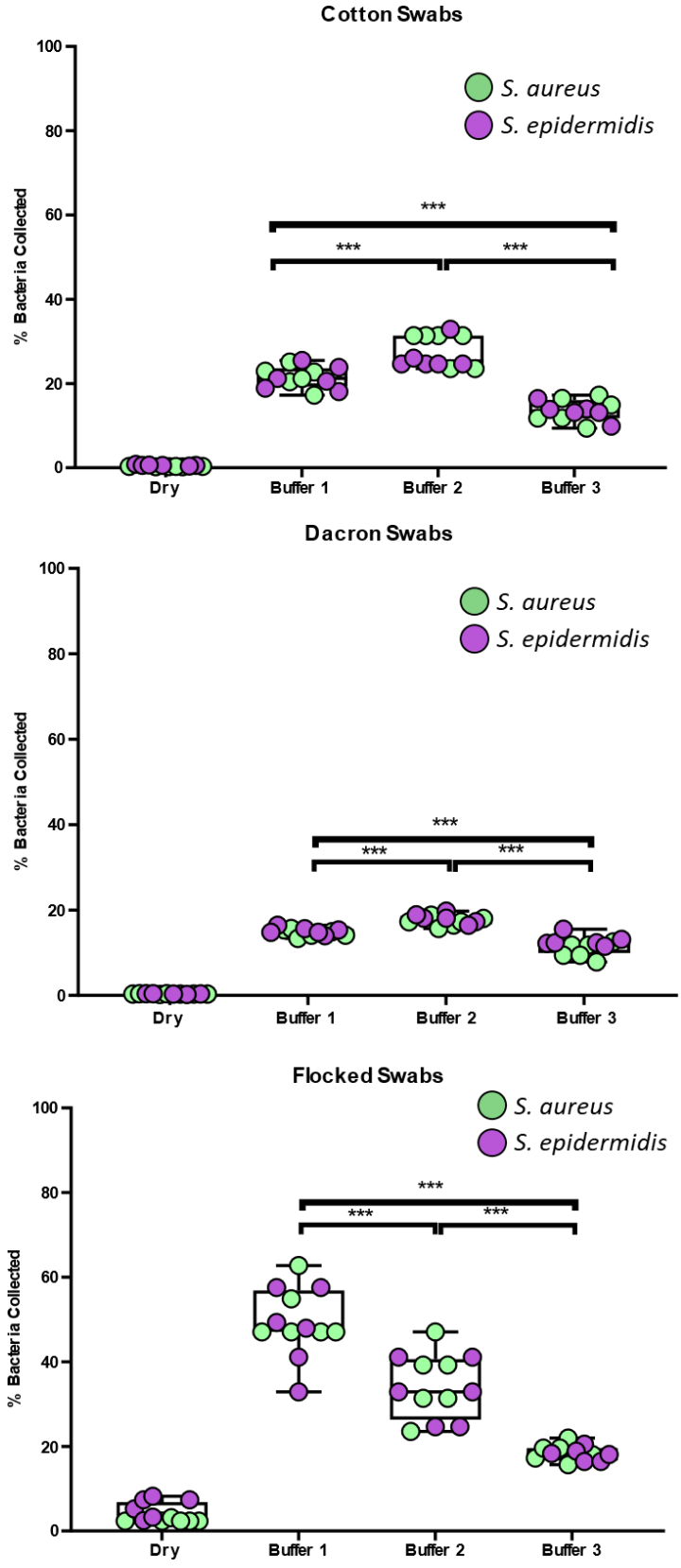


Figure 38. Percentage of Co-Culture Bacteria Collected from Each Swab Type. Green represents *S. aureus* percentage and purple represents *S. epidermidis* percentage. Again, while buffer 2 collected the most bacteria when used with cotton or Dacron, buffer 1 significantly outperformed when used with the flocked head. Again, using any buffer collected significantly more than a dry swab.

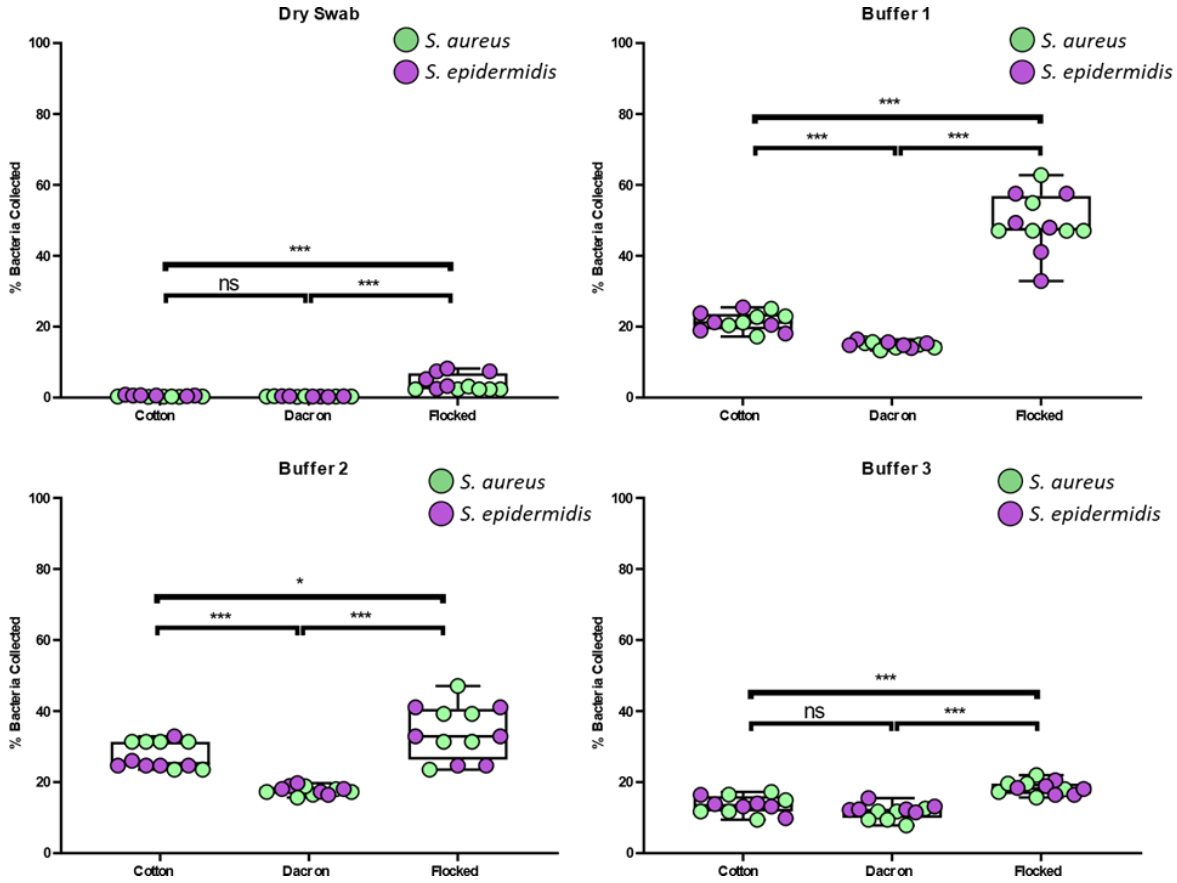


Figure 39 .Comparison of Buffer Composition and Swab head Type on Co-Culture Bacterial Collection. Green represents *S. aureus* percentage and purple represents *S. epidermidis* percentage. While a dry flocked swab collected significantly more than any other dry swab, it was still significantly ($P<0.05$) less than any swab type with a buffer. In this experiment, flocked swab collected significantly more bacteria than both cotton and Dacron when used with any buffer type. Cotton collected significantly more than Dacron with both buffer 1 and 2, but there was no significance when buffer 3 was used.

6.20 Discussion

To collect microbes from the gut in a non-invasive method, a faecal sample is the accepted protocol (Tang et al. 2020b). However, to collect a non-invasive microbial sample from the skin, there is no clear 'gold standard' and several previous studies have used a range of buffers and swab head types (Keyworth et al. 1990; Huttenhower et al. 2012; Ogai et al. 2018). The above experiments have shown that buffer type and swab head type have an impact on the quantity of bacteria collected from a skin model. Therefore, we can reject the null hypothesis, which stated, swab head type or buffer composition have no significance on the quantity of bacteria collected from skin.

6.21 Moistening the Tip of the Swab Improves Recovery of Bacterial Biomass from the Skin

Using a buffered solution to wet the swab before collecting a sample, significantly increased the quantity of bacteria removed from the skin model. Any combination of the three buffers with any swab head type always collected more than using a dry swab alone (Figure 36, Figure 38). Prior work that collected bacterial samples from various environmental surfaces have also found that using a wetting agent to premoisten swabs have increased collection sensitivity (Landers et al. 2010). Dry swabs are not highly utilised in skin microbe studies due to the repeated reduced biomass collected by them (Kong et al. 2017). However, a moistened swab head has been shown not to significantly alter the quantity of bacteria collected when sampling from a mucosal zone, such as inside the nasal cavity (Hagiya et al. 2013). Therefore, use of a wetting agent, to premoisten a swab, is likely only required when collecting from areas of skin which are not excessively moist like the inside the nose or the inner vulva.

6.22 Importance of Buffer Composition When Collecting Bacterial Biomass from the Skin

The composition of the solutions used in these previous papers include a non-ionic surfactant and an osmotic/pH buffering base. The addition of the non-ionic surfactant increases contact of the swab with the sample surface, the decreased surface tension also helps remove adhered cells and eventually release them from the swab (Moore and Griffith 2007). The solutions used in which the highest quantity of bacteria was collected across all the experiments were buffers 1 and 2 (Figure 36, Figure 38). Buffer 1 contained 0.1% v/v of the Triton-x-100, while buffer 2 contained 0.1% v/v Tween 20. Both non-ionic surfactants can cause damage to bacterial cell membranes and Triton-x-100 can be particularly potent against gram-negative bacteria (Helander and Mattila-Sandholm 2000; Johnson 2013). Tween 80, for example, has been shown to permeate membranes at a concentration as low as 0.1% (Nielsen et al. 2016).

As there was no overall significance of total bacteria collected between the two buffers, the non-ionic surfactants were unlikely to have caused serious membrane damage at the concentration

used. Buffer 3, however, significantly, and consistently collected the lowest number of bacteria from the porcine skin. While buffer 3 also contained Tween 20, the concentration was five times higher than found in buffer 2. The buffer may have initially removed the same quantity of the *Staphylococcus* spp., however, cell membrane damage could have reduced the number of viable cells. For future research into whether surfactant concentration has an effect on cell viability, the buffer compositions could have their surfactants switched, e.g., Buffer 1 made with 0.5% v/v Tween 20 rather than 0.1% v/v Tween 20.

6.23 The influence of Swab Head Types on Viable Bacterial Cells Collection

The number of viable bacterial cells collected from the skin was significantly affected by the swab head type used. Flocked swab heads collected, and deposited, significantly more bacteria when using buffer 1 or 2, across all experiments than either cotton or Dacron (Figure 37, **Error! R eference source not found.**). While not as consistently, flocked swabs still collected more than cotton or Dacron swabs using buffer 3, even without buffer. The results correspond with the published literature, which reports that flocked swabs are more efficient at uptake and release of biomass than traditional swab types (Daley et al. 2006; Dalmaso et al. 2008; Hernes et al. 2010; Finazzi et al. 2016). The design of flocked swabs differs to the traditional, as they have individual protruding nylon fibres which increase both surface area and surface tension. Individual strands and a lack of core also mean that the microbiota collected are less likely to be trapped amongst the fibres and therefore released with more ease (CopanDiagnostics 2010; Faoagali 2010; PuritanMedical 2019; Manoj et al. 2021).

While using flocked, the swab type consistently collected and released the most biomass, the same consistency could not be replicated with the cotton or Dacron swabs. Many of the experiments returned no significance between the two swab types, and neither regularly outperformed the other (Figure 37, **Error! Reference source not found.**). Previous work has found t hat while Dacron swabs were overall more efficient at absorption, extraction and recovery, many of these properties also overlapped with cotton swabs (Bruijns et al. 2018). Cotton swabs possess fatty acids which, if exposed long enough, can be toxic to certain bacteria (Zain and Bradbury 1995; Faoagali 2010). Irradiating the cotton swabs to sterilise them can also cause leaching of these fatty acids and exacerbate the issue (Dadd et al. 1970). The Centres for Disease Control and Prevention actively advise against the use of cotton swabs as their materials can inhibit PCRs (CDC 2017), while their high DNA content which could also cause issues in creating false results (Probst et al. 2010). Dacron swabs are still used for research and, when flocked swabs are unavailable, are an adequate substitute (Kline et al. 2021). However, due to their fibrous design, bacteria can become trapped and unable to be released. Nevertheless, Dacron swabs have been shown to

recover proteins better than other swab types and therefore would likely be more suited for experiments involving secreted bacterial toxins, for example (Zasada et al. 2020).

One of the reasons however that cotton and Dacron swabs were so inconsistent was the difficulty in repeating the swabbing technique. While the protocol remained the same, the pressure used in collection is extremely hard to replicate. Discrepancy between swabbing pressure has been noted in several studies that swabbing technique can impact the collection of viable bacteria and that other methods may be better suited (Ogai et al. 2018).

6.24 Conclusion

This study has highlighted the inconsistencies between previous research papers and how they may not have successfully created an accurate depiction of skin microbiota. While swabbing itself may not paint the most accurate microbial picture, there will always be a need for a non-invasive protocol involving swabs. Therefore, correct buffer and swab type selection could potentially increase the accuracy of microbial representation and it is imperative that the practise of skin swabbing becomes more standardised to help ensure consistent reproducibility across the research. The above results clearly show that using flocked swab head types in conjunction with buffer 1 recovered a significantly larger quantity of bacteria than any other combination. It also shows that regardless of swab type, a wet swab was always better than a dry swab.

7 Choosing a Suitable Swab Site for Skin Microbiome Experiments to Limit Background Contamination Interference

7.1 Introduction

Bacterial mass on skin is low compared to other areas of the body, collection of enough bacteria to produce an accurate picture of the microbial inhabitants can be difficult (Byrd et al. 2018). As discussed in the introduction, the skin has varying pHs, topography, temperature and dryness which results in the microbial fingerprint varying from region to region (Yousef et al. 2020; Zhou et al. 2020). If one were to design an experiment, it would be ideal to sample from areas in which the most bacterial DNA can be recovered. A logical continuation from the results of Section 6 would be to use the best swab and buffer combination to recover bacteria from the skin. Future general cosmetic studies could focus investigations on the areas that could give the biggest bacterial return and limit the relative abundance of background contamination which could skew results.

7.2 Microbial Inhabitants Differ from Person-to-Person

While different areas of the body will have different microbial communities, individuals themselves will differ in microbial biomass and inhabitants. Influences on skin's microbes include individual hygiene, sex, hormones, age, location, partners, and pets (Song et al. 2013; Ying et al. 2015; Gupta et al. 2017; Vandegrift et al. 2017). There's conflicting data as to whether men or women have more abundant or varied species on their skin (Marples 1982; Fierer et al. 2008), but cohabiting couples often have overlapping communities (ASM 2017; Ross et al. 2017). Pets also influence the microbiota that live on skin, with humans often harbouring microbiota more closely associated with their pets (Song et al. 2013; Wetzels et al. 2021). With such differences, you cannot say whether one community fingerprint is the 'best' as each microbial skin community is different. However, there are microbial signs in which someone's skin may be unhealthy (Liu et al. 2020a). Individuals with atopic dermatitis for instance often have a higher abundance of *S. aureus* (Ogonowska et al. 2021), or people with seborrheic dermatitis may have elevated levels of the fungal genus *Malassezia* residing on their scalp (Kim 2009).

7.3 Cosmetic Testing on Skin

Studies into how cosmetics affect skin often use the volar forearm as the testing location, called the forearm-controlled application technique (FCAT) (Ertel et al. 1995; Farage 2000; GSK 2017). The volar forearm has a large surface area, a second arm for control, a lack of interfering hair or secretions and can easily be covered up should any unsightly reactions to the product occur. It has also been shown to be a decent comparison for testing facial cosmetics as it has equivalent hydration and biochemical properties (Bazin and Fanchon 2006). However, if one were to investigate microbial composition of the skin and any changes which may occur, the volar forearm may not be the best location for such cosmetic investigations. Being classed as a 'dry' area of the skin (Grice et al. 2008), the bacteria which reside there would unlikely be comparable products

which are designed for oily or moist regions (Grice and Segre 2011). The microbial biomass available would be minimal (Breton 2018) and background contamination could dominate the samples, leaving the results with very little useful information (Salter et al. 2014; Glassing et al. 2016; Eisenhofer et al. 2019).

7.4 Background Contamination of Microbial Samples

DNA extraction from a microbial sample will always have background contamination, whether it be from the sampling equipment, extraction kit, the processing laboratory or simply from the environment which surrounds the collection site (CDC 2003). Steps can be taken to remove the background contamination through multiple negative controls including multiple negative controls of the sampling devices, surroundings and extraction methods and removing these from the samples using bioinformatics software (Grogan et al. 2019). However, many of the background contaminating bacteria are also normal inhabitants of the collection site and therefore are difficult to identify as a contaminant (Glassing et al. 2016). Contamination in low biomass samples have also been shown to have skewed data by dominating the sample and therefore inaccurately portrayed the microbial community (Hasrat et al. 2021). When choosing a site on skin to conduct a microbiome study, choosing a site with greater biomass will help reduce spikes in background contamination (Minich et al. 2019).

7.5 16S rRNA gene Sequencing to Identify Microbial Communities

The ribosome in prokaryotes have the same function as eukaryotes, they are the site of protein synthesis (Adams 2014). Prokaryotes 70S ribosome have a small 30S unit, which contains the subunit 16S rRNA, and a larger 50S unit, which contains the subunits 23S rRNA and 5S rRNA (Fox 2010). The 16S rRNA gene has more than 1500 base pairs and consists of nine hypervariable regions which are unique to each species (Bukin et al. 2019).16S rRNA gene sequencing has been used for many years to help identify microbial communities of different habitats, most research focuses on certain hypervariable regions which can usually identify down to genus level, but if the whole gene is sequenced then species can be identified (Janda and Abbott 2007). Amplification of different regions can influence the results of the communities (Soriano-Lerma et al. 2020), generally for identification of skin bacteria the V3-V4 region is used (Castelino et al. 2017). Previous work has shown that amplification of the V3-V4 region had high species richness and identified known skin species in abundance (Ross et al. 2018; Stehlikova et al. 2019).

7.6 Sequencing reads and Operational Taxononomic Units (OTUs)

16S rRNA gene sequencing recover total sequencing reads, taxon sequencing reads, unclassified sequencing reads, and unique sequencing reads from each sample. Total sequencing reads are the number of effective sequencing reads, taxon sequencing reads are the sequencing reads which can be attached to a taxon, unclassified sequencing reads are the that are not associated with

taxa and unique sequencing reads are sequencing reads which only one occur in one sample. Sequencing reads with an identical sequence of 97% or more are called operational taxonomic units (OTUs) which help determine the bacterial diversity present.

7.7 Diversity Indices

7.7.1 Alpha Diversity Indices

Alpha diversity (α-diversity) indices evaluate the species diversity within a sample group based on either measurement of abundance of specific species or distribution within the sample, or as a combination (Willis 2019).

The Shannon diversity index uses the abundance and evenness of species within a sample to calculate how unpredictable a random species plucked from the sample would be. For example, if there are only 2 species within a sample at a 1:1 ratio then the diversity is low guessing a randomly picked species would be highly likely. However, if a sample has thousands of species at different proportions, then diversity would be high and the chances of guessing a random species would be highly unlikely. Therefore, the higher the resulting number, the higher the diversity within a sample (Thukral 2017). The Simpson diversity index is similar to the Shannon diversity index, but measures the probability that two randomly picked individuals from a sample were from the same grouping (Hunter and Gaston 1988). As 0 would be high diversity and 1 would be no diversity, the data is often represented as '1 – Probability Result' to maintain the 'high number equals high diversity' theme, this is called the Gini-Simpson index (Caso and Gil 1988).

While the Shannon and Simpson's diversity indices measures abundance and evenness throughout the sample, both CHAO1 and Abundance Based Coverage Estimator (ACE), estimate the richness of a sample by abundance of different species present. CHAO1 weights the estimation on rarer species within a sample, only occurring once or twice, to approximate the number of missing species. ACE also calculates estimation of diversity and missing species through the using rare species, but these are classed as species occurring less than 10 times within a sample (Kim et al. 2017a). The Good's coverage calculates the coverage of a dominant species within a sample by measuring the number of rare OTUs with one appearance over the total OTUs within a sample (Good's Coverage = (1- frequency of rare OTUs/Total OTUs)), so the greater the result the less diversity within a sample or high domination by abundant species (Fouts et al. 2012). The phylogenetic diversity measures the total branch length of one group, the higher the total length the greater the diversity within a sample (Faith 1992).

7.7.2 Beta Diversity

Beta diversity (β-diversity) measures similarity between samples as a distance between 0 to 1. Weighted unifrac distance and unweighted unifrac distance measure the dissimilarity coefficient between paired samples. The higher the unifrac distance the greater the diversity between samples, this is measured on 0-1 scale. Weighted unifrac calculates diversity by including the

abundance of species/groups, while Unweighted unifrac only calculates using true or false, meaning simply do both samples contain this species/group (Lozupone et al. 2007). Both α-diversity and β-diversity indices are commonly used for skin studies (Silva et al. 2018).

7.8 Null Hypothesis

That the location site of skin swabbing, for the collection of bacteria, does not affect the relative background contamination.

7.9 Aims

- Investigate different swab sites and whether they are appropriate for cosmetic microbiome studies
- Choose a swab site with greater viable cells to limit background contamination
- Compare percentage of background contamination from swabs and extraction kit
- Compare the presence of bacterial taxa at each swabbing location and between participants

7.10 Materials

7.11 Swab type and Buffer

Section 6.2 highlighted how different swab head types and buffer compositions can retrieve significantly differing quantities of bacteria from skin. Using those results, this experiment used the flocked swabs (4N6FLOQSwabs™, Life Technologies, Carlsbad) and Buffer 1 (0.075 mol litre-1 of phosphate buffer [pH 7.9] containing 0.1% v/v Triton X-100) combination to recover bacteria from the skin.

7.12 Swabbing Participants

One Caucasian male, age 34, and one Caucasian female, age 27, volunteered to be swabbed. Both individuals cohabited and owned two cats at time of participation. As the samples processed were microbial in nature and removed from skin cells they are not considered 'relevant material' under the Human Tissue Act and therefore no need for a license (HTA 2021). Swab heads which may have accidentally removed skin cells were discarded at the earliest convenience after use. Signed consent was obtained from both participants (See Appendix). Due to Covid restrictions and social distancing measures, the swabbing had to take place in the participants home environment, which was not ideal. Surfaces were cleaned with antimicrobial sprays and clean gloves were worn to take the sample. However, background contamination can come from anywhere, laboratories included (Weyrich et al. 2019). The number of participants would be classed as too low for robust study. If this experiment was to be repeated, possibly to investigate the background contamination of microbiological laboratories, then the number of participants would ideally be increased to between 10-20 partakers.

7.13 Methods

7.14 Swabbing Technique

The locations were chosen due to their differing dryness, the forehead is oily, the volar forearm is dry, the axilla is moist (Introduction, Figure 1). The volar forearm is also used in many cosmetic studies. The navel was chosen because its topography differs from the rest, as it can concave into or convex out of the skin (Fahmy 2018). Due to the differing topography and size of the location swabbed, a single technique was difficult to achieve. This work also wanted to be able to remove the highest quantity of DNA possible to achieve a more accurate representation of the microbial community. Swabbing took place first thing in the morning, before showering. A single swab was fully dipped into the buffer solution for 5 seconds and samples were taken from as much of the chosen area as possible. Figure 40 highlights the direction and area of the body that was swabbed. To swab the left axilla, the arm was raised and swabbed from the top furthest left to the bottom furthest right, swab direction was vertically downwards, the head was rotated after each line was completed. For the left volar forearm and axilla, the area was swabbed from the top left to the bottom right, one-way vertical strokes away from the wrist, with the head rotated a quarter turn after each line was completed. Care was taken not to swab the antecubital fossa. For the forehead, the same technique was repeated, but with horizontal strokes, left to right, care taken not to swab the hairline or eyebrows. Due to the shape of the navel, the swab was inserted until the bottom of the navel was achieved and the perimeter swabbed in a clockwise motion 10 times. Once the sample was collected, the swab head was removed from shaft and placed into an Eppendorf™ (Eppendorf, Hamburg) polypropylene DNA Lo-bind polypropylene microcentrifuge tube ready for extraction.

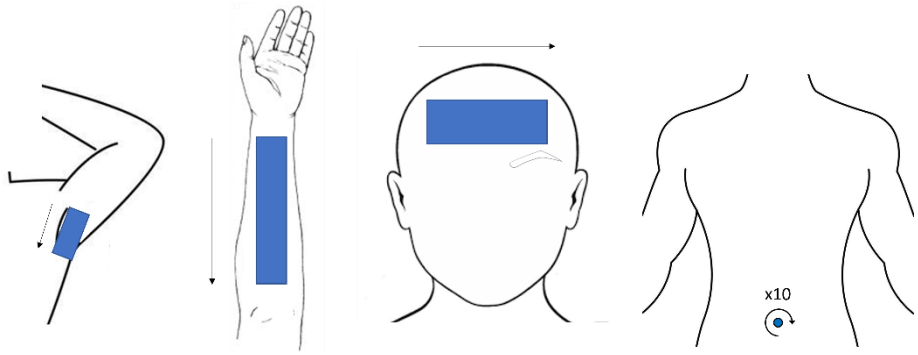


Figure 40. Body Parts Swabbed. Blue indicates the areas swabbed. The axilla (left) the arm was raised and swabbed from the top furthest left to the bottom furthest right, swab direction was downwards. The left volar forearm (second from left) was swabbed away from the wrist in repetitive vertical strokes that covered the whole area. The forehead was swabbed horizontally left to right. The navel was swabbed once the swab hit the bottom, ten times in a clockwise rotation.

7.15 Extraction of Bacterial DNA from Swabs

Bacterial DNA was extracted from the swabs using the PureLink™ Microbiome DNA Purification Kit (Invitrogen, Massachusetts). The protocol was modified slightly to achieve greater yield. The swab heads were added to a bead tube containing the lysis buffer and lysis enhancer, the tubes were placed into the MP Biomedicals™ FastPrep-24™ 5G Instrument and lysed with a velocity of 6.5 m/s twice for 45 seconds with a 5-minute intermission. After a 10-minute incubation at 65 °C, the tubes were placed back into the FastPrep-24™ at the same settings. From that point the protocol was followed until the DNA was eluted. RNase Free Water (Severn Biotech Ltd, Kidderminster) replaced the kit elution buffer. This was done as elution buffers have the potential to disrupt further applications. DNA was quantified using the Qubit 4 Fluorometer (Invitrogen, Massachusetts) using the protocol designed for the Qubit™ dsDNA High Sensitivity assay. To confirm that downstream applications would not be affected by the chemicals used in the extraction, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers 341F (5'->3') CCT AYG GGR BGC ASC AG and 806R (5'->3') GGA CTA CNN GGG TAT CTA AT. Products were run on a 1.5% agarose gel to confirm that bacterial DNA was present.

7.16 16S rRNA Amplification and Sequencing

Genomic DNA extracted from the samples were sent to Novogene Co (China) for 16S rRNA amplicon metagenomics sequencing. Due to the low DNA concentration, 30,000 reads were undertaken per sample (~150k raw reads per sample). Again, the PCR was used to amplify the V3-V4 hypervariable regions using the same primers. The PCR products went through quantification and mixing, purification, library preparation and sequencing. Amplicon was sequenced on Illumina paired-end platform to generate 250 bp paired-end raw reads (Raw PE), and then merged and pre-treated to obtain Clean Sequencing reads. The chimeric sequences in Clean Sequencing reads were detected and removed to obtain the Effective Sequencing reads which can be used for subsequent analysis (Liu et al. 2019).

7.17 Statistics

To study the microbial community composition in each sample, Operational Taxonomic Units (OTUs) were obtained by clustering with 97% identity on the Effective Sequencing reads of all samples, and then identified. The OTU analysis included species annotation, distribution, α-diversity, β-diversity, and species variance statistics. To test for significance of differences between groups for both α-diversity and β-diversity, this involved Wilcox and Tukey tests. Bacterial diversity analysis was performed using QIIME software (version 1.7.0). One-way ANOVA and t-tests were also carried out on the abundance of 'hits' sample groups and individual samples had on overall OTUs, using GraphPad Prism (version 9.3.1). The β-diversity weighted and unweighted unifrac calculations were visualised into non-metric multidimensional scaling using the Vegan and Mass packages in R (version 4.0.5) and illustrated using GraphPad Prism (version

9.3.1) The contamination percentage was achieved by removing the number of OTUs found in the male swab control, female swab control and kit control from the sample groups, this was performed in Excel (Version with Windows 11).

7.18 Results

7.19 Sequencing reads Recovered from Samples

The kit blank control returned 10,118 sequencing reads, in which 98.6% could be classified into 245 OTUs. This part of the results Section is in reference to Figure 41.

7.19.1 Male Participant's Results for Total, Usable, Percentage, Unique Sequencing reads and OTUs

For the male samples (Figure 41), the samples with the highest total sequencing reads were the axilla 83,488 of which 99.3% could be classified into 318 OTUs, 611 sequencing reads were unique to the sample. The forehead returned 81,474 sequencing reads, with 98.3% classified into 461 OTUs, 1204 sequencing reads were unique to the sample. The volar forearm returned 61,555 sequencing reads with 96.9% classified into 940 OTUs, 1879 sequencing reads were unique to the sample. The navel sample returned 32,735 sequencing reads with 97.7% being classified into 733 OTUs, 751 sequencing reads were unique to the sample. The male swab blank control also returned 14,802 sequencing reads in which 94.6% could be classified into 672 OTUs, 805 sequencing reads were also unique to that sample.

7.19.2 Female Participant's Results for Total, Usable, Percentage, Unique Sequencing reads and OTUs

For the female samples (Figure 41), the sample with the highest total sequencing reads was the forehead with 92,063 sequencing reads, of which 99.0% could be classified into 251 OTUs, 641 sequencing reads were unique to the sample. The axilla 64,942 of which 97.8% could be classified into 833 OTUs, 1406 sequencing reads were unique to the sample. The navel sample returned 34,470 sequencing reads with 95.5% being classified into 819 OTUs, 1539 sequencing reads were unique to the sample. The forearm returned 22,958 sequencing reads, with 94.7% classified into 770 OTUs, 1199 sequencing reads were unique to the sample. The female swab blank control also returned 17,200 sequencing reads in which 96.7% could be classified into 208 OTUs, 235 sequencing reads were also unique to that sample.

7.20 T-tests and One-way ANOVA of OTU abundance

One-way ANOVA analysis of the all-individual OTU abundance in all sample groups resulted in no statistical difference between the male-female-blank controls (P=0.3895). There was no statistical difference in the abundance of all individual OTUs when comparing male-female sample groups (P=0.5901), nor between male-blank controls (P=0.1298), nor female-blank controls (P=0.3002). Overall, while not statistically different, the male sample group had more dissimilar overall

individual OTU abundance than the blank controls than between the blank and female controls. However, using a t-test to analyse between two comparable individual groups did result in differences. Between male and female samples, the only statistical difference between overall individual OTU abundance was between the forearm samples (P=0.002). All the other comparable groups between male and female were not significantly different e.g., the male axilla did not have a significantly different individual OTU abundance than the female navel sample (P=0.9405). Only the male axilla sample was not significantly difference to both the male swab blank and kit blank controls (P>0.05), the rest were significantly different to their controls (P<0.03). Only the female forehead sample was not significantly different to the kit blank control (P>0.05), while the other female samples were significantly different to the kit blank control (P>0.05). Only the female axilla sample was significantly different to the female blank swab control (P<0.002), the other samples were not significantly different to the swab control (P>0.05). None of the blank controls were significantly different to each other (P>0.30).

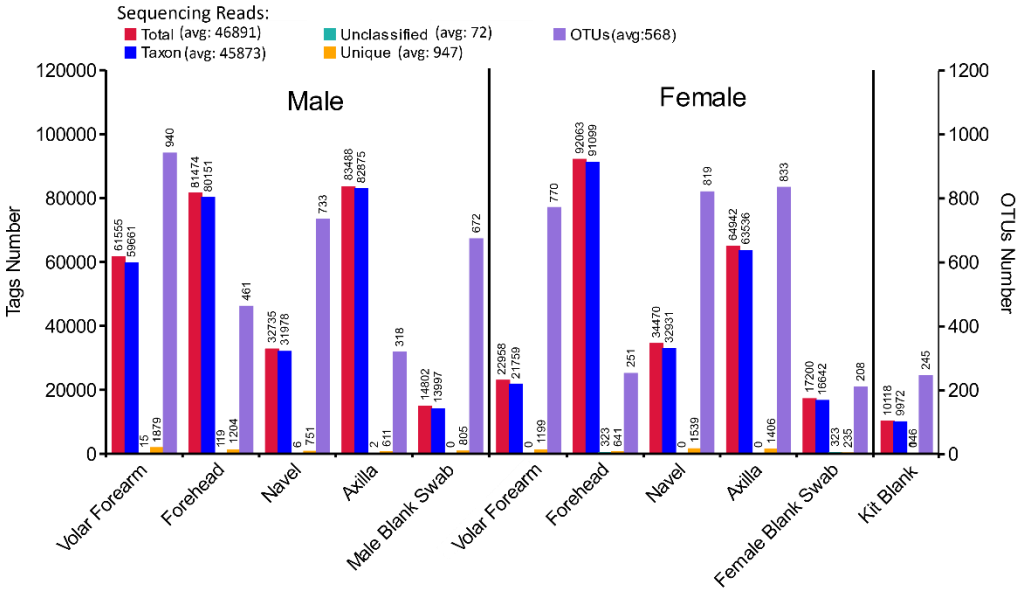


Figure 41. Number of Sequencing reads and OTUs from Each Sample. The most annotated sequencing reads from a male sample were from the axilla with 82,975, the lowest amount not including the blank was from the navel which was 31,978. The blank swab used when collecting male samples still resulted in 13,997 annotated sequencing reads. The most annotated sequencing reads from a female sample were from the forehead with 91,099, the lowest amount not including the blank was from the volar forearm which was 21,759. The blank swab used when collecting female samples still resulted in 16,642 annotated sequencing reads. The extraction kit itself produced 9972 annotated sequencing reads.

7.21 Diversity Indices Results

7.21.1 Alpha Diversity of Samples

These results refer to the top half of Table 10. The α-diversity for the male's samples taken from body sites shows that the number of observed species mostly correlates with diversity. For example, the volar forearm had 634 observed species and the highest Shannon's (6.35), Gini-Simpson's (0.96), Chao1 (851) and ACE (927) results and the least amount of domination by one species of Good's Coverage (0.976). However, phylogenetic diversity measurement of the branch length was the second longest at 58.5 units compared to the forehead which was 81 units. The forehead sample was less diverse compared to the volar forearm with every other measurement. The blank swab, although it had the lowest number of useable taxonomic sequencing reads (Figure 41), had a high number of OTUs and individual species and therefore diversity results were high. Again, the most diverse of the female samples was the volar forearm with 607 observed species, it had the highest Shannon diversity (7.04), Gini-Simpson's diversity (0.981), Chao1 (730), ACE (742) and longest phylogenetic diversity unit (45.2). Only the navel sample had less coverage or dominance by the abundant species, at 0.979 compared to 0.984. Both the female blank swab control and kit blank control had less diversity compared to the male blank swab control; all other results can be seen in Table 10. Due to lack of replicates, comparison between samples is statistically difficult to achieve but comparison of overall diversity of the male and female total samples are possible. Although there were differences at sample levels of diversity, there was no statistical difference between male and female for all the α-diversity indices (Table 11).

Participant	Sample Name	Number of Observed Species	Shannon Diversity Index	Gini-Simpson's Diversity Index	Chao1 Index	ACE Diversity Index	Good's Coverage	Phylogenetic Diversity
Male	Volar Forearm	634	6.354	0.960	851.107	926.519	0.976	58.495
	Forehead	296	4.202	0.849	501.857	514.764	0.987	81.091
	Navel	521	6.272	0.958	660.567	686.401	0.984	43.985
	Axilla	147	2.096	0.561	190.966	200.165	0.995	18.001
	Male Swab Blank	589	7.065	0.982	640.739	680.359	0.988	47.454
Female	Volar Forearm	607	7.040	0.981	730.367	741.897	0.984	45.227
	Forehead	94	1.240	0.393	183.438	215.605	0.995	16.477
	Navel	574	5.192	0.892	748.762	802.280	0.979	43.410
	Axilla	495	6.074	0.964	691.265	718.553	0.982	39.609
	Female Swab Blank	172	3.452	0.804	235.000	242.446	0.994	25.043
Kit Control	Kit Blank	245	4.426	0.917	328.133	344.657	0.991	23.787

Table 10. Alpha Diversity Index of All Samples

Table 11. Tukey and Wilcox Statistical results of the Overall Alpha Diversity Indices of the Male and Female Participants

Alpha Index	Tukey	Wilcox
	P-Value[10]	
Observed Species	0.9613759	1
Shannon Diversity Index	0.9945016	0.9262
Gini-Simpson's Diversity Index	0.9852550	0.7080
Chao1 Diversity Index	0.9777527	0.7689
ACE Diversity Index	0.9796687	0.6226
Good's Coverage	0.9937061	0.8400
Phylogenetic Diversity	0.5742229	0.3365

Table 12. Tukey and Wilcox Statistical results of the Overall Alpha Diversity Indices of the Female and All Blank Controls

Alpha Index	Tukey	Wilcox
	P-Value[11]	
Observed Species	0.8144609	0.7525
Shannon Diversity Index	0.9982553	0.3333
Gini-Simpson's Diversity Index	0.8340474	0.8847
Chao1 Diversity Index	0.6326259	0.3463
ACE Diversity Index	0.6335160	0.3010
Good's Coverage	0.4913804	0.2802
Phylogenetic Diversity	0.9588387	0.9027

[10] P<0.05 for a statistical difference

[11] P<0.05 for a statistical difference

Table 13. Tukey and Wilcox Statistical results of the Overall Alpha Diversity Indices of the Male and All Blank Controls

Alpha Index	Tukey	Wilcox
	P-Value[12]	
Observed Species	0.9278945	0.7525
Shannon Diversity Index	0.9878695	0.5833
Gini-Simpson's Diversity Index	0.9049446	0.6244
Chao1 Diversity Index	0.7411215	0.4925
ACE Diversity Index	0.7372359	0.5451
Good's Coverage	0.5458487	0.3627
Phylogenetic Diversity	0.4646718	0.4358

7.22.1 Beta Diversity Between Samples

The weighted unifrac measured diversity based on shared species abundance, while the unweighted unifrac measured diversity based on whether both samples shared a species. The unifrac scores were converted into non-metric multidimensional scaling (nmds). The closer the samples were together on Figure 42, the less diversity between samples. The below results are all in reference to Figure 42.

The weighted unifrac samples were evenly spread across the graph, with both the female and male volar forearms and navels having the least diversity between samples. The four closest samples had more OTUs in common and abundancy with the male swab blank control sample than any other blank control group. The male axilla and female swab blank control were most like the kit blank control, but diverse from each other. The unweighted unifrac however had both much more distant diversity between samples, as well as samples which were so similar, they overlapped. The samples that were so similar they overlapped included the male swab blank control, female forearm, and female axilla. The male navel sample was very close to the overlapped trio of samples and around the perimeter of the cluster included female blank swab control, kit blank control and male volar forearm. The three outlier samples were the female forehead, male forehead, and male axilla samples. The female forehead had more OTU in common with the male axilla than the male forehead.

[12] P<0.05 for a statistical difference

Due to a lack of replicates, a comparison between samples was statistically difficult to achieve, but comparison of overall diversity of the total male and female samples was possible. There were no statistical differences between the diversity between all the sample groups. However, though not significant there was more diversity between male and female groups than either male and the blank controls or female and the blank controls. (Table 14). All female samples and male samples had a diversity more alike the blank controls than each other.

Table 14. Tukey and Wilcox Statistical Differences between all Sample Groups

Sample Comparison	Tukey P-Value[13]	Wilcox P-Value
	Weighted-Unifrac	Unweighted- Unifrac
Male-Female	0.3277	0.2996
Female-Blank Controls	0.9794	0.9943
Male-Blank Controls	0.5753	0.4912

[13] P<0.05 for a statistical difference

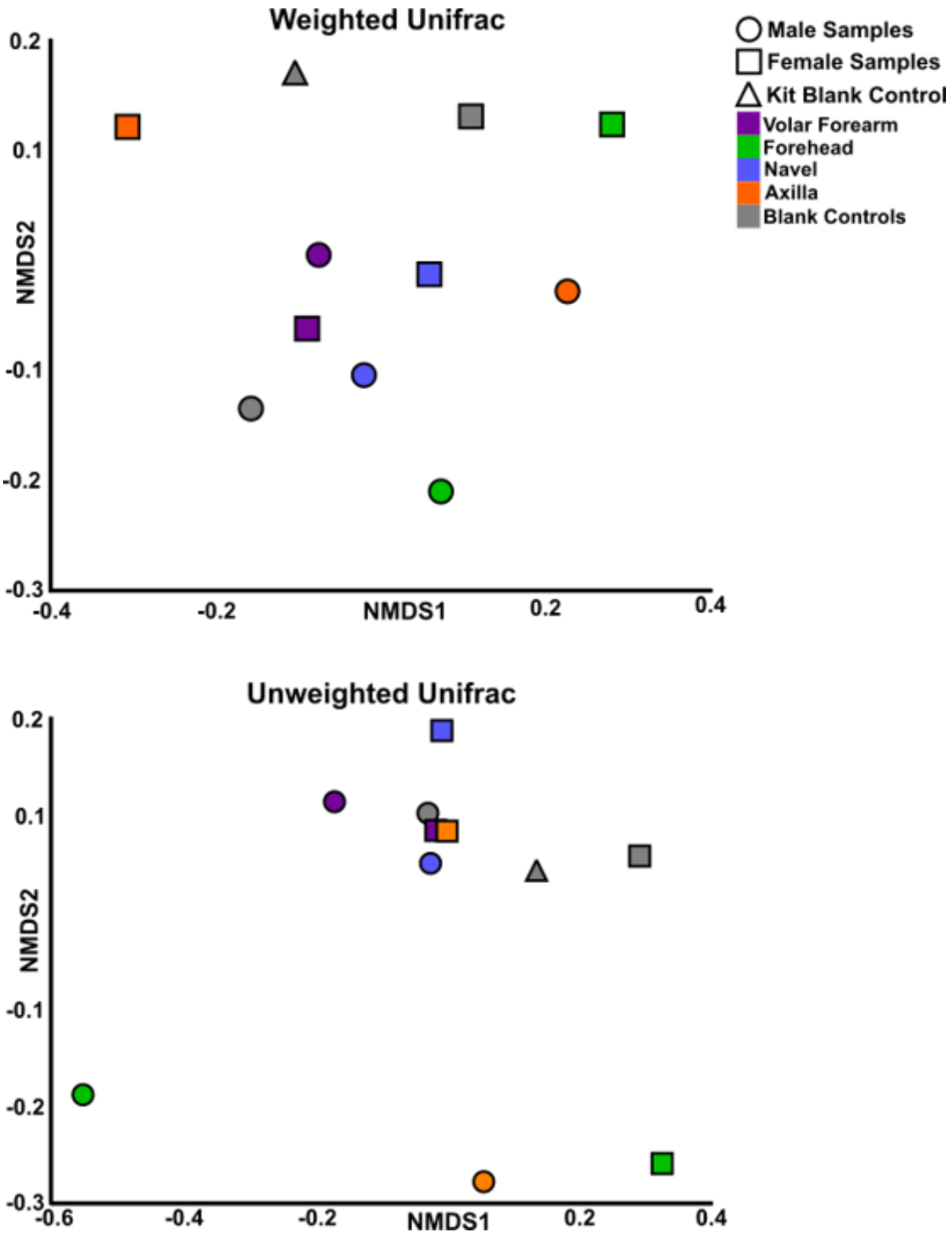


Figure 42. NMDS of Weighted-Unifrac and Unweighted-Unifrac. The closer the samples are together the less diversity between those samples. The weighted unifrac, which uses abundance and presence of OTUs, appears to a much more even spread of diversity from sample to sample than the unweighted unifrac. Both female and male navels and forearms were close in diversity, while the male and female foreheads and axilla were not so closely diverse. The unweighted unifrac only considers the binary of whether both samples contain an OTU. In the bottom of the unweighted, the male axilla and both sexes forehead samples are much farther away from the centre cluster. The centre cluster contains the samples with the low sequence reads and blanks controls.

7.23 Top 10 Isolated Genera from the Samples

Figure 43 shows the top ten genera isolated from all the samples, these include the three common skin genera *Cutibacterium*, *Corynebacterium* and *Staphylococcus*. The kit blank control was made up of over 50% of the top ten genera, with the most abundant genera being *Cutibacterium*, *Staphylococcus*, *Enhydrobacter*, *Acidovorax* and *Bacillus*.

7.23.1 Male Samples Genera Abundance

All the male samples were low in *Cutibacterium* abundance but were largely dominated by the genera *Corynebacterium* and *Staphylococcus*. Dominance from these two genera was most obvious from the axilla sample, where over 75% of the genera abundance was made up of *Staphylococcus* and *Corynebacterium*. The only other sample in which the three common skin genera made up over 50% of the relative abundance was from the forehead. The navel sample had over 25% abundance of the three common skin genera, with *Kocuria* being the next most prominent named genus. Both the volar forearm and the navel were dominated by the mixed, unnamed genera, others. The volar forearm only had 25% of its relative abundance made up of the three common skin genera, but *Kocuria* was just as prominent in abundance as *Cutibacterium,* and *Corynebacterium* combined. The male blank swab had the top 10 genera even distributed to over 25% of the relative abundance, but most of the relative abundance consisted of mixed, unnamed genera.

7.23.2 Female Samples Genera Abundance

The female forehead was an abundance of 75% *Cutibacterium*, with the next most common genus being *Staphylococcus*, very little *Corynebacterium* was isolated from here. More than 50% of the abundance of genera isolated from the navel was dominated by the three common skin genera, while this dropped to under 25% for the volar forearm, and very little of the axilla was made up of the three. Both the volar forearm and axilla were dominated by the unnamed mixed genera, the volar forearm also had a good proportion on *Lactobacillus* isolated, while the axilla had a large abundance of *Diaphorobacter.* The female blank swab control was dominated by *Cutibacterium* and around 50% was made up of the three common skin bacteria, there was also a large abundance of *Chryseomicrobium, Acidovorax, Kocuria* and *Lactobacillus.*

7.23.3 The Unnamed Genera Group

Figure 44 shows the evolutionary tree of all the genera isolated from all the samples, including the unnamed genera from Figure 43. This Figure highlights the unexpected genera in the background contaminations, such as *Helicobacter, Pantoea, Parapusillimonas* and *Muribaculaceae*.

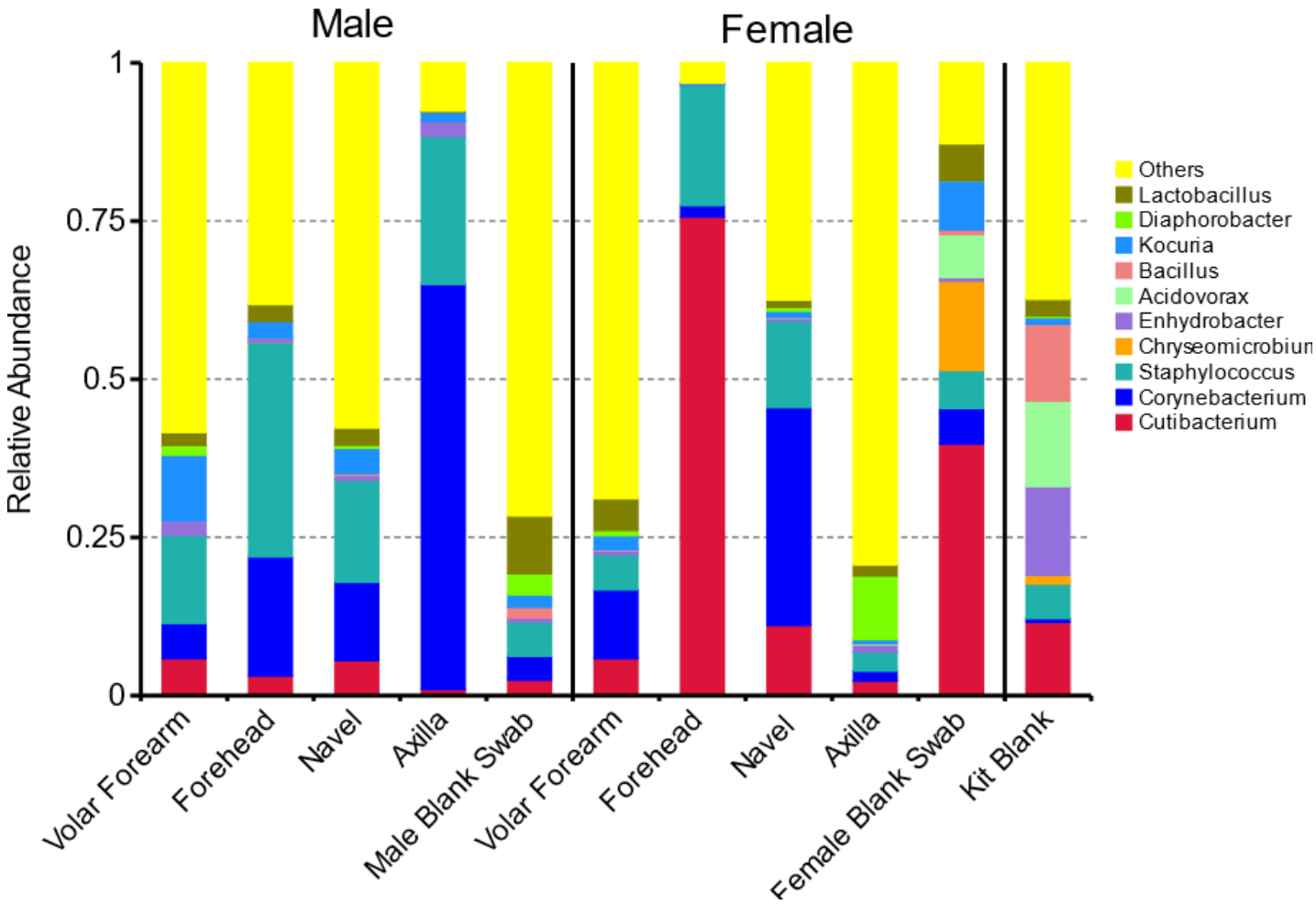


Figure 43. Top 10 Genera Found in Samples. The classic skin genera *Cutibacterium*, *Staphylococcus* and *Corynebacterium* were found within the top 10 genera found in samples. Others found in the top 10 were questionable as to whether they were found on the skin or simply background within the blank controls. The genus *Chryseomicrobium* for example often have species isolated from soil or earthworms rather than human skin (Saha et al. 2018)

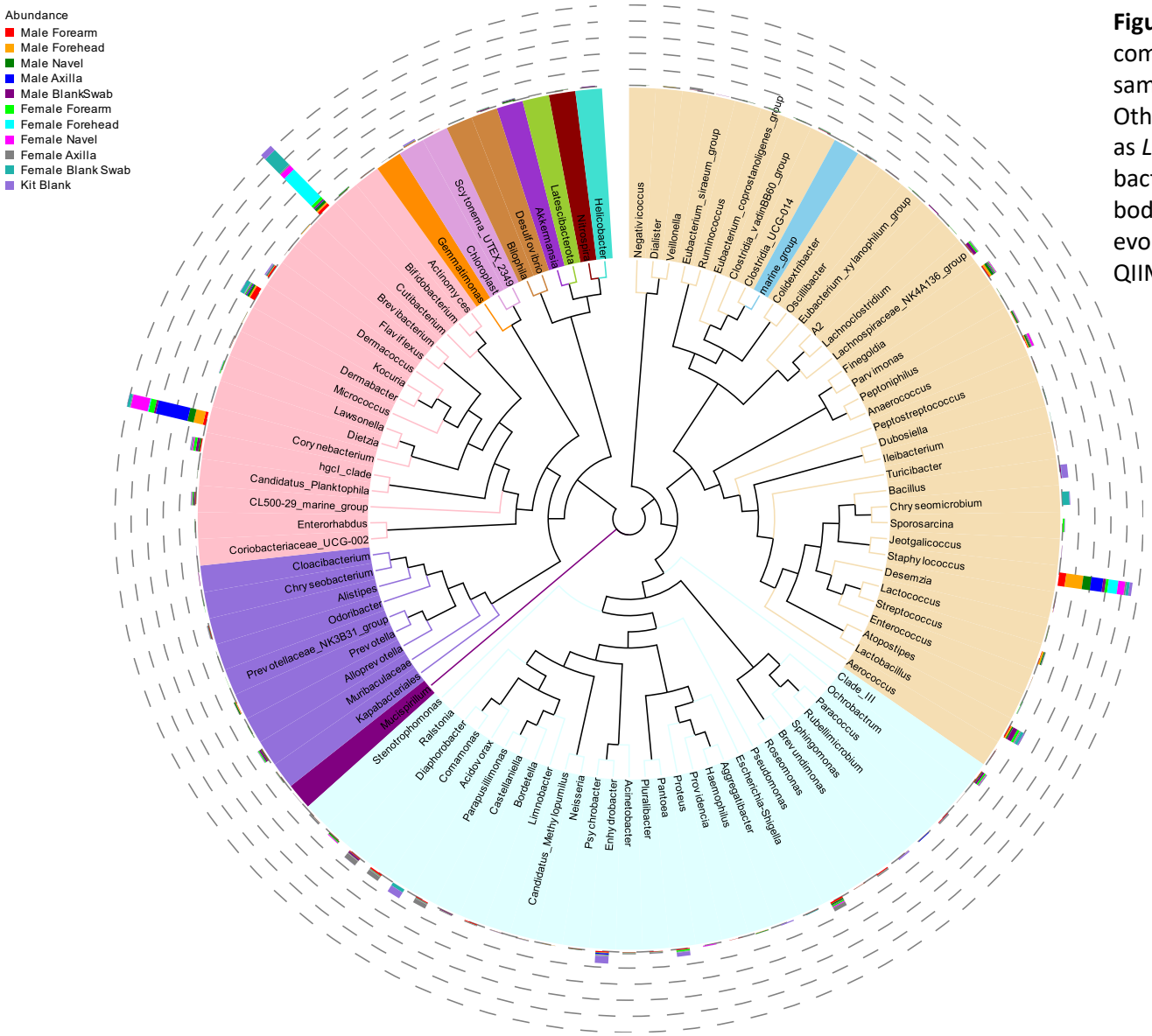


Figure 44. Evolutionary Tree of Genera from All Samples. Three common skin genera dominated the total abundance of all samples, *Cutibacterium, Staphylococcus* and *Corynebacterium*. Other genera also have a fair abundance of representation such as *Lactobacillus* and *Kocuria*. Many of the blank controls have bacteria which would be unexpected anywhere on the human body, let alone the skin. A stated in Section 7.17, analysis and evolutionary tree of bacterial diversity was produced using QIIME software (version 1.7.0).

7.24 Percentage of Background Contamination and Likely Contaminants

In the appendix, is the list of OTUs and abundance of each OTU found in the sample. The percentage of contamination differed depending on which and how many control abundances were removed from the groups. If all controls were removed from the samples (male swab blank, female blank swab, and kit blank control), the most contaminated female sample was the Forearm sample at 62.1% and the most contaminated male sample was the navel at 46.3%. For all contamination percentages please see Table 15**.**

Figure 45 shows the phylogenetic tree of all samples, from kingdom all the way to identifiable species. Of the bacteria isolated nearly 57% were from the phylum Actinobacteriota, 33.3% were from the phylum Firmicutes and 9.77% were from the phylum Proteobacteria. While the phyla Actinobacteriota and Firmicutes were isolated from all samples, Proteobacteria were isolated from predominantly the kit blank control, the female swab blank control and the female axilla. This control group domination of isolated Proteobacteria filters through to the orders, families, genera and the one species identified, *Moraxella osloensis*. The only exception is the genus *Diaphorobacter* which was predominantly isolated from the female axilla. While the Firmicutes were evenly isolated from all samples, the order Bacillales was predominantly isolated from both the kit blank control and female blank swab control. The family and subsequent taxa of Bacillaceae was predominantly recovered from the kit blank control, while the family and subsequent taxa Planococcaceae were isolated from the female blank control. There were other taxa groups which were predominantly recovered from the control groups, *Lactobacillus ingluviei, Lactobacillus johnsonii* and *Lactobacillus aviarius* were predominantly isolated from the male blank swab control, while *Lactobacillus mucosae* was only isolated from the female blank swab control and kit blank swab control. Therefore, if a large percentage of recovered bacteria from a sample contain taxa which were predominantly isolated from a control group, then it could be deduced that these were likely from background contamination.

7.24.1 The Sample from the Female Forehead was not Highly Contaminated

With the different control OTUs removed from the sample, the forehead was the least contaminated of the female samples, between 2.35-12.9% of the original sample was contaminated (Table 15). The abundance of phyla recovered from the female forehead included 80.3% Actinobacteriota, 19.7% Firmicutes and 0.02% Proteobacteria (Figure 46). The small percentage of Proteobacteria would have likely come from the kit blank control, as only the order Pseudomonadales was identifiable which was predominantly found in the kit. From the phyla Firmicutes, neither the likely contaminant species *Lactobacillus mucosae* nor any of the order Bacillales were detected in the sample.

7.24.2 The Sample from the Female Volar Forearm had Higher Contamination Levels

The percentage of the volar forearm contamination was between 18.1-62.1% contaminated, the highest contamination percentage of any sample, male or female (Table 15). The sample from the female volar forearm had a higher percentage, 5.02%, of Proteobacteria identified from the sample Figure 47. This included the species *Moraxella osloensis* and genus *Acidovorax*, both predominantly isolated from the female blank swab control and kit blank control. From the phylum Firmicutes, the order Bacillales was also detected in the sample, likely to have come from the kit blank control as both *Bacillus thermoamylovorans* and *Bacillus lentus* were identifiable. Other samples with high Proteobacteria percentage included the female axilla (54.8%), the male forearm (9.1%) and the female forearm (5.02%), for all other phylogenetic trees *see appendix*.

Table 15. Percentage of Sample Contaminated after Removal of Controls

	Sample Percentage Contaminated							
Controls Removed	**Male Forearm**	**Male Forehead**	**Male Navel**	**Male Axilla**	**Female Forearm**	**Female Forehead**	**Female Navel**	**Female Axilla**
Male Control	17.3%	5.70%	27.7%	4.11%	42.4%	2.35%	23.2%	15.3%
Female Control	14.2%	6.93%	18.4%	4.29%	22.2%	9.15%	13.7%	8.45%
Kit Blank Control	10.2%	3.79%	11.5%	4.22%	18.1%	2.35%	9.47%	8.29%
Male + Kit	26.8%	8.77%	37.3%	7.73%	56.5%	4.28%	30.0%	22.7%
Female + Kit	22.0%	8.84%	24.3%	7.15%	30.7%	11.2%	18.3%	14.0%
Male + Female + Kit	37.5%	13.5%	46.3%	10.2%	62.1%	12.9%	37.1%	26.2%

Figure 45 Phylogenetic Tree for all Samples.
Phylogenetic tree for all species highlights the groups which come from background contamination. Majority of the phylum Proteobacteria come from the contamination in the kit, while the order Bacillales is likely a joint contamination from the kit and the female blank swab. Some of the Lactobacillus spp. contaminants came from the male blank swab.

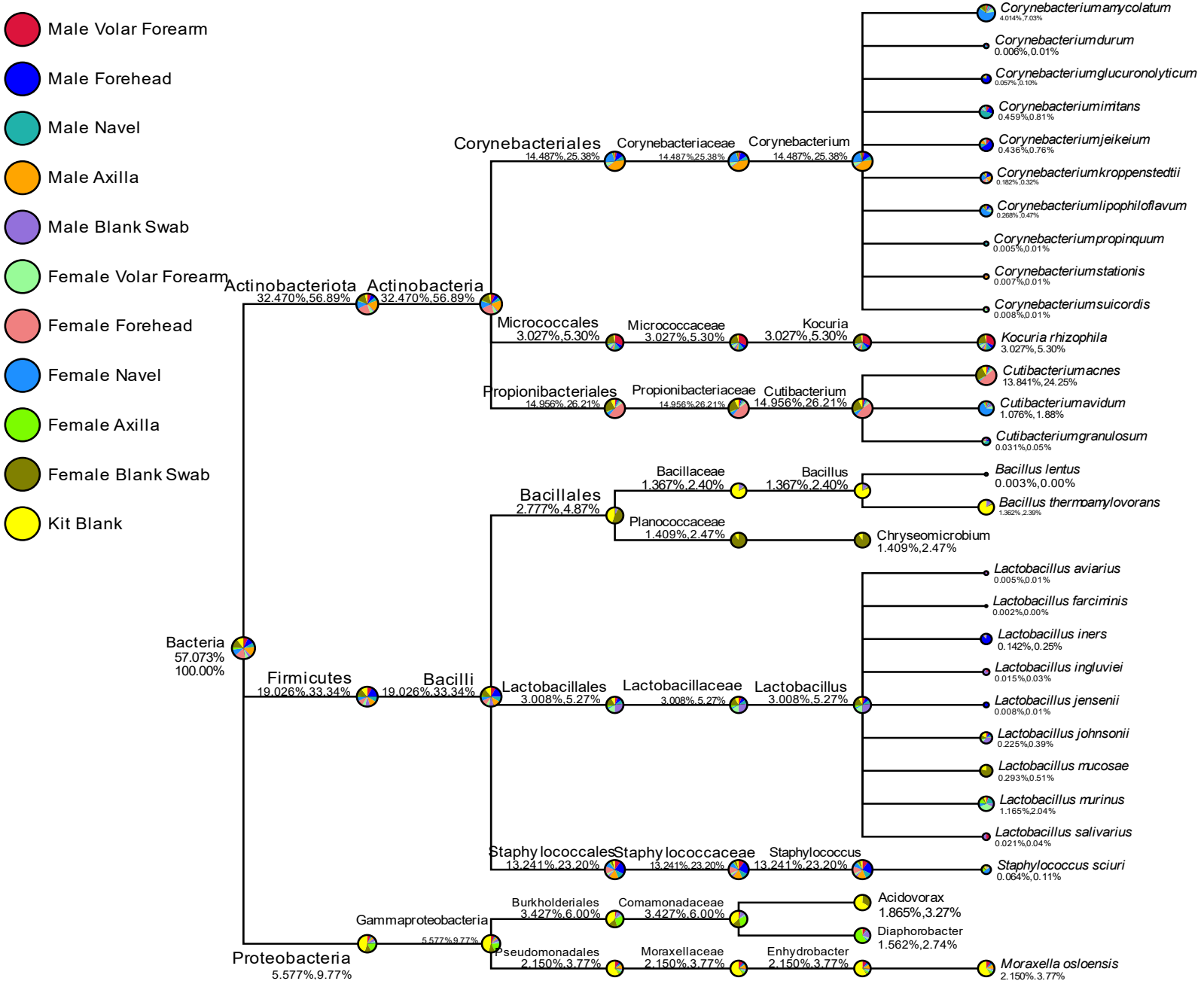


Figure 46. Phylogenetic Tree of the Identified Bacteria from the Female Forehead. The female forehead contained the least abundance of, the likely to be the main contaminant phylum Proteobacteria of any of the samples. Taxa in red were not detected in this sample.

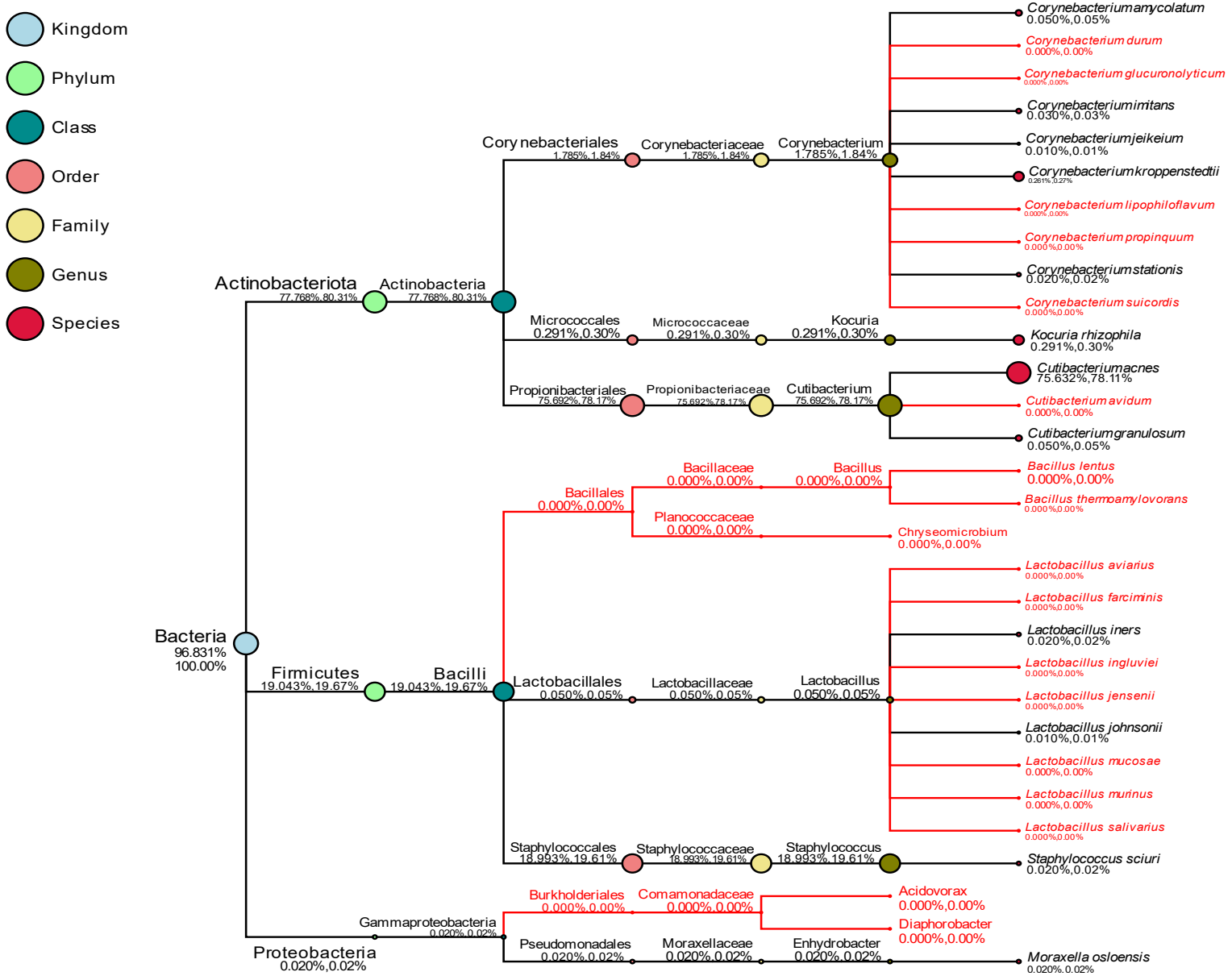
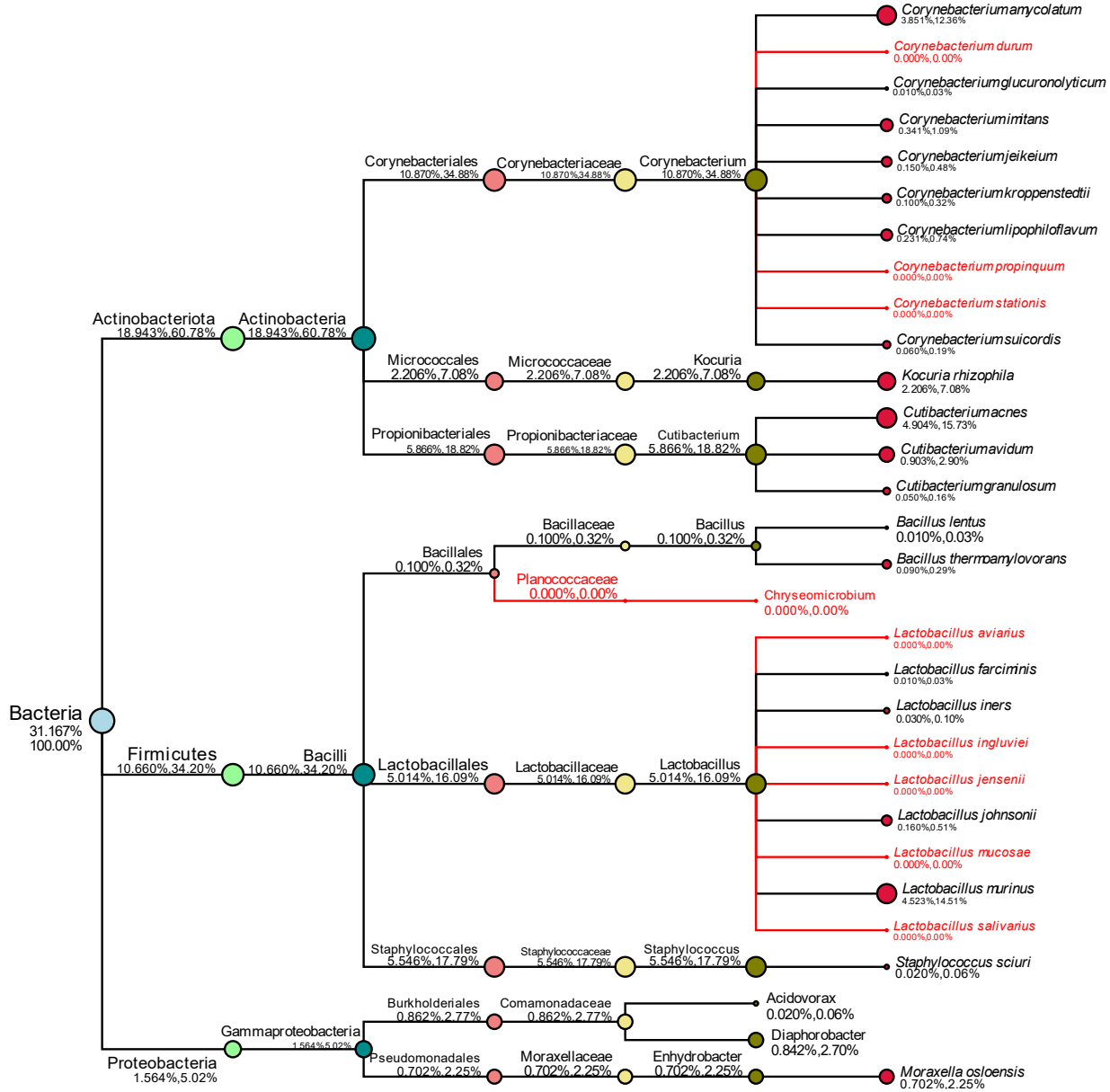


Figure 47. Phylogenetic Tree of the Identified Bacteria from the Female Forearm. The female forearm had 250 times more percentage of Proteobacteria isolated compared to the female forehead. The order Bacillales was also identified which was likely another background contaminant. Taxa in red were not detected in this sample.



7.25 Discussion

The site of swabbing does affect the relative background contamination and therefore the null hypothesis can be rejected.

7.26 Ideal Number of Volunteers

Due to the pandemic regulations, the initial samples were taken in a non-laboratory setting and with only two volunteers. While undertaking the study would have been more appropriate in a laboratory setting, the ideal participant number was not so obvious. A range of issues have to considered when deciding the correct sample size for a skin studies including logistical, ethical, budgetary and time constraints (Martínez-Mesa et al. 2016). As this was a small study, with one person undertaking the sampling and initial protocol steps, then the volunteer numbers required would be lower, this would also allow for the time constraints and small budget. However, too small of a participant size could hinder statistical analysis and not provide a suitable conclusion to the study (Faber and Fonseca 2014). A prior study, comparing swabbing and tape-stripping to retrieve skin microbes, was able to conclude their results having used only three men and four women (Ogai et al. 2018), while another study, which had investigated skin microbes at different body sites, was able to successfully draw conclusions using 17 participants (Timm et al. 2020). Therefore, a participant size of around 10 would have been appropriate for this study.

7.27 Likely Background Contaminants

There are always going to be background contaminants when working with microbial communities, even more so in low-biomass habitats like skin. Contamination of samples can come from the collection devices, the air around, the reagents in the kit and further along the process (CDC 2003). *Acidovorax, Enhydrobacter, Lactobacillus* and *Bacillus* were genera in which detection was predominantly found in the male blank swab control, female blank swab control or kit blank control (Figure 45), they have also been isolated from other negative control groups from previous microbiome studies (Eisenhofer et al. 2019). However, just because the contaminants mentioned are found predominantly on the control samples, it is possible that they can reside on skin, previous studies have found Proteobacteria such as *Enhydrobacter* and *Acidovorax* are naturally a component of the microbial community (Cosseau et al. 2016). If Proteobacteria were present on skin naturally, then the levels may have been too low compared to the controls and the dominant skin genera. While there are no records of background contamination by *Chryseomicrobium* spp., this genus is most likely to be isolated from aquatic ecosystems, deserts and from soil ejected from an earth worm's digestive tract (Shivaji et al. 2014; Saha et al. 2018), and therefore unlikely to part of the skin microbial community. The genera that also were isolated from the control samples included known skin bacteria, with over 50% of the genera isolated from the female blank control swab being *Cutibacterium*, *Staphylococcus* and *Corynebacterium*. Again, these common skin genera have been found in numerous experimental controls, all three have

been linked to being high abundance contaminants of DNA extraction and amplification kits (Salter et al. 2014; Glassing et al. 2016) and unsurprisingly contamination stemming from microbial laboratories (Weyrich et al. 2019).

7.28 Were Transient Bacteria Present?

Some of the bacteria found on skin during this study may not be classed as normal skin bacteria. These taxa may also not have come from the background controls, and instead could be classed as temporary visitors to the skin, also known as transient bacteria (Kong et al. 2012). Transient species do not proliferate on skin but can multiply if the conditions are favourable, but due to sequencing limitations, these organisms could also be dead (Byrd et al. 2018). Transient species can be transferred to skin from other areas of the body, the surrounding environment or even from pets (WHO 2009). *Staphylococcus sciuri*, was found in the control samples (Figure 45) but there is little research on it as to its contamination status and was present in the sample which collected most bacterial mass (Figure 46). The largest abundance of *S. sciuri* were found at the female axilla and female navel. *S. sciuri* is predominantly part of several animal's microbiota and has been detected in the oral mucosa of cats, an animal which both participants are in direct contact with (Shida 2009; Otto 2010). *Lactobacillus iners* was also detected on the male forehead at a relative abundance of 2.22% (see appendix, Section 10.3), this species is more closely associated with the vaginal microbial community (Jakobsson and Forsum 2007; Petrova et al. 2017). *Kocuria rhizophila* was predominantly isolated form the male forearm as well as the female blank swab control. While the genus *Kocuria* has many species which are resident bacteria, *Kocuria rhizophila* is more associated with the soil, but has been isolated from mostly the skin of males (Takarada et al. 2008; Khayyira et al. 2020). *K. rhizophila* could possibly be classed as both transient and residential on human skin (Johnson et al. 2002), very similar to how *S. aureus* was previously classed (Cogen et al. 2008; Otto 2010; Bondurant et al. 2020).

7.29 Contaminants influenced the Diversity Indices

Due to the low biomass collected from the skin, the overall samples from both male or female were not individually significantly less or more diverse than the blank control group (Table 11,

Table 11. Tukey and Wilcox Statistical results of the Overall Alpha Diversity Indices of the Male and Female Participants

Table 12, **Error! Reference source not found.**). Also, although not significant from each other or the blank controls, the diversity difference between the male and female groups were more

Alpha Index	Tukey	Wilcox
	P-Value	
Observed Species	0.8144609	0.7525
Shannon Diversity Index	0.9982553	0.3333
Gini-Simpson's Diversity Index	0.8340474	0.8847
Chao1 Diversity Index	0.6326259	0.3463
ACE Diversity Index	0.6335160	0.3010
Good's Coverage	0.4913804	0.2802
Phylogenetic Diversity	0.9588387	0.9027

diverse than between both sexes' groups and the blank control groups (Table 11).

Background contamination likely skewed the diversity results for the female axilla sample. The observed number of species for the male axilla was 147, while the female axilla observed 495. The male axilla was made up of more than 75% of the genera *Corynebacterium* and *Staphylococcus* (Figure 43), while more than 75% of the female axilla is made up of the least abundant genera grouped as others. The abundance of this 'other' group skewed the α-diversity indices to result in higher diversity within the female axilla sample compared to the male sample. The skewed results are backed up by previous work, in which less than 20% of the phylum Proteobacteria, which many of the 'other' genera are classed, were found at the axilla site of both sexes (Li et al. 2019b). Less obviously, were the skewed evenness of taxa found at the site of both sexes' volar forearms. As a dry location, more diversity is to be expected at the volar forearm than perhaps elsewhere (Table 10). While the distribution of represented phyla was alike previous work, with less Proteobacteria if anything, the relative abundance of the top genera differed. Figure 43 shows the detected genera of *Cutibacterium*, *Staphylococcus* and *Corynebacterium*, together covered around 25% of the male volar forearm abundance and a little less for the female sample. The same three genera of previous work averaged an abundance coverage of 46.3% (Gao et al. 2007). Therefore, the reduced relative abundance of the expected top genera projected a more even distribution of taxa and therefore more diverse samples.

7.30 Removal of Controls May Not Be So Easy

Using a multitude of negative controls and even positive controls can help eliminate the background contamination of samples (Kim et al. 2017b). Both males and female samples were taken together, but the decision was to use two blank swab controls dipped into the buffer for the study. Had this been an in-depth investigation, this would have very useful for the removal of contamination as there were differences in the numbers of OTUs and observed species for the male blank swab control was 672 and 589 respectively while the female blank swab control only had 208 OTUs and 172 observed species (Figure 41, Table 10). The diversity distance between the samples was also one of the longest with an Unifrac distance of 0.426 for weighted and 0.725 for unweighted. Removal of the different controls resulted in different contamination percentage, while the male axilla had a relatively small range of percentage contaminations, between 4.11-10.2%, the female axilla sample had a much larger percentage range between 8.29-26.2%. However, as there were such differences between the controls, this leaves questions as to whether the background contamination of the sample swabs so different. Many papers do not give in-depth descriptions of negative controls and previous work has presented results in which the data was not clearly distant from the contaminations. One of the main problems with microbial sampling is the lack of what is classed as true negative control e.g. a swab waved in the air is not really an accurate air control, or one swab's microbial composition might not reflect all swabs (Hornung et al. 2019). Kit contamination was also recorded from the above investigation (Figure 43). Salter (et al. 2014) found that not only do different kits have varied contamination, but that different batches of the same kit can have significant differences which can influence the microbial community resolution of low-biomass samples. Further potential issues come from choice of bioinformatics, problems reported included background contaminants missed, data skewed and contradictory outcomes (Glassing et al. 2016; Goig et al. 2020; Bharti and Grimm 2021) The way to reduce these problems would be to use multiple negative and positive controls, training for consistent technique, limit differing consumable batches and where possible, aim for sample sites with the greatest biomass available (Harris 2008; Eisenhofer et al. 2019).

7.31 Microbial Communities and Biomass at Different Swab Sites

Both the female and male navel samples had high background contamination due to the low biomass collected from the site, up to 46.3% of the samples had contamination from the control groups. The microbial composition of the navel has previously been labelled as diverse yet 'predictable' (Hulcr et al. 2012). Domination by the classic skin genera of *Corynebacterium* and *Staphylococcus* were seen in the results (Figure 43), as well as the presence of not-so-commonly found on skin *Finegoldia* and *Anaerococcus* (see appendix). The species which were found in this study were all consistent with prior research into the bacterial composition of the navel (Hulcr et al. 2012). If considering a study of the navel microbial community, be sure to use plenty of

background controls to cover the contaminating OTUs. Biomass retrieval from the axilla varied somewhat, with contamination of the male axilla being the lowest of all the sample at between 4.11-10.2%. The female axilla, while still relatively low, contamination for the sample was more contaminated than the male equivalent 8.29-26.2% (Table 15). The male axilla was dominated by the genus, *Corynebacterium* (Figure 43) while the female axilla was not and had much higher diversity. Men have been found to harbour more *Corynebacterium* than women, a genus associated with axillary odour (Troccaz et al. 2015). Women are more likely to shave their axilla (Basow 1991) and hair removal of the axilla have been shown to reduce malodour, which could explain the reduced *Corynebacterium* (Lanzalaco et al. 2016). Female axilla also had *Lactobacillus* present, which has also been previously identified in female axilla (Anukam et al. 2020). Many past cosmetic studies have used the FCAT technique, as it was assessed that the volar forearm was interchangeable with the face (Ertel et al. 1995; Farage 2000). This may have been true for hydration retention and biochemical processes but as the above in 7.23, and prior research have shown, the lack of biomass and bacteria recovered were not akin to the microbial community of the face. As the volar forearm is a dry area of skin, this would make it much more diverse compared to the oily face (Grice and Segre 2011). Prior work found that the forehead genera was dominated by either *Cutibacterium*, as seen with the female forehead sample (**Figure 43**), or has a large percentage of *Corynebacterium*, as seen with the male forehead sample (**Figure 43**). While the sampling of individuals' forearms resulted in much more variable ratios of different taxa, including larger proportions of Lactobacillales and Proteobacteria, as seen in this study with both male (see appendix) and female samples (Figure 45) (Staudinger et al. 2011). The biomass collected from the sampled areas also differed. Both the male forehead sample, and female forehead sample resulted in many more useable sequencing reads than the corresponding volar forearm samples (Figure 41). The volar forearm samples also contained a higher relative abundance than the forehead samples of background contamination (Table 15), the female volar forearm sample had the highest contamination of any sample, between 18.1-62.1%. The male forearm sample also had a high abundance of background contamination, between 10.2-37.5%. If future cosmetic studies wish to investigate a facial product, while the volar forearm could be used for traditional formulation testing, any microbial community investigations should be done on the area in question or more research done into areas with similar biomass and composition.

7.32 What about Shotgun Metagenomic Sequencing?

Staphylococcus spp. are widely regarded as top inhabitants of the skin (Otto 2010; Grice and Segre 2011), yet only *S. sciuri* was identified to species level using the 16S rRNA gene sequencing. This amplification technique has its limits with such closely related species and cannot always distinguish between *Staphylococcus* spp. which reside on the skin (Ghebremedhin et al. 2008). Again, in the above study *Corynebacterium* spp. constituted 25% of the bacteria present, when

only 9.5% were identified to species level (Figure 45). There can also be different representation of microbial communities dependent on hypervariable region covered or primer choices (Johnson et al. 2019) 16S rRNA sequencing is still a useful tool in small microbial community studies, to have an idea of what a mixed sample contains, or studies that are not human-associated microbiome based (Poretsky et al. 2014; Ames et al. 2017).

Shotgun Metagenomic Sequencing fragments all the given genomic DNA, sequences it and compares to a reference genome database. This technique covers all genetic information across the three domains so studies can include fungi and archaea instead of focusing on bacteria as with 16S rRNA sequencing (Durazzi et al. 2021). The taxonomic resolution can determine species or strain level, metabolic functions can also be investigated or identification of antimicrobial resistance genes (Hamady and Knight 2009). There are a few disadvantages to this technique, as a reference database is required to compare sequences to, if the genome is not available there would be a high chance of false positive species. Also, due to the cross-domain identification, there is a risk of host DNA interfering with the results (Cattonaro et al. 2018). There is also cost to consider, the cost of shotgun metagenomic sequencing can be two to three times more costly than 16S rRNA sequencing, which can get expensive if there is a large sample size.

7.33 Conclusion

The skin has low microbial biomass, but different areas are better for bacterial recovery than others. For instance, oily areas may be the least diverse, but the bacterial mass is much higher than dry or moist areas. Using areas with higher bacterial biomass for skin research can help reduce the relative background contamination from the surroundings, equipment, and consumables. To help identify background contaminants for skin experiments, multiple controls would be required, this includes equipment, kit, and consumable controls as well as a positive control, something scarcely used previously.

Previous skin research techniques may not translate well for studies which would include microbes. The forearm-controlled application technique (FCAT), for example, uses the forearm as a suitable control for the face and rest of the body, while in terms of microbial mass and species found on the forearm can be significantly different to the face.

Future microbial studies would also need to decide how in-depth the study should be, species level recovery can be limited with 16S rRNA sequencing, especially between closely related species like found in the *Staphylococcus* genus. There are more in-depth and accurate culture-independent techniques but the cost increases with accuracy.

8 Conclusions of Sections 2-7

8.1 Introduction

The microbial community of skin is a recent area of research compared to other areas of the human body. With the increased prevalence of skin disorders, cosmetic companies have also started to investigate and formulate products which claim to either sustain the microbial balance or promote good bacteria. However, many of these products contain ingredients which could both provide nutrients and prevent growth of the skin residents. Therefore, many cosmetic products could be counterintuitive and imbalance a healthy microbial community. The promotion of 'good' bacteria could also be problematic, *S. epidermidis* can help reduce the adherence and infection of its more pathogenic cousin *S. aureus*, but itself is an opportunistic pathogen and can cause serious nosocomial infections. Before labelling these products with any microbe-related claims, thorough investigations into their potential interactions with skin microbiota is vital. This thesis focused on cosmetic proteins, peptide rich moisturising agents, and their interactions with common skin bacteria, however many of these in-vitro experiments could use different formulation ingredients. The work started with creating laboratory-based studies which used minimal nutritional medium and investigated how the cosmetic proteins affect the fundamentals of the species, including both planktonic and sessile growth. Then moved onto experimental design using tissue culture, a popular alternative to the out-dated animal trials. Before finally investigating suitable devices for retrieving bacteria from skin and locations for commencing human panel studies.

8.2 Laboratory-based Studies

Lab-based work was never going to accurately create conditions which reflect the skin but were useful to study how cosmetics affect the fundamentals of skin species. Lactose-free skimmed medium helped identify species isolates that could be promoted if the cosmetic proteins contained large peptides. The use of a minimal medium with a pH lowered to 5 created an environment more akin to skin compared to the commonly used high nutritional media. The minimal medium also balanced the conditions between the faster *Staphylococcus* spp. and slower growing *C. jeikeium*, as well as downregulating the pathogen *S. aureus*, which favours neutral pH. Section 3 highlighted how using a minimal medium to investigate skin bacteria 'levels the playing field' and allows for slower growing organisms to compete with the faster organisms. Unfortunately, a minimal medium agar (1-2% w/v) was either not able to support consistent growth or only small unusable colonies were formed on the surface (Section 2.8). Therefore, a minimal medium agar could be something in which future investigations could attempt. The reduced water content and air interface would increase the homology to the skin environment.

Preliminary work for Section 4 investigated if the preservative as found at formulation concentrations could have bactericidal or bacteriostatic effects on skin bacteria, as many of these species are also targeted as products contaminations. The cosmetic proteins were preserved with

different combinations of common preservatives, many of the are used to target skin species which are also formulation contaminants. While the minimum inhibitory concentration was unlikely to be used in products, the low concentration of preservatives still had detrimental effect on the skin species. Sodium benzoate and EDTA were the most effective at inhibiting the growth of all species but worryingly so, the pathogenic *Staphylococcus* spp. was less effected than the commensal *S. epidermidis*. Future research into cosmetic proteins could investigate whether dry, and therefore unpreserved, proteins affect this skin microbes. The lack of preservation of the dry cosmetic proteins could increase the total nutrition available, reduce the environmental stresses and cause imbalances by promoting the faster growing species.

Removal of the casamino acid supplement from the minimal medium and replacement with the varied active level of cosmetic proteins showed whether the bacteria could utilise the cosmetic proteins as a source of nutrition and nitrogen for both planktonic and sessile growth. Without in-depth proteomic analysis, peptide size could not be determined, but the two extracellular protease possessing *Staphylococcus* spp., grew with more of the cosmetic protein at a range of active levels. The variation between cosmetic proteins was highlighted in this thesis, as each individual cosmetic protein had a varying effect on the growth of individual species. However, it was clear that Crosilk LPC, the protein with the highest concentration of preservatives, had a negative effect on all three species tested. Similar results were seen when the two *Staphylococcus* spp. were grown together, the species which grew best with a certain cosmetic protein was inclined to dominate in a mixed culture supplemented with that specific cosmetic protein. Future work could delve into producing more varied culture mixes, expand into different common genera on skin. Further investigations could also consider the expression of the extracellular proteases in planktonic growth when supplemented with cosmetic proteins.

The degree of biofilm formation did not necessary reflect the planktonic growth. At pH 5, *S. aureus* could grow well planktonically, but biofilm formation was poor. The poor biofilm formation of *S. aureus* was highlighted when co-cultured with *S. epidermidis,* in which the commensal dominated the biofilm percentage when grown with the minimal medium and when supplemented with cosmetic proteins. Again, further investigations could include producing biofilms with more skin microbiota involved and studying gene expression when in biofilm formation. The biofilm quantification experiments could also be moved from microwell plates onto a material more akin to skin.

8.3 Tissue Culture

The skin community does not just start and end with its microbial residents, but interactions with the skin cells and the immune response. Section 5 covered the experiments which involved with tissue culture. The skin naturally expresses a low level of immune responses to help recognition of

commensal species and be on alert for invading pathogens. HaCaT cells expressed these low levels of immune markers without being extra stimulated by the exposure to cosmetic proteins or bacteria or both. The addition of *S. epidermidis* however lowered the concentration of immune markers expressed by the HaCaT cells in the surrounding supernatant, sometimes to a negligible result. Exposure of *S. epidermidis* to the higher active level concentrations reduced the number of adhered cells, and the concentration of immune markers increased. However, the concentration of immune markers in the supernatant whether the HaCaT cells were alone or grown with *S. epidermidis*, were insignificant compared to when HaCaT cells were grown with *S. aureus*, in which the immune marker concentration significantly increased. The increased immune marker concentration was also noted with a co-culture of *Staphylococcus* spp. However, the number of adhered cells was so much lower, suggesting that *S. epidermidis* had now switched from reducing, to promoting these immune markers to help the cells with the *S. aureus* infection. This work also highlighted the importance of a low pH on the skin, if one species dominated the biofilm formation of the mixed co-culture, it was *S. aureus*. In previous Section 4.12*, S. epidermidis* was likely to dominate a mixed biofilm, but the pH was lower. Future work could investigate either lowering the pH of the experiment or taking it a step further and create a liquid air interface which would be much more akin to the skin. Again, increasing the genera in the mixed species experiments, including slower growing organisms such as *C. jeikeium* or species who thrive on lower oxygen levels like *Cutibacterium acnes*.

8.4 Designing a Human Panel Study

While not the most efficient way of recovering bacteria and bacterial DNA from skin, there will always be a need for swabbing skin as a non-intrusive method. Over the years different studies have used different swab types and buffers to remove bacteria and bacterial DNA from skin. In Section 6, a porcine skin model was inoculated with known quantities of *Staphylococcus* spp. before being recovered by three different swab and three different buffer combinations. Using any wet swab was better at recovering bacteria than any dry swab. Using a flocked swab recovered the most bacteria compared to a dacron or cotton swab.

Many cosmetic studies use the volar forearm as a comparable skin site to the face to test facial products. However, while comparable for hydration and moisturising studies, this may not be so for microbial studies. Using the best buffer and swab composition, microbes were removed from several different skin sites and sequenced using 16S rRNA. The volar forearm, a dry area of the skin, had some of the highest background contamination from both the swab controls and kit controls. The forehead samples, an oily area of the skin, had some of the least amount of background contamination. Both areas also had different dominating genera of bacteria. Section 7

highlighted that cosmetic studies will likely need to change their testing processes, as the volar forearm is not a good equivalence to the face for microbial composition and mass.

8.5 What Have all the Sections from this Thesis achieved?

With cosmetic companies moving into more studies involving microbes on the skin, new protocols are required to take these experiments from the fundamental lab work all through to skin panel studies. The basic, but essential investigations into both planktonic and sessile growth can help identify possible problematic ingredients that may promote pathogenic species or negatively affect commensal species. The tissue culture experiments are an alternative to the now very unpopular animal models, after all the cosmetics are going on the skin and the microbiome involves the cells which react to both bacteria which reside on the skin and cosmetics which are added. Finally, skin panel studies are used throughout industry to test the skin's properties after exposure to cosmetic ingredients, however, many of these practices do not translate well into microbial investigations. Areas of the skin which may have similar hydration properties may have completely different microbial inhabitants and biomass available. The final Sections emphasized which swabs and buffers, used in prior studies, were able to collect the most bacteria from skin, as well as where to target skin studies and how to overcome background contamination.

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10 Appendix

The following appendix is regarding Section 3 and 7. It contains the statistical information of the minimum inhibitory concentration from Section 3.21. It also contains the complete taxa trees for all samples, the complete list of abundance of OTUs per sample and signed consent forms from Section 7.

Appendix Contents

10.1 MIC Tables[14]

Table 16 A, B and C. Minimum Inhibitory Concentration Tables for A) *Staphylococcus aureus* B) *Staphylococcus epidermidis* and C) *Corynebacterium jeikeium*

A

Protein	Crosilk Protein Complex Preservatives (0.5% sodium benzoate + 0.01% disodium EDTA)				Hydroavena HPO Preservatives (0.5% sodium benzoate + 0.3% potassium sorbate)				Hydrolactin Liquid PE Preservatives (1% phenoxyethanol + 0.2% disodium EDTA)				Hydrosativum PE Preservatives (1% phenoxyethanol + 0.3% potassium sorbate)				Hydrosolanum PE Preservatives (1% phenoxyethanol + 0.3% potassium sorbate + 0.2% disodium EDTA)				Prolevium Preservatives (1% phenoxyethanol + 0.3% potassium sorbate + 0.2% disodium EDTA)			
Active level	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD
4%	99.3	100	99.7	0.33	92.5	93.6	93.2	0.51	88.9	99.6	70.5	5.36	13.4	25.1	18.6	5.96	62.3	67.7	64.6	2.80	60.7	69.6	64.9	4.48
2%	89.4	91.4	90.3	0.88	57.7	62.7	59.4	2.28	64.6	80.2	19.3	8.44	12.5	20.0	15.2	4.19	18.4	23.4	20.8	2.51	17.6	25.7	21.6	4.05
1%	39.7	45.4	43.5	2.60	19.1	21.2	20.2	0.93	13.5	28.0	16.3	7.65	10.5	17.0	12.9	3.54	15.7	20.4	18.1	2.35	16.5	20.1	18.2	1.82
0.5%	13.0	17.4	15.2	2.13	12.1	13.0	12.5	0.43	12.9	22.9	14.3	5.72	7.40	15.8	11.0	4.34	10.4	18.0	15.0	4.05	11.3	18.9	14.9	3.81
0.25%	8.04	12.9	10.6	2.12	9.83	10.1	10.0	0.13	11.6	19.2	11.4	4.23	4.40	13.5	8.93	4.55	7.9	14.5	11.8	3.48	8.9	15.8	12.9	3.58
0.125%	3.88	7.85	3.97	6.26	7.22	8.25	7.50	0.50	9.82	14.4	6.30	2.59	1.90	10.2	5.70	4.19	5.80	10.8	8.40	2.51	5.9	9.7	7.8	1.90
0.0625%	2.47	4.54	2.07	3.68	3.53	5.45	4.29	0.90	0.00	0.00	0.00	0.00	0.00	5.20	2.37	2.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

B

Protein	Crosilk Protein Complex Preservatives (0.5% sodium benzoate + 0.01% disodium EDTA)				Hydroavena HPO Preservatives (0.5% sodium benzoate + 0.3% potassium sorbate)				Hydrolactin Liquid PE Preservatives (1% phenoxyethanol + 0.2% disodium EDTA)				Hydrosativum PE Preservatives (1% phenoxyethanol + 0.3% potassium sorbate)				Hydrosolanum PE Preservatives (1% phenoxyethanol + 0.3% potassium sorbate + 0.2% disodium EDTA)				Prolevium Preservatives (1% phenoxyethanol + 0.3% potassium sorbate + 0.2% disodium EDTA)			
Active level	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD
4%	100	100	100	0.00	97.7	99.1	98.3	0.62	97.4	98.3	97.7	0.50	20.5	23.3	21.6	1.50	93.9	95.6	94.7	0.84	93.5	95.9	94.3	1.39
2%	95.7	100	97.6	2.08	61.5	65.3	62.9	1.63	34.1	51.0	42.5	8.41	11.6	13.2	12.5	0.86	38.3	49.6	44.7	5.81	44.2	50.1	47.4	2.97
1%	42.3	52.1	46.3	4.16	15.0	18.3	16.9	1.42	21.4	28.8	25.3	3.72	8.34	12.3	10.4	1.97	13.9	20.4	17.8	3.48	18.7	22.2	19.9	2.03
0.5%	10.8	14.5	12.7	1.54	7.83	11.5	9.38	1.62	12.7	19.1	16.7	3.46	6.22	11.4	8.88	2.57	1.72	15.2	9.19	6.87	5.55	9.88	7.32	2.27
0.25%	3.31	6.10	4.56	1.16	3.80	5.71	4.77	0.78	11.8	15.4	13.9	1.90	4.43	10.8	8.06	3.28	0.00	9.47	3.16	5.47	1.45	4.78	3.29	1.69
0.125%	1.01	3.97	2.60	1.38	2.34	4.08	1.74	0.81	10.1	12.8	11.5	1.38	3.60	10.4	6.40	3.56	0.00	7.86	2.62	4.54	0.75	2.43	1.43	0.88
0.0625%	0.00	2.71	1.23	1.24	0.68	1.54	1.03	0.40	7.59	9.43	8.27	1.01	2.15	9.48	5.29	3.78	0.00	6.45	2.15	3.73	0.00	0.00	0.00	0.00

C

Protein	Crosilk Protein Complex Preservatives (0.5% sodium benzoate + 0.01% disodium EDTA)				Hydroavena HPO Preservatives (0.5% sodium benzoate + 0.3% potassium sorbate)				Hydrolactin Liquid PE Preservatives (1% phenoxyethanol + 0.2% disodium EDTA)				Hydrosativum PE Preservatives (1% phenoxyethanol + 0.3% potassium sorbate)				Hydrosolanum PE Preservatives (1% phenoxyethanol + 0.3% potassium sorbate + 0.2% disodium EDTA)				Prolevium Preservatives (1% phenoxyethanol + 0.3% potassium sorbate + 0.2% disodium EDTA)			
Active level	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD	Min inhibition	Max inhibition	Mean inhibition	SD
4%	84.0	89.9	86.3	3.13	85.3	90.7	87.4	2.90	64.4	70.6	67.3	2.83	68.2	71.4	69.9	1.38	46.1	62.8	55.6	8.57	30.5	40.4	34.2	5.54
2%	37.7	44.7	41.4	3.5	43.2	50.8	46.4	3.95	39.9	66.8	48.3	12.4	31.6	46.2	42.2	7.12	39.2	57.1	45.3	10.2	29.2	31.6	30.4	1.22
1%	21.9	42.1	31.0	10.3	35.6	45.4	39.7	5.11	36.7	42.3	39.5	2.37	31.1	44.7	41.0	6.56	33.0	52.8	41.2	10.4	18.6	23.0	21.2	2.27
0.5%	18.3	36.5	27.6	9.11	28.2	39.1	33.8	5.47	29.9	38.3	34.1	3.46	11.1	41.5	32.7	14.5	31.5	37.7	34.3	3.17	17.8	20.2	19.4	1.39
0.25%	16.0	31.1	22.5	7.80	25.1	30.1	27.1	2.68	28.7	37.8	31.6	4.16	8.33	20.7	12.8	5.50	23.0	35.3	28.4	6.27	14.9	17.4	16.1	1.26
0.125%	7.08	9.93	8.74	1.48	11.8	17.5	14.3	2.87	22.7	29.3	27.0	2.98	2.91	7.57	5.3	2.29	14.1	21.0	17.4	3.50	11.7	15.5	13.3	2.02
0.0625%	0.00	4.81	2.28	2.41	6.11	9.69	7.96	1.79	11.1	22.7	15.2	5.37	0.35	2.97	1.56	1.15	4.63	19.3	12.2	7.34	3.15	7.90	5.91	2.45

[14] The red numbers indicate where the minimum inhibitory concentration had been achieved

10.2 Consent Forms

CONSENT FORM

Title of research project:
Chapter in PhD Thesis
Microbial Communities of Different Swab Sites

Name of Chief/Principal Investigator:
Supervisor: Julian Marchesi
PhD Candidate: Hannah Salvage

	Please initial box
I confirm that I have understand how the research will be undertaken and how I need to participate.	HS
I confirm that I have understood the information provided for the above research project and thatI have had the opportunity to ask questions and that these have been answered satisfactorily.	HS
I understand that my participation is voluntary, and I am free to withdraw at any time without giving a reason and without any adverse consequences (e.g. to medical care or legal rights, if relevant). I understand that if I withdraw, information about me that has already been obtained may be kept by Cardiff University.	HS
I understand who will have access to personal information that I will have provided, how the data willbe stored and what will happen to the data at the end of the research project.	HS
[IF RELEVANT] I understand that after the research project, anonymized data may be made publicly available via a data repository and may be used for purposes not related to this research project. I understand that it will not be possible to identify me from this data that is seen and used by other researchers, for ethically approved research projects, on the understanding that confidentiality will be maintained.	HS
[IF RELEVANT] I consent to being audio recorded/ video recorded/ having my photograph taken for the purposes of the research project and I understand how it will be used in the research.	N/A
[IF RELEVANT] I understand that anonymized excerpts and/or verbatim quotes from my [INTERVIEW/QUESTIONNAIRE ETC] may be used as part of the research publication.	HS
I understand how the findings and results of the research project will be written up and published.	HS
I agree to take part in this research project.	HS

Version xx [DATE]

[redacted]

PhD Candidate
Role of person taking consent
(print)

THANK YOU FOR PARTICIPATING IN OUR RESEARCH

Figure 48. Copy of Consent Form for the Female Participant

CONSENT FORM

Title of research project:
Chapter in PhD Thesis
Microbial Communities of Different Swab Sites

Name of Chief/Principal Investigator:
Supervisor: Julian Marchesi
PhD Candidate: Hannah Salvage

	Please initial box
I confirm that I have understand how the research will be undertaken and how I need to participate.	DT
I confirm that I have understood the information provided for the above research project and thatI have had the opportunity to ask questions and that these have been answered satisfactorily.	DT
I understand that my participation is voluntary, and I am free to withdraw at any time without giving a reason and without any adverse consequences (e.g. to medical care or legal rights, if relevant). I understand that if I withdraw, information about me that has already been obtained may be kept by Cardiff University.	DT
I understand who will have access to personal information that I will have provided, how the data willbe stored and what will happen to the data at the end of the research project.	DT
[IF RELEVANT] I understand that after the research project, anonymized data may be made publicly available via a data repository and may be used for purposes not related to this research project. I understand that it will not be possible to identify me from this data that is seen and used by other researchers, for ethically approved research projects, on the understanding that confidentiality will be maintained.	DT
[IF RELEVANT] I consent to being audio recorded/ video recorded/ having my photograph taken for the purposes of the research project and I understand how it will be used in the research.	N/A
[IF RELEVANT] I understand that anonymized excerpts and/or verbatim quotes from my [INTERVIEW/QUESTIONNAIRE ETC] may be used as part of the research publication.	DT
I understand how the findings and results of the research project will be written up and published.	DT
I agree to take part in this research project.	DT

Version xx [DATE]

[redacted]

PhD Candidate
Role of person taking consent
(print)

THANK YOU FOR PARTICIPATING IN OUR RESEARCH

Figure 49. Copy of Consent Form for the Male Participant

10.3 Taxonomic Trees

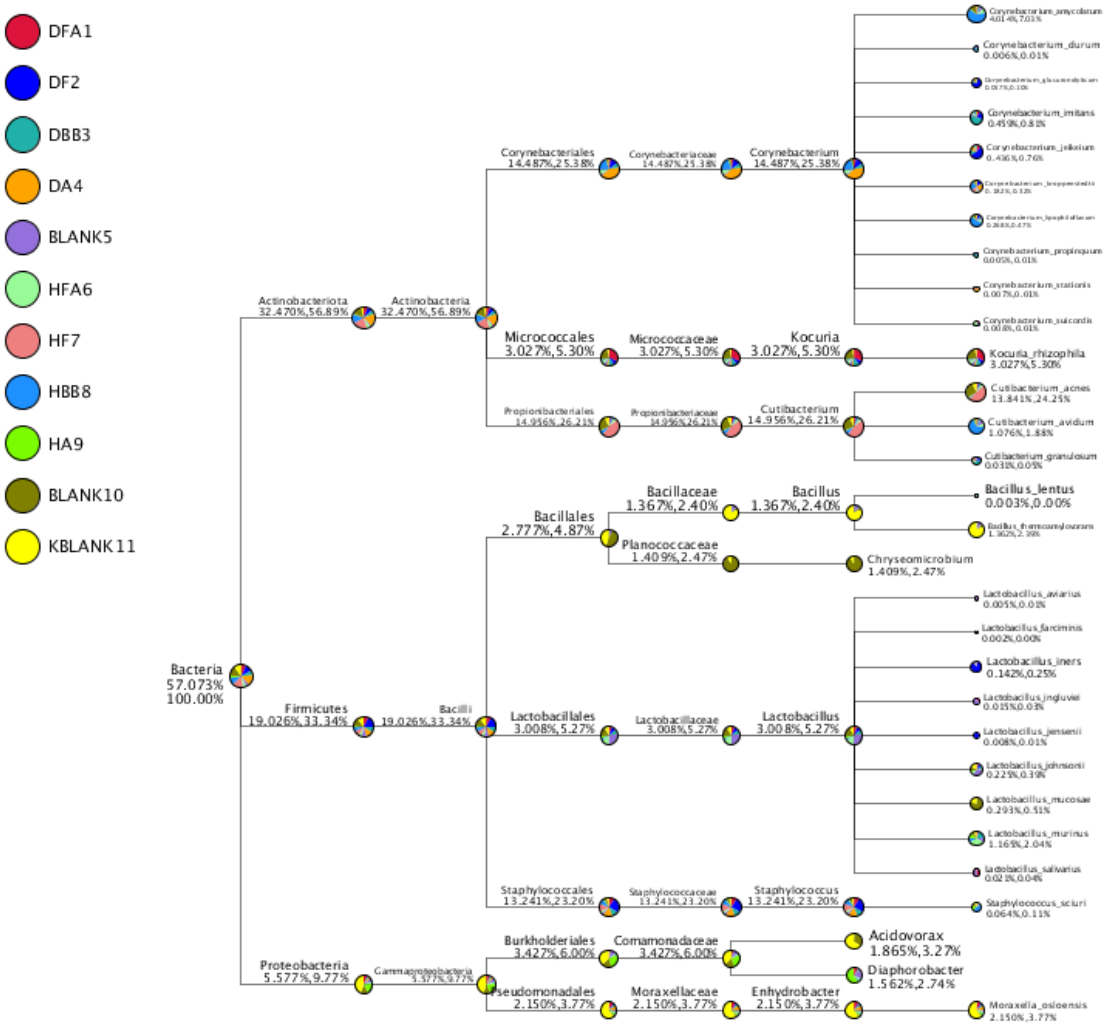

Figure 50. Taxa Tree of all Samples.

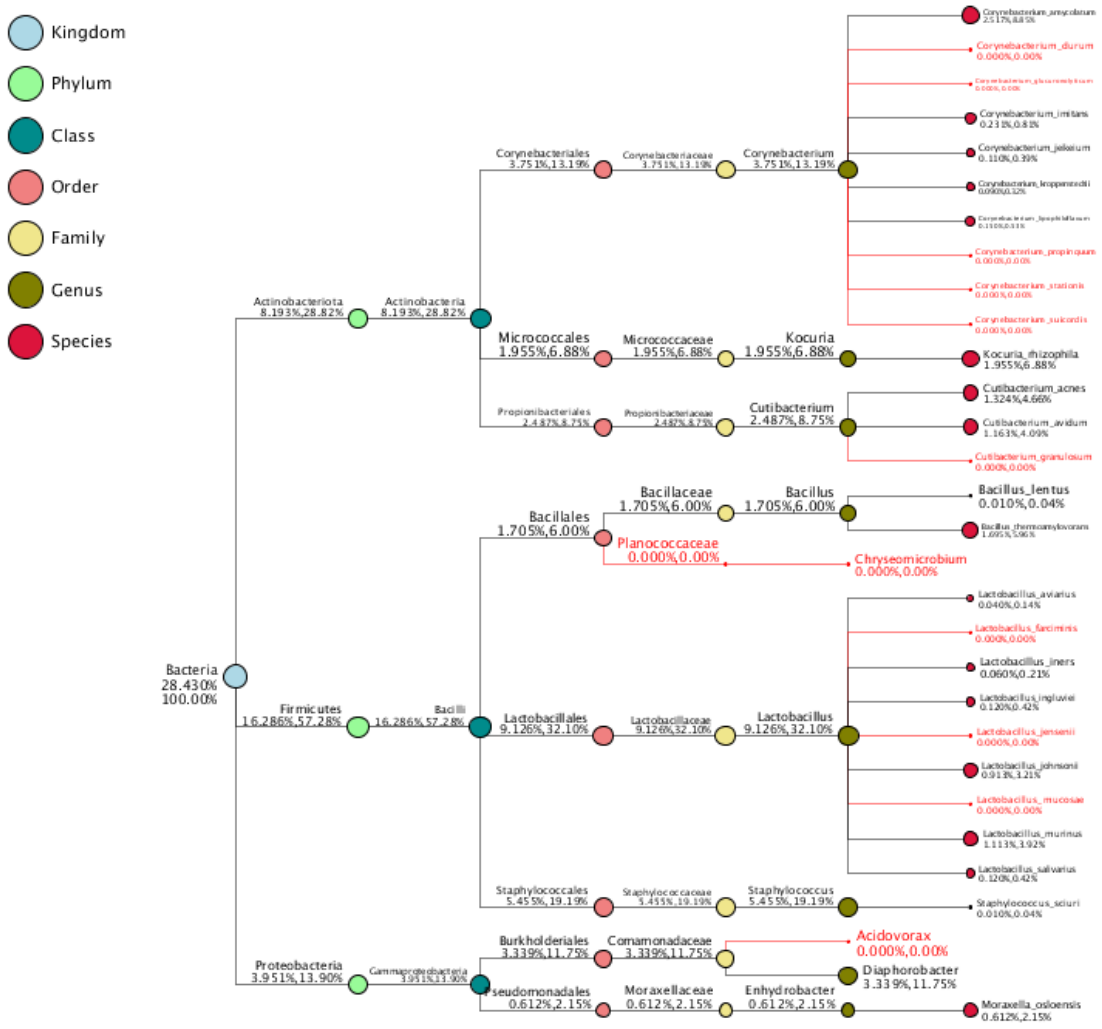

Figure 51. Taxa Tree for the Male Blank Swab Control.

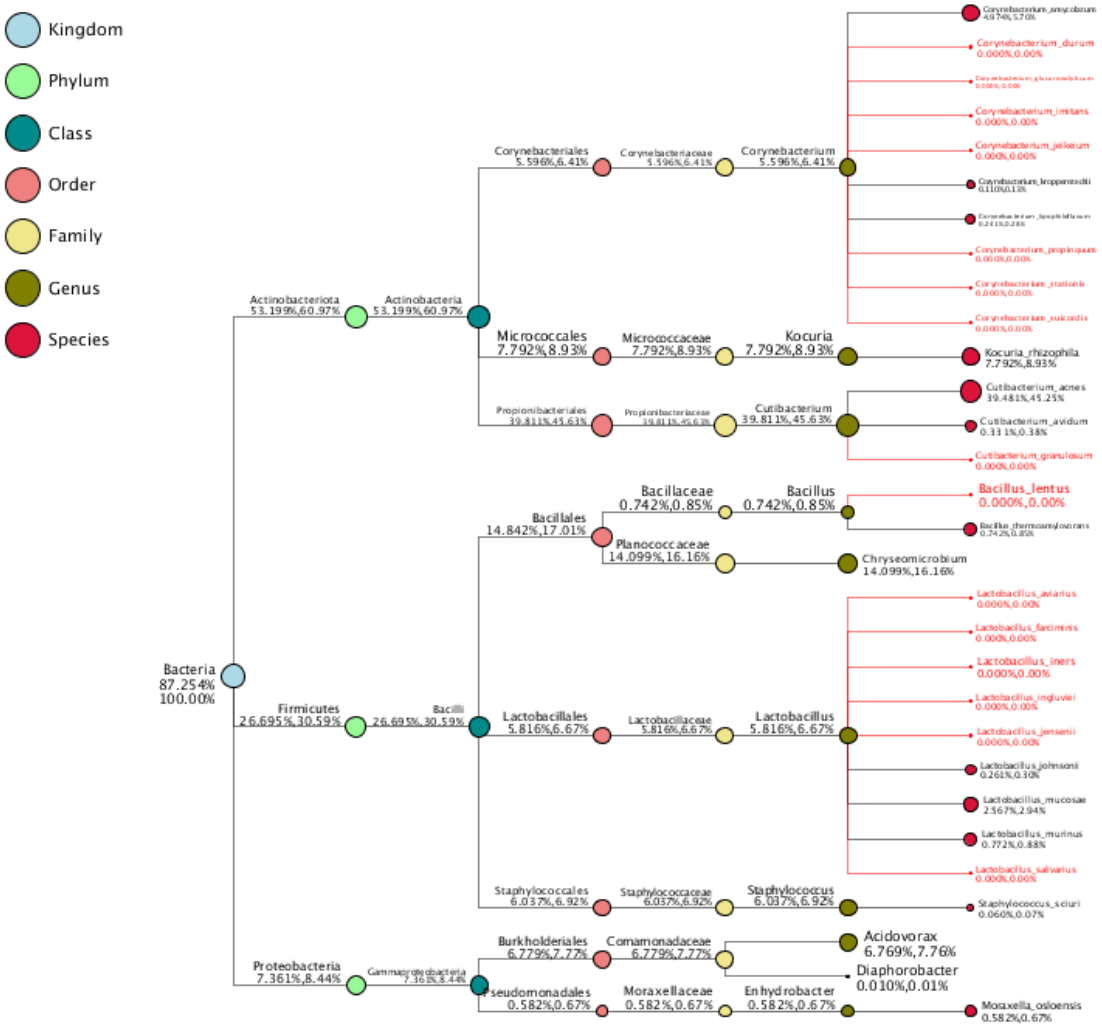

Figure 52. Taxa Tree for the Female Blank Swab Control.

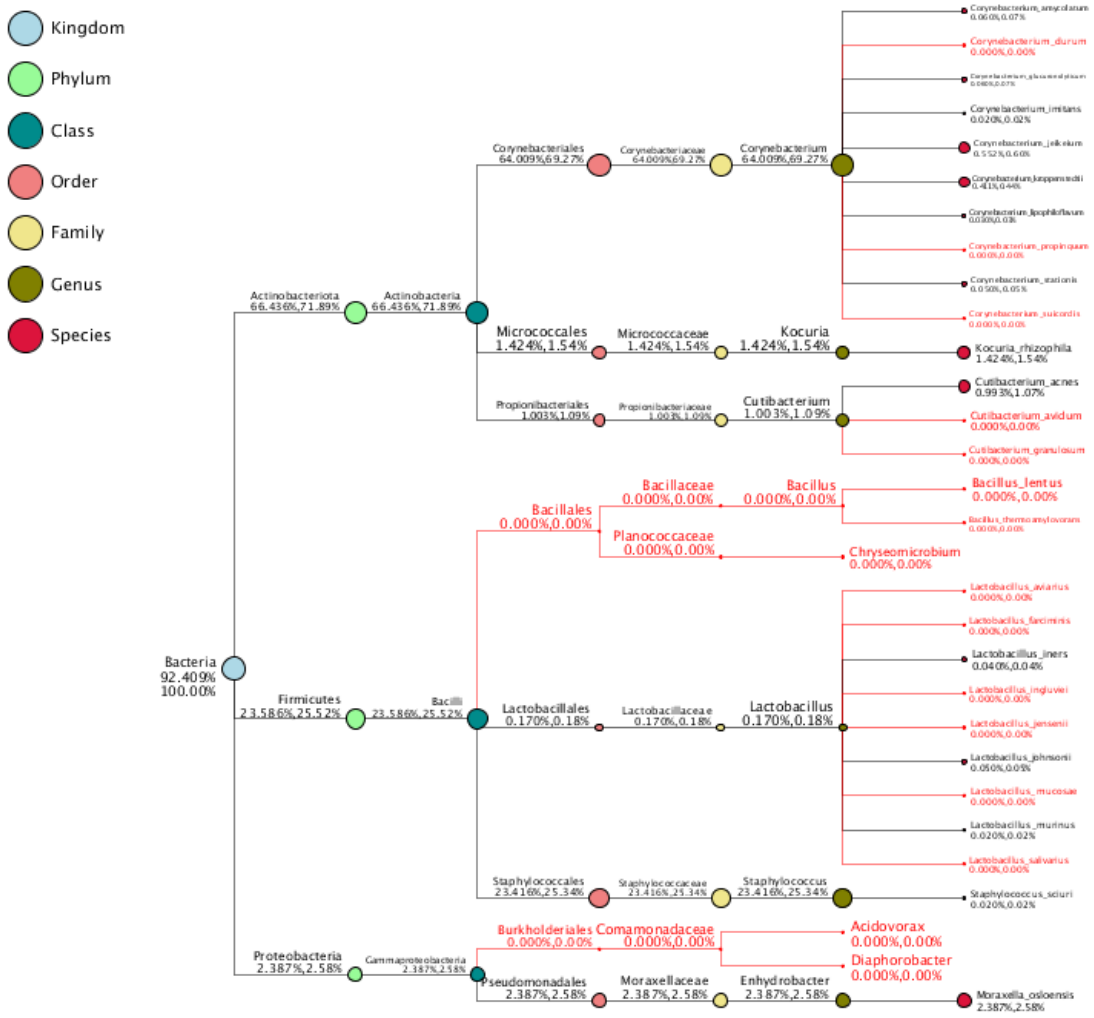

Figure 53 Taxa Tree for the Male Axilla Sample.

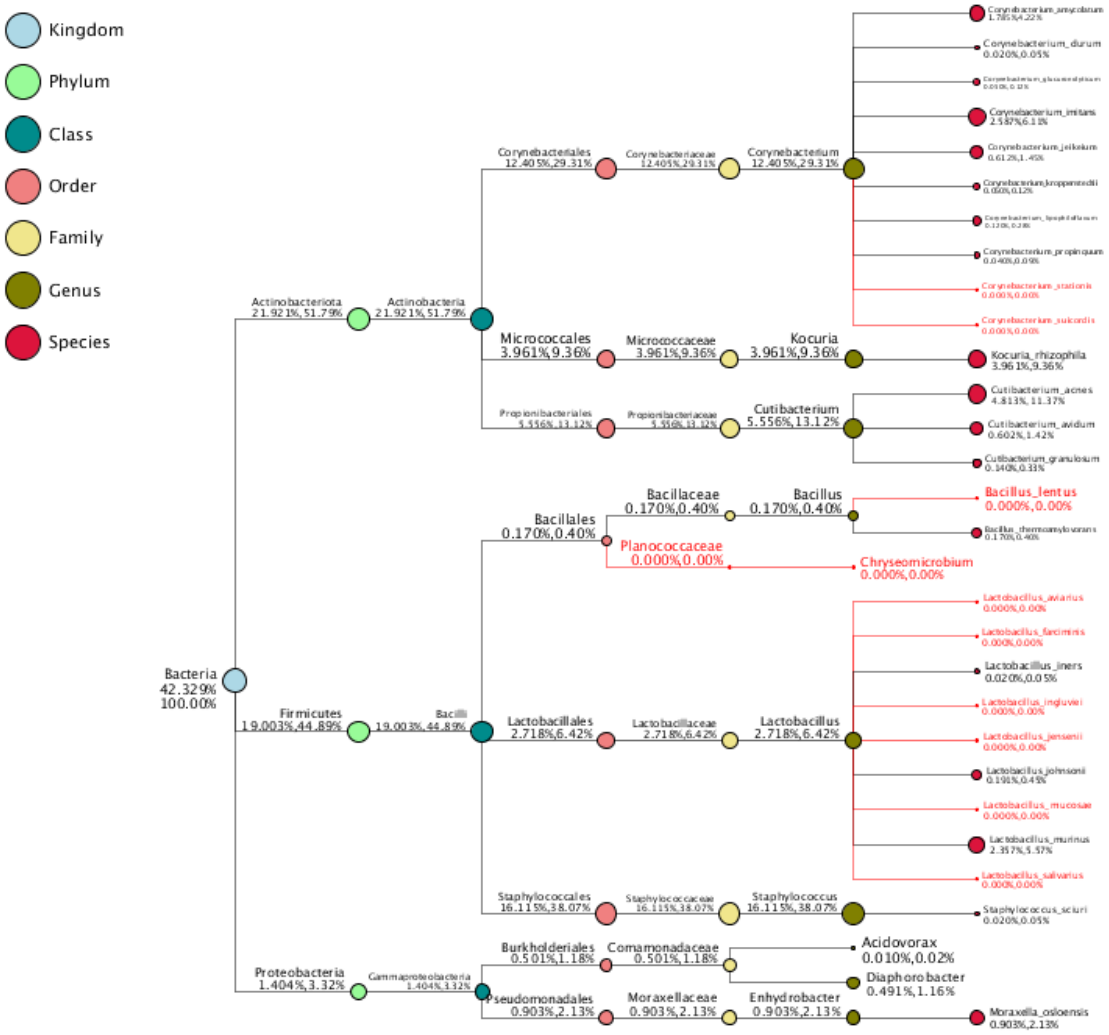

Figure 54. Taxa Tree for the Male Navel Sample.

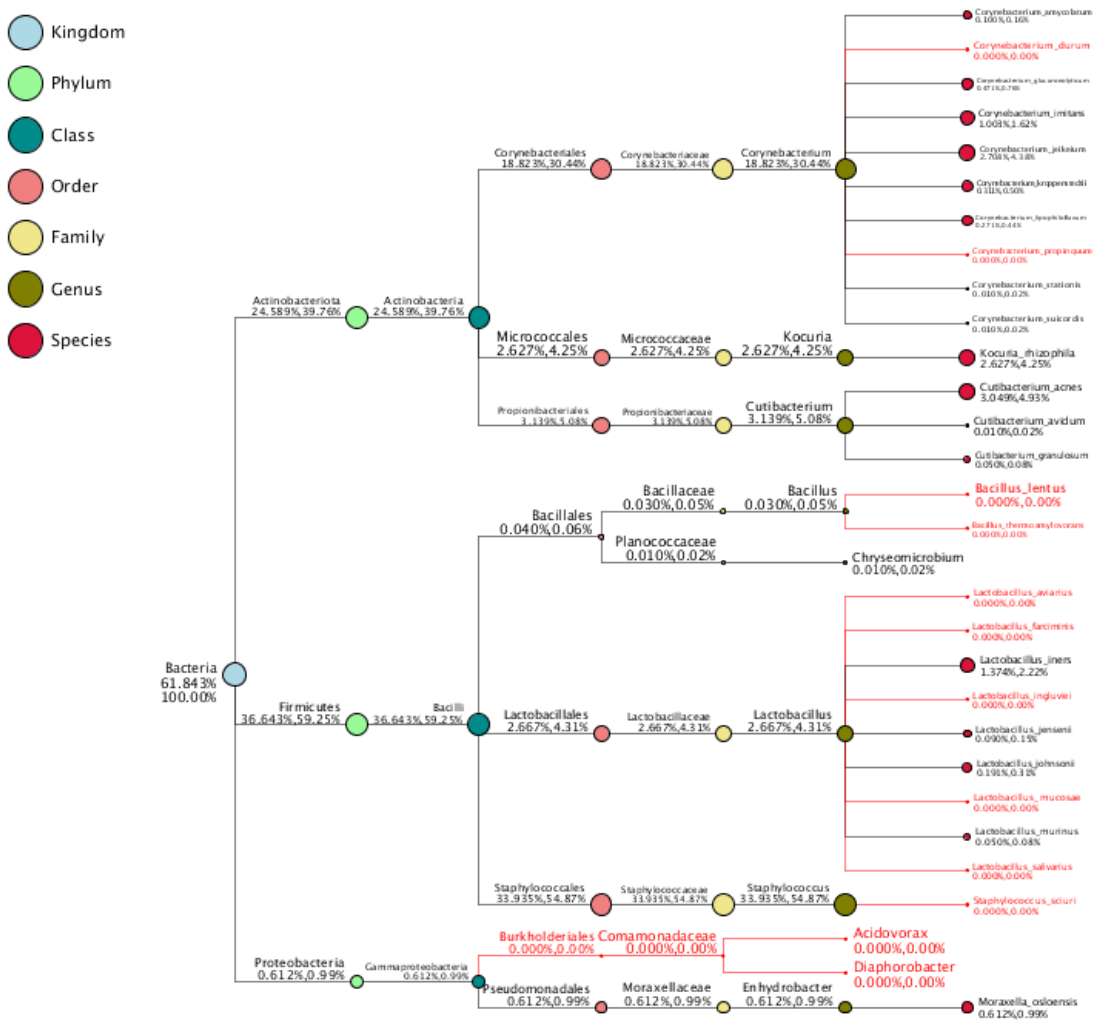

Figure 55. Taxa Tree for the Male Forehead Sample.

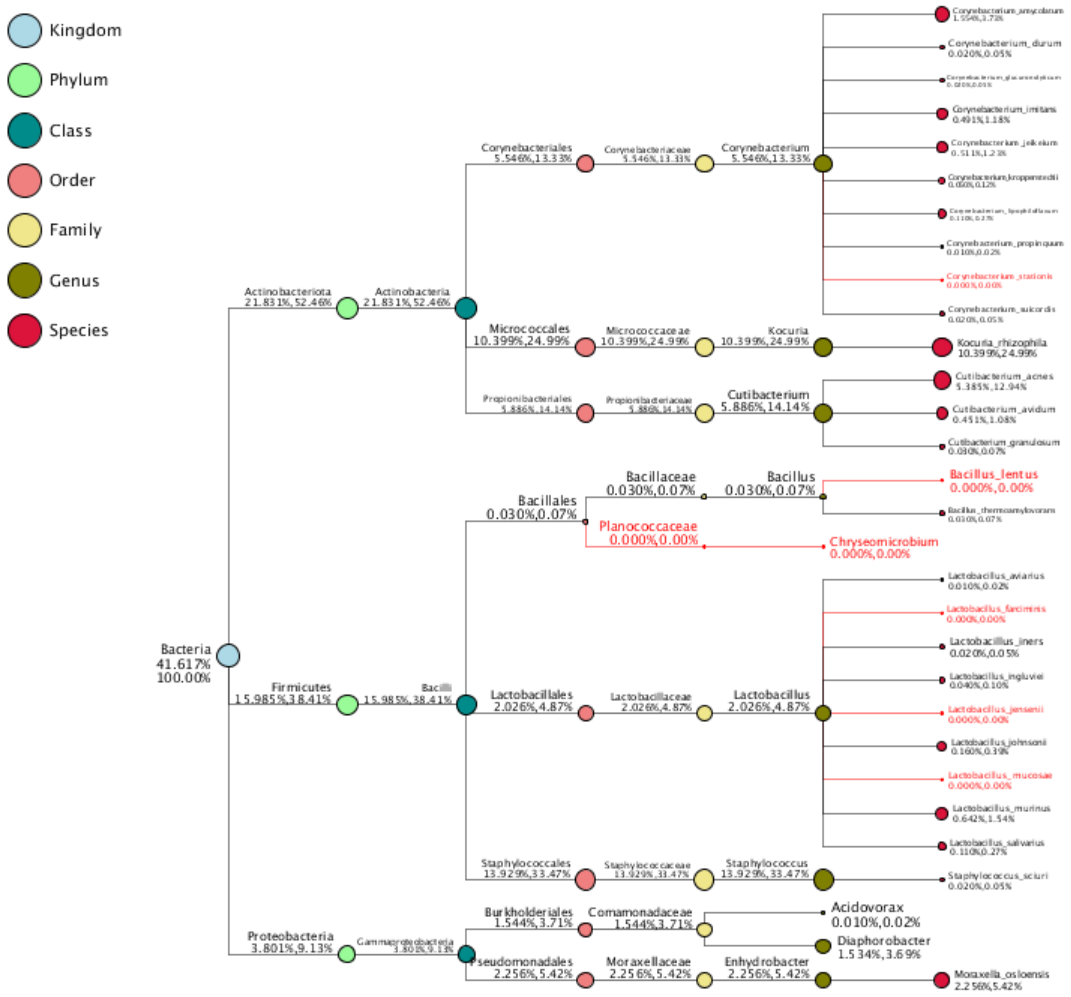

Figure 56. Taxa Tree for the Male Forearm.

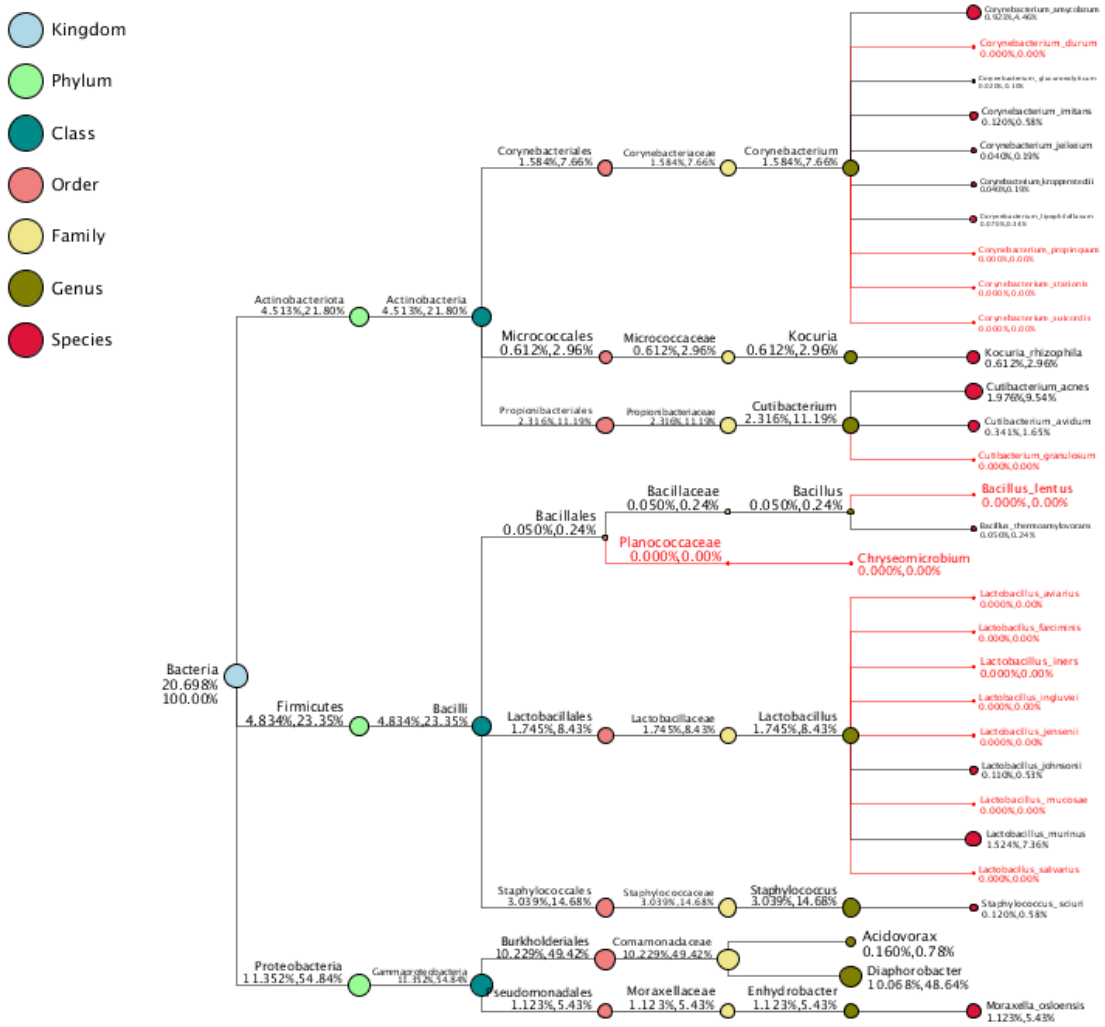

Figure 57. Taxa Tree for the Female axilla.

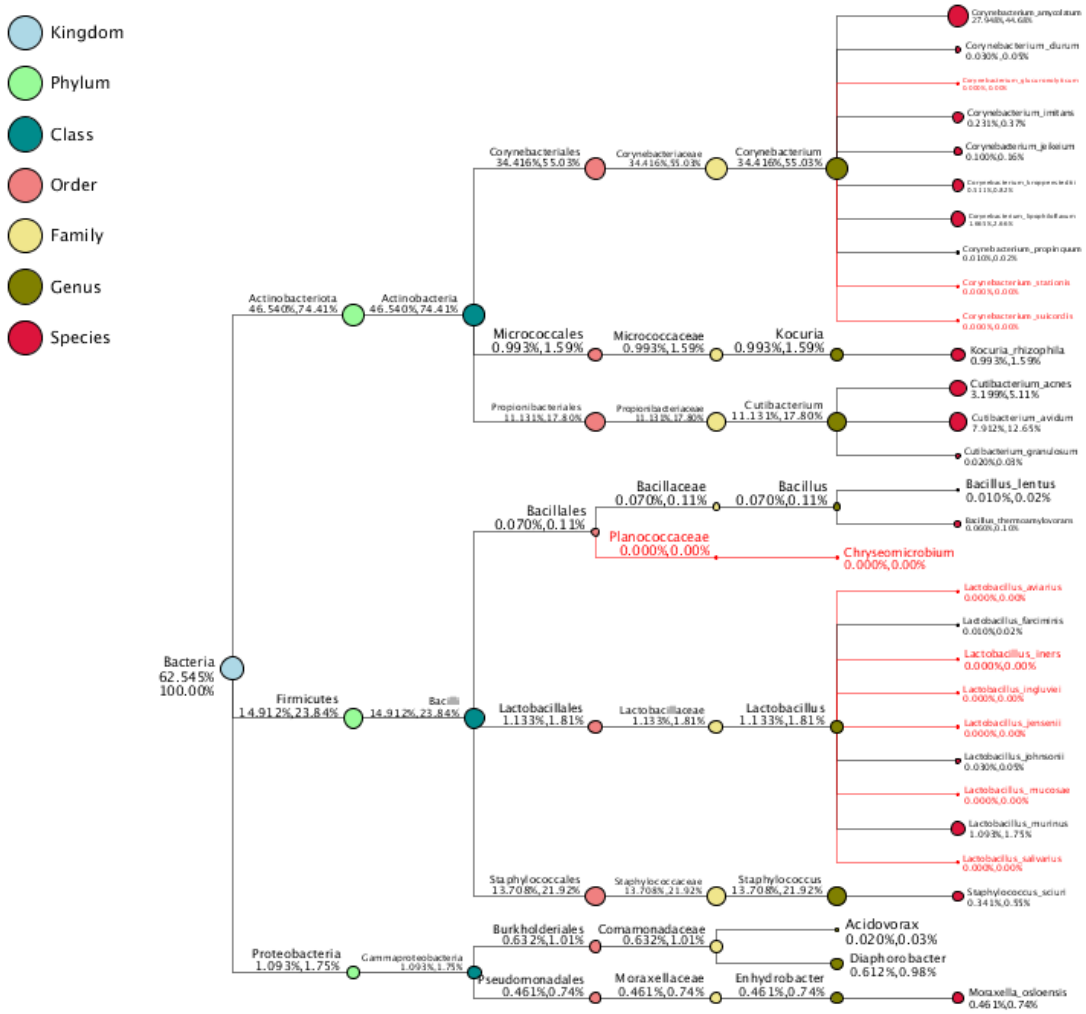

Figure 58. Taxa Tree for the Female Navel.

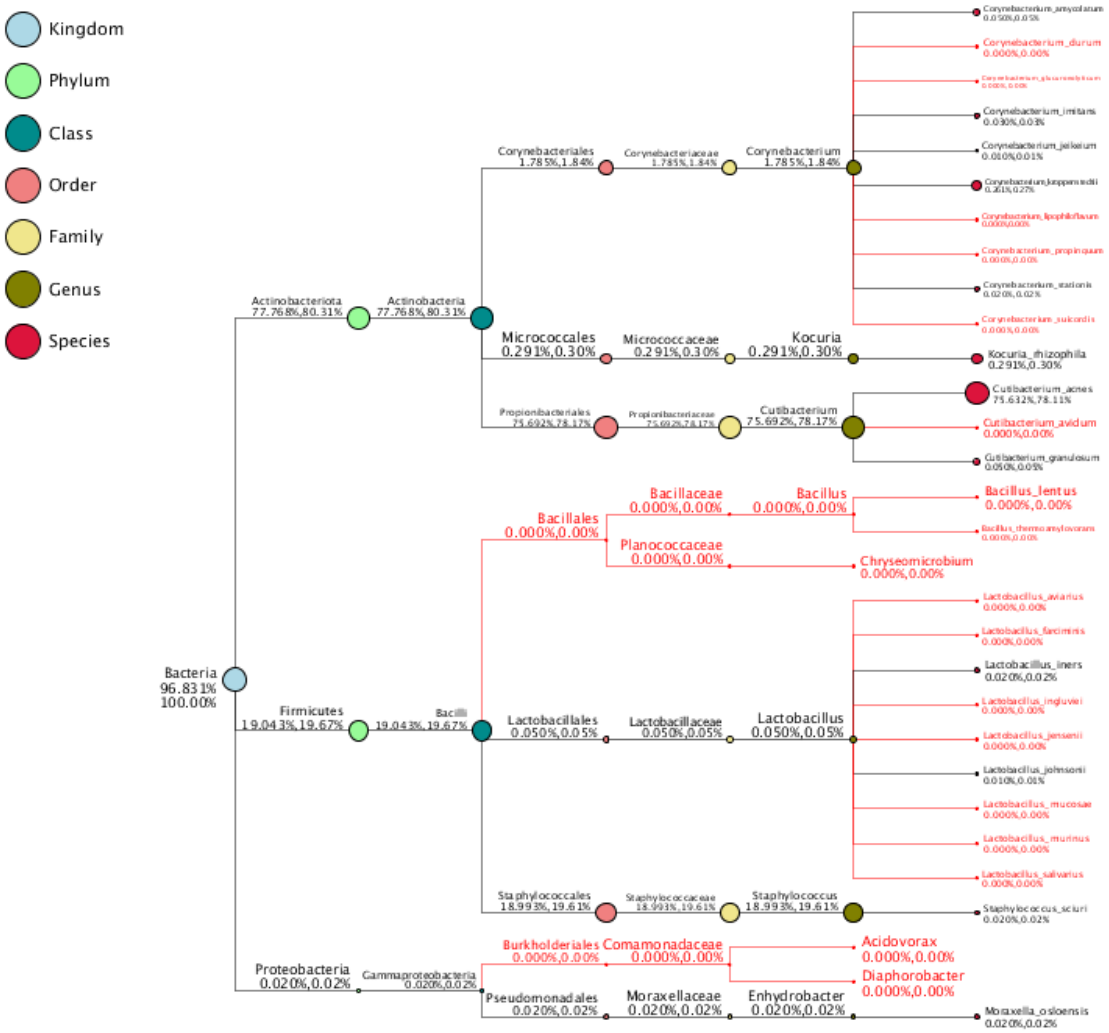

Figure 59. Taxa Tree for the Female Forehead.

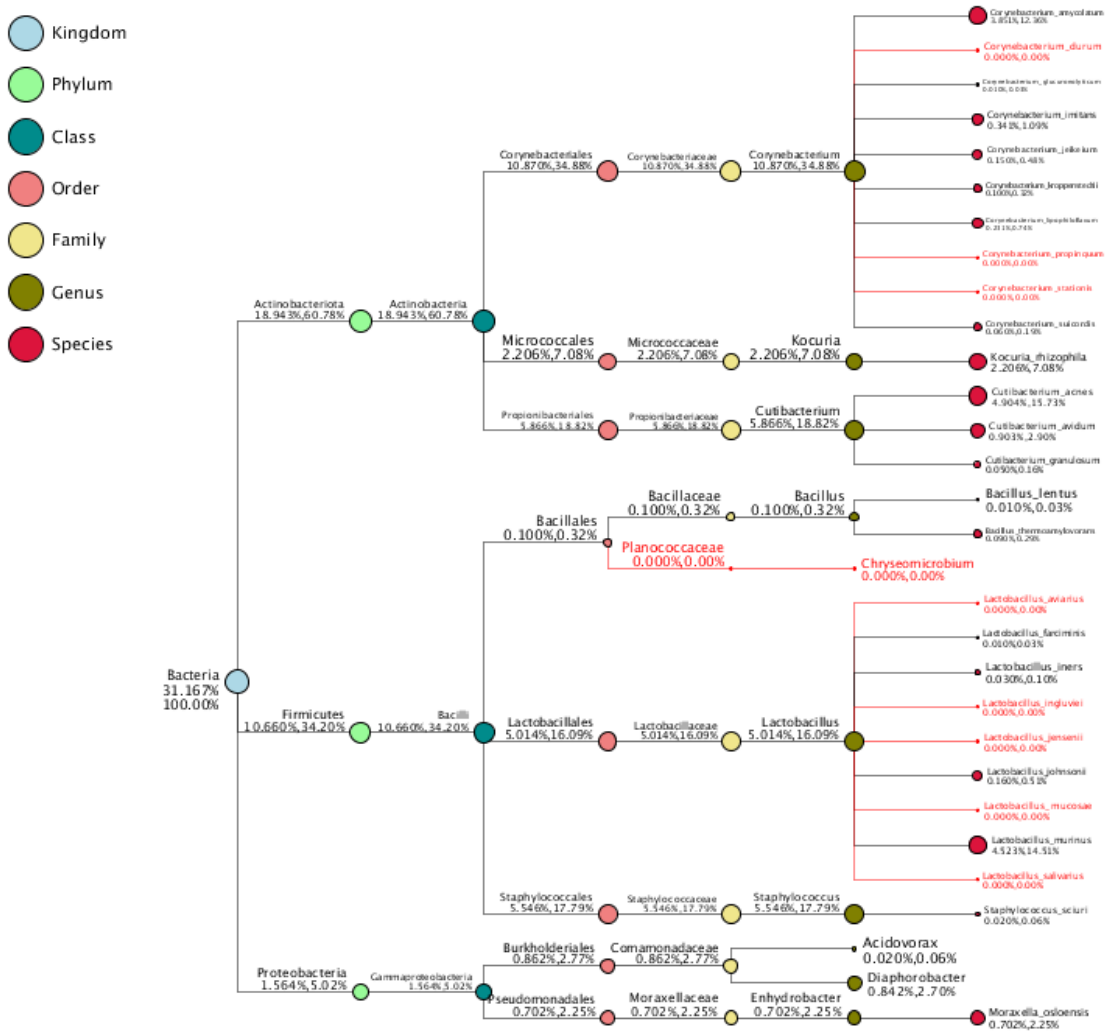

Figure 60. Taxa Tree for the Female Forearm.

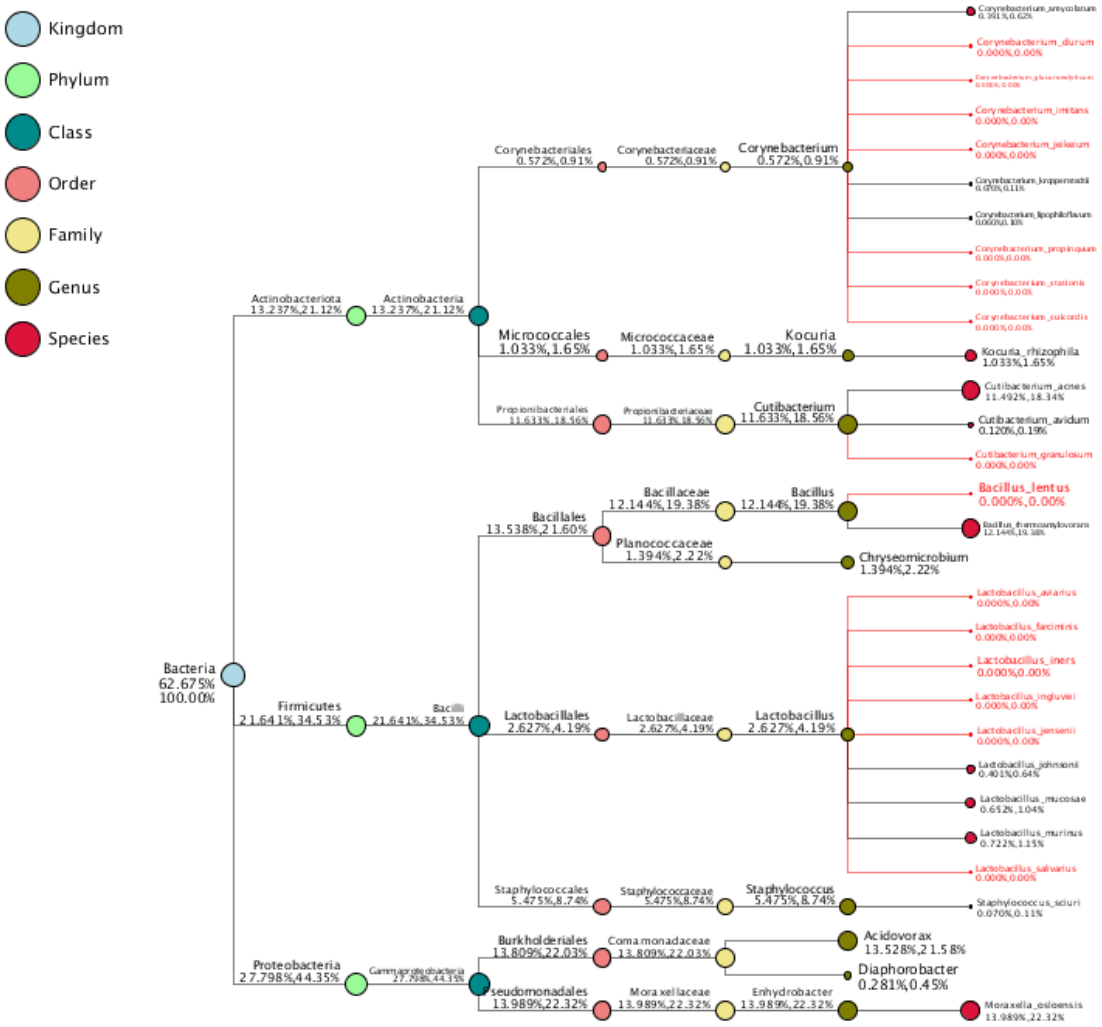

Figure 61. Taxa Tree for the Kit Blank Control.

10.4 OTUs Abundance of All Samples

Table 20.

	Forearm	Forehead	Navel	Axilla	Blank	Forearm	Forehead	Navel	Axilla	Blank	Kit		
	61555	81474	32735	83488	14802	22958	92063	34470	64942	17200	10118	Total	Male
	1879	1204	751	611	805	1199	641	1539	1406	235	146	Unique	Female
	59661	80151	31978	82875	13997	21759	91099	32931	63536	16642	9972	Taxa	Kit Blank
OTU#	15	119	6	2	0	0	323	0	0	323	0	Unclas Taxonomy	
1	3215	2436	1540	821	186	1067	69149	1056	1255	6696	1146	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Cutibacterium;s__Cutibacterium_acnes	
2	221	1706	39	51307	14	65	38	75	31	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium	
3	8255	27191	5129	19914	752	1186	17120	4370	1818	1015	536	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Staphylococcaceae;g__Staphylococcus	
4	929	88	570	50	353	840	55	9203	590	842	40	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_amycolatum	
5	77	12313	30	326	404	30	0	20	35	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__;s__	
6	6206	2116	1267	1177	274	481	275	329	386	1322	103	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Kocuria;s__Kocuria_rhizophila	
7	1293	3347	1574	437	249	412	15	558	442	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Finegoldia	
8	986	0	136	0	126	215	0	186	5442	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Parapusillimonas	
9	917	0	160	0	468	184	0	203	6397	3	28	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Diaphorobacter	
10	1377	0	147	0	63	174	0	123	4434	1	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Comamonas;s__Comamonas_denitrificans	
11	1349	499	288	1979	87	151	11	150	711	100	1392	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Enhydrobacter;s__Moraxella_osloensis	
12	1567	0	267	0	149	276	4	247	4258	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	
13	867	0	512	0	589	737	0	487	880	48	24	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__SAR11_clade;f__Clade_III;g__Clade_III;s__	
14	455	5217	187	405	18	38	37	26	30	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium	
15	310	2168	196	448	16	33	12	28	25	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_jeikeium	
16	385	42	755	21	157	981	3	360	962	130	72	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_murinus	
17	766	4229	2012	338	47	1183	1215	1180	176	47	5	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium	
18	1227	0	53	0	44	96	0	70	1317	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Brevundimonas	
19	1860	845	381	629	70	132	60	115	137	23	225	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Dermacoccaceae;g__Dermacoccus;s__Dermacoccus_sp.	
20	1462	6	100	11	61	173	0	120	1766	607	637	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae	
21	4	10	0	0	0	0	0	3	0	2392	139	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Chryseomicrobium;s__	
22	294	809	829	21	32	75	15	76	78	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_imitans	
23	427	46	637	1	0	311	0	537	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Ralstonia;s__Ralstonia_pickettii	
24	556	672	2	21	829	3	9	1	1	0	12	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	
25	297	642	692	115	74	159	5	317	147	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Peptoniphilus;s__Peptoniphilus_grossensis	
26	974	0	47	0	25	61	0	48	699	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Bordetella	
27	112	28	759	59	218	92	141	19	21	12	21	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia;s__Akkermansia_muciniphila	
28	289	181	123	12	47	116	0	56	1301	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;s__Acinetobacter_johnsonii	
29	235	39	59	3	41	58	14	45	1230	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Micrococcus;s__Micrococcus_luteus	
30	879	0	633	0	456	761	0	532	913	61	79	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__	
31	38	533	78	20	4	7	856	1	14	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__;s__	
32	130	0	43	0	162	39	0	54	1076	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Castellaniella;s__Castellaniella_defragrans	
33	23	0	24	0	4	10	0	11	1419	0	0	k__Bacteria;p__Latescibacterota;c__Latescibacterota;o__Latescibacterota;f__Latescibacterota;g__Latescibacterota;s__	
34	297	0	148	0	187	257	0	170	319	16	10	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__	
35	333	1269	488	144	70	76	1	31	130	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus_dysgalactiae	
36	92	16	33	0	31	59	0	35	2443	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Veillonella	
37	869	95	56	334	57	72	4	62	1110	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	
38	16	1103	7	37	7	8	16	2	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_iners	
39	195	0	96	0	53	86	0	82	2118	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas;s__Stenotrophomonas_acidaminiphila	
40	249	1050	164	58	16	31	12	16	47	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	
41	74	34	516	0	4	265	2	373	176	7	6	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	
42	168	349	345	15	30	36	0	18	36	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__Prevotella_disiens	
43	0	83	10	1015	0	2	0	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rubellimicrobium;s__	
44	271	0	172	0	149	243	0	152	300	25	20	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__Candidatus_Nanopelagicus	
45	89	346	386	20	12	39	0	22	51	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus	
46	165	111	91	4	5	295	489	161	1	29	1	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Lawsonella;s__	
47	208	90	269	8	97	145	0	1091	196	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Anaerococcus	
48	90	578	90	20	17	24	15	10	22	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Brevibacteriaceae;g__Brevibacterium;s__Brevibacterium_sp.	
49	22	0	56	0	237	18	0	20	34	126	1211	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus_thermoamylovorans	
50	326	783	289	35	33	52	7	26	90	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Anaerococcus;s__Anaerococcus_mediterraneensis	
51	135	12	56	4	220	163	2	31	184	20	8	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio;s__	
52	1	0	0	0	1	63	0	539	132	1	26	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Morganellaceae;g__Proteus	
53	327	444	372	29	36	36	2	15	43	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__Prevotella_bivia	
54	155	0	78	0	69	106	0	88	191	3	12	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Methylophilaceae;g__Candidatus_Methylopumilus;s__	
55	202	0	144	0	92	142	0	68	195	0	8	k__Bacteria;p__Bacteroidota;c__Kapabacteria;o__Kapabacteriales;f__Kapabacteriales;g__Kapabacteriales;s__	
56	295	3	91	0	4	5	2	39	108	166	6	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia-Shigella;s__Escherichia_coli	
57	57	128	4	146	8	12	0	7	275	1	8	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Staphylococcaceae;g__Jeotgalicoccus;s__	
58	0	0	0	0	0	518	0	14	140	2	21	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina;s__Sporosarcina_pasteurii	
59	190	237	25	8	1	4	0	6	13	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast	
60	16	373	17	50	0	2	0	2	6	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_glucuronolyticum	
61	29	252	18	342	12	23	225	169	19	20	7	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_kroppenstedtii	
62	0	0	0	0	0	0	0	0	0	436	66	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_mucosae	
63	155	0	98	0	77	157	0	91	213	6	4	k__Bacteria;p__Acidobacteriota;c__Holophagae;o__Holophagales;f__Holophagaceae;g__marine_group;s__	
64	0	0	0	0	0	0	0	0	0	318	0	Unknown	
65	0	0	0	0	0	1	0	0	0	34	376	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae	
66	106	0	43	0	46	66	0	51	134	1	10	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Elsterales;f__;g__;s__	
67	149	91	13	95	13	12	0	5	34	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__Aerococcus;s__Aerococcus_urinaeequi	
68	109	5	26	14	15	35	5	11	37	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Roseomonas;s__Roseomonas_gilardii	
69	64	328	52	154	22	11	0	11	24	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Desemzia;s__	
70	156	4	0	1	4	0	0	0	0	8	257	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast	
71	65	2	84	0	34	32	6	324	40	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Anaerococcus;s__	
72	70	16	333	0	5	103	18	34	76	2	21	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium_pseudolongum	
73	3	1	9	27	441	19	0	1	322	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__	

74	79	0	9	0	7	14	0	7	333	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Flaviflexus;s__
75	60	9	20	10	25	23	1	15	263	4	3	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__;s__
76	67	95	44	21	116	63	10	3	70	376	74	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus
77	21	21	60	34	157	67	0	4	30	11	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__;s__
78	124	6	181	0	37	32	0	21	47	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Peptostreptococcus
79	70	0	85	0	4	13	0	10	15	48	216	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas
80	259	517	273	118	31	57	8	27	77	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Anaerococcus;s__Anaerococcus_vaginalis
81	283	137	168	75	14	33	3	33	60	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Psychrobacter;s__Psychrobacter_faecalis
82	527	1	19	8	40	52	2	45	14	3	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__
83	83	150	63	45	178	32	18	12	71	45	40	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_johnsonii
84	112	184	103	45	6	20	7	22	17	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Dermabacteraceae;g__Dermabacter
85	96	12	28	6	37	115	0	70	10	13	5	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
86	20	1	97	2	18	23	1	17	134	5	10	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
87	1	88	14	3	0	70	0	4	150	4	2	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__Lactococcus_lactis
88	70	37	109	2	24	20	0	20	23	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Parvimonas;s__
89	4	1	40	1	13	49	0	5	158	0	12	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NK4A214_group;s__
90	7	4	4	4	2	18	0	10	221	3	9	k__Bacteria;p__Deferribacterota;c__Deferribacteres;o__Deferribacterales;f__Deferribacteraceae;g__Mucispirillum
91	81	9	137	3	59	20	0	14	9	0	2	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Alloprevotella;s__
92	217	61	0	5	6	2	76	0	35	28	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Turicibacter;s__
93	37	146	45	1	10	13	0	1	16	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales
94	36	0	135	0	20	18	1	17	33	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Peptoniphilus;s__Peptoniphilus_coxii
95	4	0	110	1	0	22	0	14	130	7	8	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
96	81	0	46	0	36	67	0	46	77	2	3	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group
97	14	232	39	4	2	0	0	2	3	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Dialister;s__Dialister_invisus
98	64	270	47	89	4	6	0	9	12	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Brevibacteriaceae;g__Brevibacterium;s__
99	87	0	46	0	41	55	0	46	111	0	5	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Limnobacter;s__
100	7	4	0	0	8	5	0	6	187	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Ruminococcus
101	0	0	0	0	0	0	296	0	0	0	0	Unknown
102	327	0	0	0	1	0	2	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae
103	1	0	11	0	55	116	0	0	47	1	5	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__;s__
104	144	0	85	0	77	108	0	61	146	11	20	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
105	0	0	7	1	260	43	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__A2;s__
106	0	0	2	0	0	0	0	4	310	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
107	77	0	32	0	32	41	0	42	79	1	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Polynucleobacter
108	247	3	19	4	22	23	3	8	60	7	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
109	40	0	18	0	52	82	0	15	75	6	2	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
110	1	11	15	10	0	12	0	3	208	3	7	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Bilophila;s__
111	4	0	5	0	18	88	2	5	85	1	16	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Eubacterium_coprostanoligenes_group;g__Eubacterium_coprostanoligenes_group;s__
112	0	0	0	0	0	0	0	0	0	13	201	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Aggregatibacter;s__

113	14	0	41	3	7	15	0	10	85	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__Alistipes_inops
114	2	0	8	110	0	0	18	0	53	0	8	k__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Helicobacter;s__Helicobacter_typhlonius
115	21	2	60	3	45	35	1	9	21	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
116	82	0	4	0	11	20	0	10	193	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Ochrobactrum;s__Ochrobactrum_intermedium
117	1	4	30	2	196	6	18	41	25	4	3	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae
118	195	0	32	1	72	20	0	0	23	0	3	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
119	32	10	2	49	11	24	0	2	126	0	3	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
120	14	82	69	1	2	1	0	1	6	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium_adolescentis
121	18	5	95	0	14	13	0	4	23	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Ruminococcus;s__Ruminococcus_bicirculans
122	15	0	91	0	158	0	1	0	1	6	0	k__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Helicobacter
123	12	0	12	0	146	12	0	8	8	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Cloacibacterium;s__
124	45	1	58	0	36	43	2	20	20	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
125	58	57	3	3	4	3	11	23	12	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus;s__
126	194	2	5	0	3	5	0	5	11	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Nostocaceae;g__Scytonema_UTEX_2349;s__
127	50	0	27	0	37	40	0	25	31	4	3	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
128	8	0	4	0	0	6	0	7	100	1144	1349	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax
129	60	0	51	0	31	53	0	30	72	6	14	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae
130	19	125	1	0	0	0	1	0	0	0	0	k__Bacteria
131	28	16	26	10	35	52	4	12	0	1	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotellaceae_UCG-001;s__
132	6	0	7	0	1	3	0	8	139	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingobium;s__Sphingobium_amiense
133	141	15	42	8	73	112	5	48	66	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
134	143	0	7	0	13	14	0	5	5	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus
135	129	21	2	0	68	56	11	34	5	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
136	232	0	8	0	4	8	0	7	16	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Morganellaceae;g__Providencia;s__Providencia_stuartii
137	5	0	4	0	2	5	0	3	162	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Schlegelella;s__
138	93	0	6	2	8	56	2	11	45	3	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Eubacterium_coprostanoligenes_group;g__Eubacterium_coprostanoligenes_group;s__
139	9	2	20	8	15	10	0	3	103	1	9	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__;s__
140	0	10	0	109	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Subdoligranulum;s__
141	58	64	138	0	26	17	64	1	211	14	5	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Dubosiella;s__
142	15	8	11	3	18	29	2	5	55	1	5	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter;s__
143	3	21	2	0	1	175	0	67	40	0	58	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina;s__
144	57	0	43	0	25	46	0	31	63	4	8	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
145	16	147	3	153	3	5	13	6	29	0	1	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Gemellaceae;g__Gemella;s__
146	15	4	1	22	2	3	18	115	82	9	7	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Staphylococcaceae;g__Staphylococcus;s__Staphylococcus_sciuri
147	0	0	0	0	2	65	0	5	199	16	21	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Atopostipes;s__
148	37	6	35	12	28	25	2	25	101	2	5	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Colidextribacter;s__
149	31	20	29	9	0	10	7	0	36	2	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eubacterium_fissicatena_group;s__
150	11	174	1	5	0	2	0	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__Neisseria;s__Neisseria_perflava
151	23	4	62	0	34	24	7	1	5	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__

152	105	0	6	5	9	5	4	5	14	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-006;s__
153	105	21	69	30	73	91	19	13	289	12	12	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Colidextribacter;s__
154	15	0	1	0	6	9	1	108	9	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Vellonella;s__Veillonella_ratti
155	89	0	47	0	52	82	0	42	94	3	5	k__Bacteria;p__Nitrospirota;c__Nitrospiria;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__
156	15	58	0	44	0	0	0	5	0	0	1	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__Sphingomonas_faeni
157	26	15	53	1	3	0	0	3	9	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium_bifidum
158	4	0	10	0	41	11	0	1	43	9	4	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;g__Coriobacteriaceae_UCG-002;s__
159	1	2	9	8	3	11	7	7	99	1	7	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__;s__
160	0	0	0	0	0	26	0	27	159	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Atopostipes;s__
161	8	0	79	0	3	6	0	3	6	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Segetibacter;s__
162	1	0	0	31	194	0	0	4	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
163	58	110	3	6	2	4	28	9	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Rothia;s__Rothia_mucilaginosa
164	16	9	11	4	19	52	5	23	92	1	1	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
165	16	0	16	1	13	30	0	2	51	3	2	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
166	1	100	2	4	0	3	24	0	0	0	5	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1;s__
167	0	89	0	2	0	0	0	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Turicella;s__
168	24	0	0	0	0	63	0	20	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotellaceae_NK3B31_group;s__
169	0	0	21	0	2	99	0	2	151	1	4	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
170	46	81	8	1	0	8	0	4	3	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus_salivarius
171	43	0	28	0	34	43	0	45	56	3	2	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
172	9	7	8	10	6	34	3	3	84	2	9	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
173	1	1	11	0	22	12	0	0	19	4	4	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Anaerotruncus;s__
174	16	12	40	8	19	14	18	0	2	0	7	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella;s__
175	0	0	103	0	0	0	0	0	0	3	13	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Ileibacterium;s__Ileibacterium_valens
176	2	3	8	1	2	1	0	0	104	2	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__Candidatus_Saccharimonas;s__
177	54	0	43	0	31	57	0	28	71	1	7	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__Candidatus_Planktophila;s__
178	38	0	17	0	21	20	0	19	54	2	4	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Acidibacter;s__
179	29	0	30	0	11	9	0	15	20	1	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Dinghuibacter;s__
180	29	2	28	2	14	21	0	18	16	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Terrimonas;s__
181	5	0	9	0	2	72	0	0	7	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Chryseobacterium;s__Chryseobacterium_hominis
182	3	0	7	0	16	1	0	3	62	3	5	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
183	26	0	21	0	25	15	0	14	30	3	0	k__Bacteria;p__Chloroflexi;c__SL56_marine_group;o__SL56_marine_group;f__SL56_marine_group;g__SL56_marine_group;s__
184	19	70	3	5	0	0	0	0	2	3	3	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Mitochondria;g__Mitochondria
185	27	0	4	0	4	63	0	47	14	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Negativicoccus
186	19	0	18	0	14	18	1	13	12	3	7	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae
187	1	2	24	0	2	8	1	6	55	5	6	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Rikenellaceae_RC9_gut_group;s__
188	61	0	7	0	5	4	0	2	5	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas;s__Porphyromonas_sp.
189	12	3	0	69	0	1	0	6	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Microbacterium
190	38	10	3	0	1	43	3	17	21	6	4	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Romboutsia;s__Romboutsia_ilealis
191	1	11	15	0	7	8	0	5	36	2	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Tuzzerella;s__
192	40	0	30	0	13	43	0	20	27	0	2	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
193	104	0	1	1	12	5	8	0	23	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
194	43	21	0	2	4	4	0	1	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Peptoniphilus;s__Peptoniphilaceae_bacterium
195	13	5	35	3	19	13	4	12	29	3	4	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__
196	269	7	194	0	163	196	0	2608	217	54	12	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Cutibacterium;s__Cutibacterium_avidum
197	65	2	4	2	4	2	2	0	9	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__GCA-900066575;s__
198	2	0	15	12	10	17	4	7	14	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_acidifaciens
199	23	1	13	3	4	7	0	2	49	7	4	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
200	0	58	0	45	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
201	0	80	11	0	0	0	0	0	6	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Dialister;s__Dialister_pneumosintes
202	33	4	2	0	25	33	2	3	8	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis;s__
203	3	61	1	3	1	1	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales
204	149	0	0	4	5	1	0	2	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
205	13	10	7	6	11	44	2	14	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
206	10	2	63	7	5	12	7	4	44	2	11	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
207	11	0	8	42	1	2	5	6	7	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Erysipelatoclostridium;s__
208	14	2	3	3	50	2	1	1	2	2	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__
209	7	0	57	0	0	1	0	2	6	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Rhizobacter
210	0	127	0	3	0	0	2	0	18	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
211	27	23	47	0	0	2	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Mitochondria;g__Mitochondria
212	12	42	22	0	2	1	0	1	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Planomicrobium;s__Planomicrobium_sp.
213	16	0	14	0	6	17	0	13	19	1	3	k__Bacteria;p__Chloroflexi;c__JG30-KF-CM66;o__JG30-KF-CM66;f__JG30-KF-CM66;g__JG30-KF-CM66;s__
214	0	0	0	19	0	0	94	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales
215	3	0	0	0	0	0	0	0	72	0	6	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio
216	7	0	11	0	69	16	2	15	0	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
217	35	24	0	0	2	2	0	2	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus;s__Haemophilus_parainfluenzae
218	6	1	15	0	9	0	1	2	65	8	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Faecalibaculum
219	39	9	8	67	4	7	0	5	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae;g__Gordonia
220	0	37	0	37	4	4	7	3	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
221	3	0	34	5	6	2	2	0	38	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__
222	0	0	0	0	0	0	0	0	0	49	8	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Dietziaceae;g__Dietzia
223	14	0	16	0	8	15	0	21	23	2	4	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__bacterium_enrichment
224	19	0	9	0	15	17	0	20	14	2	4	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Methylophilaceae;g__;s__
225	19	0	2	2	10	31	0	1	50	3	10	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
226	7	0	48	0	2	1	0	0	3	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__;s__
227	20	37	40	2	1	1	2	3	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces;s__Winkia_neuii
228	28	0	17	1	24	17	0	3	27	3	6	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
229	64	0	0	0	17	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_salivarius

230	6	4	1	0	3	12	2	0	90	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eubacterium_xylanophilum_group;s__
231	6	0	49	0	6	5	0	0	1	0	0	k__Bacteria;p__Deinococcota;c__Deinococci;o__Deinococcales;f__Deinococcaceae;g__Deinococcus
232	0	0	0	54	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae
233	2	2	20	1	0	0	2	0	53	2	3	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__;s__
234	15	14	1	0	0	3	0	44	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces;s__Schaalia_radingae
235	9	0	6	0	6	9	0	13	20	1	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__FukuN57;s__
236	11	38	17	0	1	4	0	1	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Rothia;s__
237	2	0	7	0	1	95	0	0	54	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Alloprevotella
238	40	8	6	26	3	1	2	1	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Microlunatus;s__
239	49	0	42	0	22	55	0	37	76	4	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__;s__
240	18	0	11	0	7	19	0	9	12	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Micavibrionales;f__Micavibrionaceae;g__;s__
241	29	0	5	0	10	17	0	6	18	0	1	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Acidobacteriae;f__Acidobacteriaceae;g__Paludibaculum;s__
242	32	0	8	0	25	17	0	4	18	1	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__
243	18	0	31	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Moraxella;s__Moraxella_lincolnii
244	56	0	6	0	3	2	0	4	5	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
245	10	0	8	0	12	26	0	12	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Odoribacter;s__
246	0	2	0	50	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Caproiciproducens;s__
247	10	37	0	3	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Subdoligranulum;s__
248	1	16	12	62	5	6	6	4	18	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_acidifaciens
249	3	0	6	0	6	47	0	2	8	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
250	63	215	41	25	21	52	3	549	49	41	5	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_lipophiloflavum
251	4	1	2	73	1	1	0	0	1	0	3	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
252	18	0	22	0	10	12	0	0	0	0	2	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
253	5	43	21	0	2	2	3	4	3	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus_anginosus
254	33	0	3	10	0	44	0	4	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eubacterium_ruminantium_group;s__
255	13	0	1	1	0	31	0	6	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
256	23	19	3	0	0	5	4	2	4	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Methylobacterium-Methylorubrum
257	0	3	0	38	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Caproiciproducens;s__
258	1	15	32	1	7	7	4	5	20	1	4	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_vulgatus
259	1	0	5	0	38	0	0	0	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__;s__Chryseobacterium_frigidum
260	23	37	21	3	2	4	0	4	10	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__Neisseria
261	0	0	2	0	1	47	0	5	40	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Atopostipes
262	21	2	6	8	40	48	3	2	11	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
263	77	0	56	0	1	0	0	0	0	0	2	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Allobaculum;s__
264	60	5	28	0	25	35	0	34	66	2	5	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
265	6	3	6	1	9	9	1	24	28	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter;s__
266	18	0	9	0	18	12	0	15	27	0	0	k__Bacteria;p__Bacteroidota;c__Kapabacteriia;o__Kapabacteriales;f__Kapabacteriales;g__Kapabacteriales;s__
267	12	0	6	0	6	10	0	2	7	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
268	25	0	33	0	0	4	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__Alkalinema;s__

269	11	0	8	0	2	13	0	9	5	1	1	k__Bacteria
270	4	49	0	5	0	0	0	1	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__Eremococcus;s__
271	12	75	0	4	0	2	2	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces;s__Schaalia_odontolytica
272	41	0	22	0	17	29	0	33	23	1	4	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__MWH-UniP1_aquatic_group;g__MWH-UniP1_aquatic_group
273	33	1	1	2	6	0	10	2	1	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Ruminococcaceae;s__
274	2	0	59	0	1	0	0	10	3	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Calidibacillus;s__
275	17	54	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium_longum
276	13	0	6	0	6	20	0	1	8	1	1	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__;g__;s__
277	20	0	5	0	8	12	0	17	21	1	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Arenimonas;s__
278	9	0	4	0	5	16	0	6	7	2	4	k__Bacteria;p__Actinobacteriota;c__Actinobacteria
279	34	0	32	0	15	36	0	19	43	4	2	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
280	40	2	5	0	1	0	1	1	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae;g__Williamsia;s__Williamsia_sp.
281	0	0	0	0	0	0	0	0	2	50	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
282	7	0	31	0	28	0	0	1	16	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
283	1001	10	121	15	30	591	7	45	670	62	800	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Erwiniaceae;g__Pantoea;s__Pantoea_ananatis
284	43	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Planctomycetota;c__OM190;o__OM190;f__OM190;g__OM190;s__
285	3	81	6	4	0	0	7	0	0	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Dialister;s__Dialister_propionicifaciens
286	3	0	23	0	0	1	0	10	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Caulobacter;s__
287	27	2	4	0	3	1	0	0	2	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Nostocaceae;g__Calothrix_PCC-6303;s__
288	16	0	9	0	6	11	0	4	12	0	0	k__Bacteria;p__Bacteroidota;c__Kapabacteriia;o__Kapabacteriales;f__Kapabacteriales;g__Kapabacteriales;s__
289	0	0	0	0	0	27	0	5	27	2	1	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Pseudogracilibacillus;s__Pseudogracilibacillus_auburnensis
290	11	2	6	2	3	16	0	5	15	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
291	5	5	0	25	10	4	7	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__
292	64	9	7	5	29	99	1	5	78	7	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
293	27	0	1	0	0	8	0	4	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Microvirga;s__Microvirga_sp.
294	8	0	8	6	0	11	1	2	20	3	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__UCG-010;g__UCG-010;s__
295	7	0	5	0	1	2	5	2	38	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
296	0	0	2	0	0	0	0	0	55	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
297	9	2	17	3	24	19	5	0	1	0	3	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
298	83	2	30	0	32	17	1	12	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
299	27	0	1	0	0	3	0	3	5	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__;s__
300	18	0	12	0	3	7	0	15	16	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas;s__Pseudomonas_peli
301	23	0	3	1	4	0	0	0	1	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Odoribacter;s__
302	1	11	8	0	8	39	0	4	5	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
303	32	0	8	7	2	0	0	2	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Butyricicoccaceae;g__Butyricicoccus;s__
304	0	0	0	0	0	0	0	38	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Chitinophaga
305	29	4	0	0	0	6	0	11	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae
306	25	3	3	1	0	8	10	4	0	0	2	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
307	88	1	1	0	0	6	0	2	9	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__;s__

308	11	0	6	0	4	4	0	3	14	1	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__Pedosphaeraceae;s__
309	24	1	26	1	15	13	1	0	20	1	7	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
310	5	37	0	0	5	2	0	1	0	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella;s__Collinsella_aerofaciens
311	16	5	9	8	10	8	1	2	8	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptococcales;f__Peptococcaceae;g__;s__
312	0	0	34	0	6	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Odoribacter;s__
313	13	32	1	0	0	0	0	2	1	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
314	0	4	2	0	14	10	1	1	46	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia;s__Lachnospiraceae_bacterium
315	56	0	1	0	0	0	2	0	27	0	1	k__Bacteria;p__Deferribacterota;c__Deferribacteres;o__Deferribacterales;f__Deferribacteraceae;g__Mucispirillum;s__
316	37	8	13	13	1	6	9	3	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
317	16	0	5	0	3	2	0	7	11	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__GOUTA6;s__
318	4	0	1	3	5	6	1	6	15	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Eubacterium_nodatum_group;s__
319	0	34	0	0	0	0	3	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Mitochondria;g__Mitochondria
320	21	0	10	0	7	10	0	3	12	6	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group
321	1	13	14	2	2	3	0	1	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
322	36	6	5	0	2	6	0	20	7	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Bradyrhizobium;s__Bradyrhizobium_elkanii
323	31	0	0	1	3	5	0	9	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__Lachnospiraceae_bacterium
324	20	63	2	2	0	2	2	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces
325	2	0	5	0	3	8	0	11	12	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__IMCC26256;f__IMCC26256;g__IMCC26256;s__Actinobacteria_bacterium
326	1	4	6	0	0	2	3	0	11	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia;s__Lachnospiraceae_bacterium
327	35	0	30	0	1	0	0	2	7	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__Aliterella;s__
328	23	0	6	0	3	22	0	10	20	0	1	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae
329	5	64	0	4	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina;s__Sporosarcina_psychrophila
330	9	3	16	2	6	10	4	8	20	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
331	22	0	16	0	14	33	0	15	45	2	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Synechococcales;f__Cyanobiaceae;g__Cyanobium_PCC-6307;s__
332	4	21	2	1	1	3	1	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae;g__Rhodococcus;s__Rhodococcus_erythropolis
333	0	47	0	3	1	0	1	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;s__Acinetobacter_lwoffii
334	11	0	12	0	6	13	0	8	13	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Methylophilaceae;g__;s__
335	6	0	7	0	8	9	0	13	23	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__TRA3-20;g__TRA3-20;s__
336	19	0	11	2	3	15	0	6	10	2	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Virgulinella_fragilis
337	14	0	0	0	5	3	0	4	6	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__Allosbacter;s__
338	16	0	9	0	5	18	0	14	11	1	1	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
339	6	11	10	4	7	16	1	6	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
340	13	0	13	0	7	6	0	2	9	0	1	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
341	2	0	0	5	0	0	0	0	24	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
342	0	0	0	28	0	0	0	3	0	0	0	k__Bacteria;p__WS2;c__WS2;o__WS2;f__WS2;g__WS2;s__
343	19	0	6	0	13	12	0	22	12	3	3	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Limnobacter;s__
344	26	0	2	0	5	2	0	0	4	0	0	k__Bacteria;p__Chloroflexi;c__Ktedonobacteria;o__Ktedonobacterales;f__Ktedonobacteraceae;g__1959-1;s__
345	15	0	13	0	5	1	0	2	9	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
346	0	28	0	0	0	0	0	0	0	0	0	k__Bacteria

347	1	0	1	11	32	0	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Eubacterium_siraeum_group;s__
348	19	0	6	0	6	18	0	12	23	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Pseudohongiellaceae;g__Bin10;s__
349	436	0	39	0	30	48	0	60	1275	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Comamonas;s__Comamonas_kerstersii
350	9	0	2	0	3	4	0	9	24	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
351	18	0	2	0	0	0	0	0	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__Conchiformibius
352	1	0	3	0	4	9	0	5	6	1	2	k__Bacteria;p__SAR324_clade(Marine_group_B);c__SAR324_clade(Marine_group_B);o__SAR324_clade(Marine_group_B);f__SAR324_clade(Marine_group_B);g__SAR324_clade(Marine_group_B);s__
353	21	6	0	0	3	1	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Acidobacteriales;f__;g__;s__
354	0	0	0	0	1	0	0	31	2	9	5	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium;s__Agrobacterium_radiobacter
355	4	0	5	0	1	4	0	7	6	0	1	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Phormidiaceae;g__Planktothrix_NIVA-CYA_15;s__
356	23	17	0	1	0	4	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__1174-901-12;s__
357	0	31	0	0	0	0	0	0	0	0	0	Unknown
358	11	0	0	0	10	3	0	5	9	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Roseomonas;s__
359	5	0	9	0	5	6	0	5	7	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sneathiellales;f__Sneathiellaceae;g__;s__
360	8	25	0	1	2	0	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Alicyclobacillales;f__Alicyclobacillaceae;g__Tumebacillus;s__
361	0	3	0	25	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Rhodanobacteraceae;g__Dokdonella;s__Dokdonella_koreensis
362	1	0	41	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Dolosigranulum;s__Dolosigranulum_pigrum
363	0	31	0	0	0	1	2	18	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__;s__
364	7	0	2	0	3	6	0	5	11	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
365	19	1	2	0	3	0	0	1	11	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
366	12	0	8	0	10	9	0	10	7	1	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__C39
367	2	0	5	0	4	0	1	4	6	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Family_XIII_UCG-001;s__
368	9	0	3	0	1	4	0	2	8	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__NS11-12_marine_group;g__NS11-12_marine_group;s__
369	10	0	12	0	10	24	0	7	24	3	0	k__Bacteria;p__Nitrospirota;c__Nitrospiria;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__
370	0	21	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__MB-A2-108;o__MB-A2-108;f__MB-A2-108;g__MB-A2-108;s__
371	10	0	9	0	9	15	0	13	13	0	1	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Candidatus_Aquiluna;s__
372	23	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Herpetosiphonaceae;g__Herpetosiphon;s__
373	14	0	13	0	8	12	0	4	9	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__;s__
374	0	0	0	0	0	16	0	9	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Monoglobales;f__Monoglobaceae;g__Monoglobus;s__
375	11	0	7	0	2	10	0	11	9	0	1	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__;s__
376	15	0	1	0	9	7	0	8	4	2	1	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__PeM15;f__PeM15;g__PeM15;s__
377	18	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Nannocystales;f__Nannocystaceae;g__Nannocystis;s__
378	14	2	6	2	9	6	0	6	10	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
379	2	1	0	1	22	17	2	1	3	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Monoglobales;f__Monoglobaceae;g__Monoglobus;s__
380	18	4	6	2	8	6	4	3	4	2	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
381	0	2	0	19	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Bdellovibrionales
382	14	0	3	0	5	0	0	2	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__;g__;s__
383	24	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Chlorosarcina_brevispinosa
384	9	0	33	5	10	9	6	8	9	4	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae;g__Parabacteroides;s__Parabacteroides_distasonis
385	18	0	11	0	5	24	0	12	17	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__Nitrosomonas

386	12	1	0	28	0	2	0	5	1	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium;s__
387	4	0	1	0	3	8	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae
388	13	9	2	0	9	0	0	4	10	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Eubacterium_brachy_group;s__
389	13	4	0	6	4	0	0	0	7	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__Clostridium_sp.
390	8	0	3	0	14	18	0	1	8	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
391	0	0	0	0	0	0	0	0	1	14	5	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__AB1;g__AB1
392	16	0	1	0	11	0	0	2	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
393	15	0	5	1	4	0	0	0	6	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
394	1	2	11	0	2	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__UCG-002;s__
395	18	0	2	0	4	0	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__IS-44;s__
396	4	0	4	0	2	4	0	13	11	1	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Rheinheimera;s__
397	5	0	4	0	10	3	0	0	11	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
398	21	0	0	0	0	0	0	1	2	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae;g__Candidatus_Udaeobacter;s__
399	28	1	1	1	0	0	0	0	0	0	4	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-001;s__
400	0	1	3	1	0	0	0	0	21	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
401	4	0	3	1	4	21	3	0	0	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
402	9	0	3	0	3	15	0	3	13	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20;s__
403	12	0	10	0	6	10	0	5	13	0	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Ga0077536;f__Ga0077536;g__Ga0077536;s__
404	1	2	3	2	2	12	1	5	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales
405	4	0	4	0	5	7	0	3	9	0	1	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20
406	7	0	7	0	4	10	0	0	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__NS11-12_marine_group;g__NS11-12_marine_group;s__
407	0	0	17	0	0	4	0	1	10	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia
408	6	1	1	0	3	2	0	4	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__;s__
409	0	0	0	0	0	1	0	0	24	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
410	1	0	2	0	0	3	0	32	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Kribbella;s__Kribbella_sp.
411	0	0	0	0	0	0	0	26	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
412	2	0	4	1	1	1	0	6	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae;g__Parabacteroides;s__Parabacteroides_goldsteinii
413	0	22	0	0	0	0	0	0	0	0	0	k__Bacteria
414	3	0	2	0	5	3	0	1	3	0	0	k__Bacteria;p__Dependentiae;c__Babeliae;o__Babeliales;f__Babeliales;g__Babeliales;s__
415	8	0	9	0	5	5	0	8	9	3	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Sphaerotilus
416	17	0	0	0	1	1	0	0	4	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Anaerotruncus;s__
417	594	0	63	11	36	120	0	61	1334	19	21	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Pluralibacter;s__Pluralibacter_gergoviae
418	4	0	0	2	5	0	0	0	14	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
419	9	0	5	0	2	8	0	4	12	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__
420	22	0	0	0	17	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_ingluviei
421	0	0	0	1	24	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
422	0	0	5	1	2	1	1	0	6	1	0	k__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Gastranaerophilales;f__Gastranaerophilales;g__Gastranaerophilales;s__
423	5	0	2	0	3	1	0	1	5	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Cyclobacteriaceae;g__Algoriphagus;s__
424	5	0	5	0	6	8	0	8	8	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__;s__
425	0	0	0	0	0	0	0	71	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Devosiaceae;g__Devosia;s__
426	12	0	0	0	0	0	0	2	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MND1;s__
427	0	0	0	18	0	0	0	0	0	0	0	k__Bacteria;p__Patescibacteria;c__Gracilibacteria;o__Candidatus_Peribacteria;f__Candidatus_Peribacteria;g__Candidatus_Peribacteria;s__
428	0	15	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Leptotrichia;s__
429	0	0	0	0	0	0	0	0	17	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__RF39;f__RF39;g__RF39
430	0	0	0	0	0	0	0	36	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Rhodanobacteraceae;g__Chujaibacter;s__
431	0	12	0	3	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;g__Atopobium
432	0	12	0	3	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rubellimicrobium;s__
433	12	0	0	0	1	1	0	9	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Caenimonas;s__
434	0	16	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__;g__
435	8	7	0	0	0	17	0	4	4	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
436	5	0	0	0	0	1	2	30	1	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__67-14;g__67-14;s__
437	22	0	2	0	6	8	0	0	2	1	6	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
438	0	0	1	0	0	1	0	0	13	0	1	k__Bacteria;p__Firmicutes;c__Clostridia
439	3	0	6	0	2	4	0	4	8	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__env.OPS_17;g__env.OPS_17;s__
440	0	0	0	0	0	0	0	0	36	0	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Paenalcaligenes;s__
441	2	0	6	0	0	6	0	1	17	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
442	2	0	0	1	0	8	0	0	9	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__UCG-005;s__
443	0	22	0	3	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Pasteurella
444	7	0	13	0	0	0	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_propinquum
445	1	8	0	1	2	0	0	1	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Oribacterium
446	4	0	1	0	0	0	0	1	8	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
447	12	0	0	0	0	2	0	0	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae;g__Rhodococcus;s__Rhodococcus_fascians
448	0	19	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Geobacillus;s__Geobacillus_thermodenitrificans
449	2	0	5	0	1	2	0	4	5	1	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia
450	2	0	1	1	3	4	0	5	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__;f__;g__;s__
451	8	1	6	8	3	17	0	15	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
452	7	0	5	0	5	5	0	3	8	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Opitutales;f__Opitutaceae;g__Opitutus;s__
453	12	3	1	0	0	0	0	6	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Arenimonas;s__
454	4	16	0	1	0	1	1	0	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__Facklamia
455	7	5	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter;s__Campylobacter_ureolyticus
456	3	0	2	0	0	3	0	5	10	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__B1-7BS;g__B1-7BS;s__
457	1	0	3	0	1	1	0	4	6	7	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae
458	26	2	2	0	1	1	0	13	3	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Weissella;s__Weissella_ciburia
459	0	12	0	0	0	0	0	0	0	0	0	Unknown
460	0	0	0	0	0	0	0	32	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Pseudolabrys;s__
461	0	11	0	2	2	0	1	94	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__
462	2	14	3	0	0	4	0	0	10	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingorhabdus
463	17	0	1	0	1	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Exiguobacterales;f__Exiguobacteraceae;g__Exiguobacterium

464	0	24	0	0	0	0	0	0	0	0	0	k__Bacteria
465	14	0	0	0	9	6	0	0	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__A2;s__
466	6	0	9	0	4	11	0	4	10	3	1	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
467	31	0	6	0	10	6	0	2	2	0	1	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
468	1	0	1	0	10	6	0	0	0	0	1	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae
469	31	0	2	0	2	7	2	35	7	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Streptomycetales;f__Streptomycetaceae;g__Streptomyces
470	9	0	2	0	0	2	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Microprunus;s__
471	0	11	0	0	0	0	0	0	0	0	0	Unknown
472	0	1	0	0	0	4	0	1	8	1	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
473	6	1	1	1	7	10	1	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Christensenellales;f__Christensenellaceae;g__Christensenellaceae_R-7_group;s__
474	9	0	2	0	5	7	0	5	16	1	1	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
475	0	14	2	0	3	0	0	1	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
476	0	0	0	0	0	0	1	28	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
477	7	1	1	3	1	0	0	0	7	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
478	4	0	3	0	7	3	0	4	2	1	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__053A03-B-DI-P58;f__053A03-B-DI-P58;g__053A03-B-DI-P58;s__
479	13	0	0	0	0	4	0	0	7	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Ureibacillus;s__Ureibacillus_thermosphaericus
480	5	0	3	0	3	7	0	3	9	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Methylacidiphilales;f__Methylacidiphilaceae;g__;s__
481	11	0	5	0	5	6	0	2	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__Candidatus_Aquirestis;s__
482	4	0	0	0	2	16	0	2	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Defluviitaleaceae;g__Defluviitaleaceae_UCG-011;s__
483	1	0	10	0	0	1	4	0	3	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Dryopteris_fragrans
484	11	0	0	0	6	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_aviarius
485	0	12	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Dependentiae;c__Babeliae;o__Babeliales;f__Vermiphilaceae;g__Vermiphilaceae;s__
486	10	0	7	4	1	6	1	5	15	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
487	0	15	4	2	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__Prevotella_timonensis
488	2	0	1	0	3	2	0	3	3	2	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__Silvanigrellales;f__Silvanigrellaceae
489	6	1	0	1	0	13	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
490	12	0	3	0	0	0	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_durum
491	3	9	2	0	0	1	1	3	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae
492	7	0	1	0	0	3	0	0	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Ferruginibacter;s__
493	3	2	1	0	0	0	0	1	3	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__;s__
494	9	0	0	0	0	0	0	1	3	0	0	k__Bacteria;p__Myxococcota;c__Myxococcia;o__Myxococcales;f__Anaeromyxobacteraceae;g__Anaeromyxobacter;s__
495	4	8	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__;s__
496	4	0	0	0	3	4	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Christensenellales;f__Christensenellaceae;g__Christensenella;s__Christensenella_sp.
497	4	0	3	0	2	5	0	7	9	1	2	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__KF-JG30-C25;f__KF-JG30-C25;g__KF-JG30-C25;s__
498	0	0	3	0	0	1	0	7	4	2	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
499	24	8	5	0	1	3	1	5	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Mycobacteriaceae;g__Mycobacterium
500	0	0	0	0	0	59	0	7	203	10	7	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Atopostipes;s__
501	2	0	6	0	1	8	0	0	7	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria
502	4	0	1	0	15	2	0	0	14	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Rikenella;s__Rikenella_microfusus

503	4	0	2	0	3	2	0	4	10	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__PLTA13;f__PLTA13;g__PLTA13;s__
504	0	0	33	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Odoribacter;s__
505	12	0	3	0	1	2	0	2	0	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
506	1	0	3	0	4	1	0	0	3	0	0	k__Bacteria
507	0	0	1	1	0	1	1	5	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Family_XIII_AD3011_group;s__
508	0	13	0	2	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Anaerococcus;s__Anaerococcus_sp.
509	2	16	2	1	0	0	7	4	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides;s__Nocardioides_sp.
510	0	0	8	0	7	6	0	0	6	0	1	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Rosiflexaceae;g__;s__
511	1	1	0	4	2	0	5	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
512	4	16	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Ruminococcus_torques_group;s__Ruminococcus_torques
513	3	0	0	0	2	5	0	6	11	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__;s__
514	0	0	10	0	0	1	0	1	1	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__Chloroplaea_SAG_10.99;s__
515	1	24	2	1	0	0	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Faecalibacterium
516	7	0	4	0	2	2	0	1	2	0	0	k__Bacteria
517	12	0	0	0	0	1	0	3	3	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__Gaiella;s__
518	11	7	11	31	5	7	5	1	20	2	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
519	1	8	1	1	0	6	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
520	1	1	14	3	3	7	11	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella;s__Burkholderiales_bacterium
521	0	1	2	0	11	0	0	1	2	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
522	5	1	8	0	6	11	0	6	5	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
523	12	0	0	0	3	7	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
524	4	0	5	0	1	1	0	0	7	0	0	k__Bacteria;p__Myxococcota;c__Myxococcia;o__Myxococcales;f__Myxococcaceae;g__;s__
525	1	0	4	0	4	8	0	1	0	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Gordonibacter;s__
526	0	12	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Kineosporiales;f__Kineosporiaceae;g__Kineococcus
527	0	0	1	0	0	5	0	4	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
528	6	0	1	0	1	0	0	3	6	3	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__211ds20;f__211ds20;g__211ds20;s__
529	6	0	2	0	2	4	0	1	7	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__env.OPS_17;g__env.OPS_17;s__
530	0	13	0	0	0	0	1	0	0	0	0	k__Bacteria
531	12	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Terrimonas;s__
532	0	0	2	5	1	10	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Marvinbryantia;s__
533	7	1	1	0	1	1	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Fenollaria;s__
534	3	4	0	0	5	10	0	0	0	11	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia;s__
535	3	1	3	2	0	5	0	1	8	0	1	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
536	4	1	2	0	0	1	1	3	10	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptococcales;f__Peptococcaceae;g__;s__
537	1	0	3	1	0	0	0	0	9	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptococcales;f__Peptococcaceae;g__Peptococcus;s__
538	15	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Acholeplasmatales;f__Acholeplasmataceae;g__Anaeroplasma;s__
539	0	2	0	0	0	9	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
540	0	0	5	0	6	3	0	8	6	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
541	16	0	1	0	0	0	2	4	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__;s__

542	0	14	2	0	0	2	0	1	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__IMCC26256;f__IMCC26256;g__IMCC26256;s__
543	12	0	3	0	5	6	0	3	18	1	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__;s__
544	1	0	6	0	0	4	0	1	4	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
545	0	12	0	0	0	0	0	0	0	0	0	k__Bacteria
546	0	12	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Aureimonas
547	23	0	3	0	2	1	0	5	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Hydrogenophaga;s__
548	0	10	0	0	0	0	0	0	0	0	0	Unknown
549	11	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__Solirubrobacter;s__
550	12	0	14	0	13	19	0	12	15	4	2	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Nocardiaceae;g__Rhodococcus;s__Rhodococcus_ruber
551	0	10	0	0	0	0	0	0	0	0	0	k__Bacteria
552	7	0	4	0	2	3	0	7	3	1	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__NS11-12_marine_group;g__NS11-12_marine_group;s__
553	6	0	1	0	3	7	0	1	1	0	2	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__;s__
554	0	7	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Bergeyella;s__
555	7	0	1	1	19	21	3	6	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
556	4	0	2	0	2	1	0	2	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__KI89A_clade;f__KI89A_clade;g__KI89A_clade;s__
557	8	14	1	0	0	0	2	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__;s__
558	6	0	2	0	2	8	0	2	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__Aeromonas;s__Aeromonas_veronii
559	0	0	0	8	0	0	1	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Ruminococcus;s__Ruminococcus_flavefaciens
560	2	0	5	2	3	3	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
561	7	0	1	0	2	0	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Azospirillales;f__Azospirillaceae;g__Skermanella;s__
562	2	0	0	0	1	0	0	0	0	2	10	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Listeriaceae;g__Brochothrix;s__Brochothrix_thermosphacta
563	10	0	0	0	0	0	1	0	1	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Acetatifactor;s__
564	0	13	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodomicrobiaceae;g__Rhodomicrobium;s__
565	4	0	1	0	1	1	0	6	7	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales;f__Reyranellaceae;g__Reyranella;s__
566	0	14	0	3	0	0	0	0	0	0	0	k__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter
567	1	0	6	0	1	1	0	1	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ethanoligenenaceae;g__Acetanaerobacterium;s__
568	0	0	0	0	0	7	0	6	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Acholeplasmatales;f__Acholeplasmataceae;g__Anaeroplasma;s__
569	9	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae
570	3	0	3	0	1	5	0	2	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Synechococcales;f__Prochlorothrichaceae;g__Prochlorothrix_PCC-9006;s__
571	7	0	6	0	6	3	0	9	3	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20
572	2	0	2	0	3	2	0	5	2	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Oscillatoriaceae;g__Planktothricoides_SR001;s__
573	0	10	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
574	0	0	0	0	2	0	0	32	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Altererythrobacter;s__
575	3	0	2	0	3	1	0	2	3	0	2	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__TRA3-20;g__TRA3-20;s__
576	0	27	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Turicibacter;s__
577	2	2	2	4	4	0	0	2	7	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
578	8	0	0	0	0	3	0	19	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__PLTA13;f__PLTA13;g__PLTA13;s__
579	3	10	7	0	0	0	3	14	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Marmoricola
580	5	0	2	0	1	7	0	3	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Azospirillales;f__Azospirillaceae;g__;s__
581	5	0	6	0	1	1	0	5	9	0	0	k__Bacteria;p__Hydrogenedentes;c__Hydrogenedentia;o__Hydrogenedentiales;f__Hydrogenedensaceae;g__Hydrogenedensaceae;s__
582	4	0	3	0	5	2	0	1	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Marvinbryantia
583	98	18	3	0	6	11	0	10	81	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter
584	3	0	0	0	1	0	0	25	3	1	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Micropepsales;f__Micropepsaceae;g__;s__
585	6	0	1	1	2	9	0	0	7	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Ruminococcus;s__
586	3	0	2	0	1	2	0	1	2	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
587	11	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Cellulomonadaceae;g__Cellulomonas;s__Cellulomonas_sp.
588	0	12	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Xylochloris_irregularis
589	6	0	4	0	3	9	2	3	9	1	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
590	13	1	0	0	0	0	0	2	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Promicromonosporaceae;g__Cellulosimicrobium;s__Cellulosimicrobium_cellulans
591	5	9	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces;s__Gleimia_europaea
592	0	8	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Bacillaria_paxillifer
593	0	11	0	0	0	0	0	0	0	0	0	k__Bacteria
594	9	0	0	0	0	0	0	0	0	0	0	k__Bacteria
595	0	10	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__Prevotella_nanceiensis
596	0	23	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Spirosomaceae;g__Dyadobacter
597	4	7	0	3	3	0	4	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
598	9	0	0	0	3	0	0	2	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae
599	6	0	10	0	4	11	0	4	9	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Sediminibacterium;s__
600	8	0	0	1	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Colidextribacter;s__
601	7	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Alkanindiges;s__
602	0	6	0	3	0	2	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__;g__;s__
603	10	0	1	0	0	0	0	1	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Hymenobacteraceae;g__Hymenobacter;s__Hymenobacter_sp.
604	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__Bauldia;s__
605	5	0	0	1	2	0	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
606	4	0	4	0	1	3	0	0	6	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__MWH-UniP1_aquatic_group;g__MWH-UniP1_aquatic_group;s__
607	0	0	0	0	0	0	0	2	0	7	3	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas
608	1	3	11	0	0	2	0	0	9	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__;s__
609	3	0	1	3	1	2	0	0	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NK4A214_group;s__
610	7	0	0	0	0	1	0	4	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Vulcanibacterium;s__
611	16	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
612	0	9	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Hydrogenophilaceae;g__Hydrogenophilus;s__Hydrogenophilus_thermoluteolus
613	2	0	1	0	1	3	0	6	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Ga0077536;f__Ga0077536;g__Ga0077536;s__
614	6	0	1	0	0	0	0	1	1	0	0	k__Bacteria;p__Deinococcota;c__Deinococci;o__Deinococcales;f__Deinococcaceae;g__Deinococcus
615	17	0	2	0	0	1	1	3	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Rathayibacter;s__Rathayibacter_tritici
616	4	9	0	0	0	0	1	0	0	0	0	k__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium;s__Fusobacterium_periodonticum
617	0	1	0	0	11	4	0	0	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Butyricicoccaceae;g__Butyricicoccus;s__
618	0	0	2	0	1	5	0	1	5	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Chromobacteriaceae;g__Vogesella;s__
619	13	0	0	0	0	1	4	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast

620	0	8	0	0	0	0	0	0	0	0	0	Unknown
621	0	0	0	0	0	0	12	0	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__RF39;f__RF39;g__RF39;s__
622	0	0	0	0	0	0	0	0	0	0	10	k__Bacteria;p__Firmicutes;c__Bacilli;o__Mycoplasmatales;f__Mycoplasmataceae;g__Mycoplasma;s__
623	12	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__mle1-7;s__
624	19	4	0	4	2	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
625	4	0	0	0	0	0	0	0	1	2	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales
626	9	0	1	0	11	3	0	3	10	1	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Spongiibacteraceae;g__BD1-7_clade;s__
627	3	11	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Varibaculum
628	31	0	16	0	3	7	0	36	7	0	4	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__Sphingomonas_koreensis
629	6	0	11	11	14	0	3	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
630	8	1	6	0	0	3	0	2	8	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
631	56	0	34	0	35	37	0	31	64	8	5	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__Sporichthyaceae;s__
632	8	0	0	0	1	1	0	4	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Paenibacillales;f__Paenibacillaceae
633	3	0	0	0	0	1	0	5	7	0	0	k__Bacteria;p__Planctomycetota;c__OM190;o__OM190;f__OM190;g__OM190;s__
634	1	0	0	0	0	4	0	2	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__Conexibacter;s__
635	0	12	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__Prevotella_corporis
636	6	0	1	0	0	2	0	1	6	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Spongiibacteraceae;g__BD1-7_clade;s__
637	0	0	2	3	2	14	3	0	0	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
638	9	0	2	0	1	0	0	0	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia;s__
639	5	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
640	4	0	0	0	3	4	0	1	7	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia
641	6	1	0	1	5	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__Clostridium_leptum
642	0	0	0	15	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Hymenobacteraceae;g__Pontibacter;s__Pontibacter_sp.
643	11	0	16	2	14	1	1	4	9	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
644	4	2	1	9	3	1	1	1	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
645	0	6	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Gallicola;s__
646	6	3	3	14	2	1	1	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__ASF356;s__
647	0	0	0	0	0	0	9	0	0	0	0	Unknown
648	2	10	0	45	0	0	18	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_stationis
649	0	11	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Oceanobacillus;s__
650	0	0	0	0	0	0	0	9	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Polyangiaceae;g__;s__
651	3	0	0	2	0	0	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__UCG-010;g__UCG-010;s__
652	25	14	0	1	5	10	0	13	15	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium
653	6	0	0	0	0	0	1	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Watanabea_reniformis
654	3	0	0	0	0	0	0	1	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__Cardiobacteriaceae
655	6	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Moraxella;s__
656	0	0	0	0	0	0	0	16	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas
657	0	0	4	0	3	1	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__A0839;g__A0839;s__
658	0	11	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae;g__Chthoniobacter;s__
659	1	0	3	0	1	2	0	0	1	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Bifidi19;f__Bifidi19;g__Bifidi19;s__
660	13	0	9	0	3	5	0	5	11	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__Ellin6067;s__
661	0	11	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Mogibacterium;s__Mogibacterium_sp.
662	49	4	1	0	2	30	0	0	20	2	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
663	7	0	5	0	1	4	0	1	11	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__Candidatus_Planktophila
664	2	0	4	0	1	1	0	2	2	0	0	k__Bacteria;p__Nitrospirota;c__Nitrospiria;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__
665	0	0	0	8	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Mycobacteriaceae;g__Mycobacterium;s__Mycobacterium_hassiacum
666	0	0	0	0	0	1	0	0	0	5	5	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Dubosiella;s__Firmicutes_bacterium
667	0	0	8	0	0	0	0	0	6	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia;s__
668	8	3	1	0	0	6	5	4	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Nakamurellaceae;g__Nakamurella
669	2	0	2	0	0	0	0	1	1	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
670	0	10	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__AKYH767;g__AKYH767;s__
671	0	9	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__;s__
672	8	1	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Pseudonocardiales;f__Pseudonocardiaceae;g__Pseudonocardia;s__
673	2	0	5	0	3	1	0	4	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Cryomorphaceae;g__;s__
674	21	7	0	2	1	1	7	4	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae
675	5	0	3	0	11	2	4	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
676	0	0	2	0	3	3	0	2	2	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__;g__;s__
677	0	9	0	0	0	0	0	0	0	0	0	Unknown
678	0	1	8	0	7	6	5	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculum;s__
679	6	0	1	0	1	5	0	3	6	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae
680	0	6	0	0	0	0	0	0	0	0	0	k__Bacteria
681	0	0	4	1	2	5	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
682	1	0	1	0	0	5	0	0	0	0	0	k__Bacteria;p__Chloroflexi
683	1	0	3	0	0	1	0	3	4	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
684	0	8	0	0	0	0	0	0	0	0	0	k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobrevibacter;s__Methanobrevibacter_smithii
685	0	0	0	0	0	0	0	9	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Micropepsales;f__Micropepsaceae;g__;s__
686	4	0	0	0	0	6	0	1	4	0	1	k__Bacteria;p__Dependentiae;c__Babeliae;o__Babeliales;f__Babeliales;g__Babeliales;s__
687	15	0	0	0	1	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
688	0	7	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales;f__Defluviicoccaceae;g__Defluviicoccus;s__
689	4	0	0	1	1	2	1	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis;s__
690	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Eubacterium_coprostanoligenes_group;g__Eubacterium_coprostanoligenes_group;s__
691	4	3	0	0	2	0	0	1	9	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
692	3	0	0	0	3	8	0	2	5	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
693	4	0	2	0	3	0	1	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Methylocystis
694	1	6	1	2	0	1	0	2	8	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_FCS020_group;s__
695	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__TM7a
696	1	0	2	4	0	0	1	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__;g__;s__Azospirillum_sp.
697	2	0	4	0	1	2	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__Sulfuritalea;s__

698	5	0	2	0	1	0	0	9	5	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
699	0	10	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__67-14;g__67-14;s__
700	1	0	1	0	2	4	0	1	2	0	2	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__;g__;s__
701	4	0	0	0	1	1	0	3	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Fodinicurvataceae;g__;s__
702	0	9	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium;s__
703	4	0	3	0	1	5	0	7	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
704	1	0	1	0	0	3	0	0	4	0	0	k__Bacteria;p__Armatimonadota;c__Fimbriimonadia;o__Fimbriimonadales;f__Fimbriimonadaceae;g__Fimbriimonadaceae;s__
705	0	0	0	2	5	1	0	0	1	0	1	k__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Gastranaerophilales;f__Gastranaerophilales;g__Gastranaerophilales;s__
706	23	2	8	5	6	9	0	1	25	1	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis;s__Clostridium_sp.
707	1	0	2	0	1	3	0	0	4	0	0	k__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales;f__Gemmataceae;g__
708	0	14	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
709	0	3	0	1	3	3	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Eubacterium_siraeum_group;s__
710	3	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Murdochiella;s__Murdochiella_asaccharolytica
711	1	0	0	0	0	18	0	0	0	2	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
712	2	0	1	0	0	0	0	0	3	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium;s__
713	11	0	17	0	0	2	0	0	1	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__;s__
714	5	0	0	0	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Christensenellales;f__Christensenellaceae;g__Christensenella;s__Christensenella_minuta
715	1	0	3	0	5	5	0	7	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MND1;s__
716	1	0	2	0	2	4	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia
717	5	0	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SJA-15;f__SJA-15;g__SJA-15;s__
718	0	0	0	0	0	0	0	0	5	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Crocinitomicaceae;g__Fluviicola;s__
719	0	0	0	12	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Haloimpatiens;s__bacterium_NLAE-zl-C463
720	7	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
721	1	7	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides
722	12	14	0	3	0	15	1	0	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_suicordis
723	0	8	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Acidothermaceae;g__Acidothermus;s__
724	89	67	222	1	16	13	0	12	14	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
725	0	8	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes
726	0	0	0	0	0	0	0	10	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Actinomarinales
727	5	10	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Geobacillus
728	2	10	1	1	0	0	0	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Intrasporangiaceae;g__Ornithinibacter;s__
729	0	5	0	0	0	0	0	0	0	0	0	k__Bacteria
730	3	0	0	0	3	2	0	3	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MND1;s__
731	4	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter;s__Oscillibacter_sp.
732	2	0	7	0	2	0	0	1	5	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__SH3-11;s__
733	0	1	2	0	1	1	0	4	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__RF39;f__RF39;g__RF39;s__
734	4	0	1	0	0	1	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Ferrovibrionales;f__Ferrovibrionales
735	4	0	2	0	3	0	0	1	3	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__Ohtaekwangia;s__
736	0	1	0	0	0	3	0	1	3	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
737	7	0	1	0	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__UCG-005;s__
738	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Cyanobacteria;c__Sericytochromatia;o__Sericytochromatia;f__Sericytochromatia;g__Sericytochromatia;s__
739	11	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio;s__Desulfovibrio_fairfieldensis
740	7	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Hymenobacteraceae;g__Hymenobacter
741	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria
742	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria
743	2	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
744	4	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
745	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__Conexibacter;s__
746	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae
747	6	0	6	0	5	4	0	5	10	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae
748	0	0	2	0	2	3	0	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__;s__
749	0	20	0	3	0	0	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus
750	3	0	1	0	0	1	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__;g__;s__
751	1	0	0	0	0	1	0	0	5	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Anaerotruncus;s__
752	1	0	1	0	1	0	1	0	3	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Enterorhabdus;s__
753	0	10	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces
754	2	0	0	0	0	0	0	17	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Mesorhizobium
755	0	0	0	0	0	0	0	11	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Hyphomonadaceae;g__SWB02;s__
756	29	44	1	1	1	5	0	1	12	1	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus_danieliae
757	2	0	3	0	0	0	0	0	0	0	0	Unknown
758	0	15	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__KD4-96;o__KD4-96;f__KD4-96;g__KD4-96;s__
759	7	0	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales
760	0	9	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Xiphinematobacteraceae;g__Candidatus_Xiphinematobacter;s__
761	7	0	0	0	1	1	0	0	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
762	0	10	0	0	0	0	0	0	0	0	0	k__Bacteria
763	0	1	0	5	0	0	15	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
764	2	0	2	0	0	4	0	0	4	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__053A03-B-DI-P58;f__053A03-B-DI-P58;g__053A03-B-DI-P58;s__
765	0	0	0	0	0	2	0	3	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae
766	2	0	4	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eubacterium_xylanophilum_group;s__
767	4	0	0	0	0	0	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__IMCC26256;f__IMCC26256;g__IMCC26256;s__
768	0	0	1	2	0	2	2	0	5	0	0	k__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Gastranaerophilales;f__Gastranaerophilales;g__Gastranaerophilales;s__
769	1	0	1	0	3	2	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Candidatus_Megaira;s__
770	7	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Polyangiaceae;g__Pajaroellobacter;s__
771	1	0	1	0	1	2	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides
772	5	0	0	0	0	1	0	1	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas
773	1	5	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Friedmanniella;s__
774	2	0	2	0	3	4	0	0	4	0	0	k__Bacteria;p__Bacteroidota;c__Kapabacteria;o__Kapabacteriales;f__Kapabacteriales;g__Kapabacteriales;s__
775	0	0	0	3	0	0	6	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_sartorii

776	13	0	1	0	0	3	0	1	1	0	0	k__Bacteria
777	0	1	4	1	1	0	0	0	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
778	9	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Citricoccus;s__Citricoccus_sp.
779	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria
780	2	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__;f__;g__;s__
781	0	0	0	0	0	10	0	0	0	0	0	k__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter;s__Campylobacter_hominis
782	0	0	0	0	0	5	0	5	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Odoribacter;s__
783	0	5	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia;f__Hungateiclostridiaceae;g__Fastidiosipila;s__Fastidiosipila_sanguinis
784	0	5	0	0	0	0	0	0	0	0	0	k__Bacteria
785	8	0	5	0	6	5	0	2	3	0	0	k__Bacteria;p__SAR324_clade(Marine_group_B);c__SAR324_clade(Marine_group_B);o__SAR324_clade(Marine_group_B);f__SAR324_clade(Marine_group_B);g__SAR324_clade(Marine_group_B);s__
786	0	10	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Hymenobacteraceae;g__Hymenobacter;s__
787	0	0	0	6	0	0	0	0	0	0	0	k__Bacteria
788	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Rosiflexaceae;g__;s__
789	5	0	1	0	2	0	0	0	3	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__;g__;s__
790	0	0	1	1	1	2	0	2	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
791	3	0	0	0	0	1	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MN01;s__
792	4	0	5	0	0	4	0	0	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae
793	1	0	1	4	2	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Butyricicoccaceae;g__UCG-009
794	1	0	3	0	2	5	0	0	8	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
795	1	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
796	2	0	2	0	2	5	0	1	1	1	1	k__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Blastocatellales;f__Blastocatellaceae
797	7	0	3	0	1	1	0	2	9	0	1	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
798	9	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Photobacterium;s__Photobacterium_phosphoreum
799	37	21	90	2	1	9	1	27	12	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae
800	1	0	2	0	1	0	0	2	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Coxiellales;f__Coxiellaceae;g__Coxiella;s__
801	0	7	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacterium;s__Sphingobacterium_hotanense
802	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Chloroflexi;c__OLB14;o__OLB14;f__OLB14;g__OLB14;s__
803	0	9	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Chloroflexi;c__KD4-96;o__KD4-96;f__KD4-96;g__KD4-96;s__
804	6	0	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio;s__
805	0	0	2	0	0	0	0	0	3	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
806	1	0	0	0	3	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Magnetospiraceae;g__;s__
807	0	0	0	0	0	0	7	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__IMCC26256;f__IMCC26256;g__IMCC26256;s__
808	0	0	3	0	0	0	0	0	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
809	6	2	1	0	0	0	0	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae
810	2	0	1	0	0	4	0	0	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__;s__
811	2	0	3	0	6	3	0	0	3	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__NS11-12_marine_group;g__NS11-12_marine_group;s__
812	3	0	0	0	1	1	0	1	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
813	4	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Acidothermaceae;g__Acidothermus
814	84	8	27	19	14	62	5	17	151	3	12	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__;s__Clostridium_sp.
815	12	0	0	0	0	0	0	0	0	5	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
816	5	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Rhodanobacteraceae;g__Pseudofulvimonas;s__
817	2	0	1	0	0	4	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales
818	0	0	0	0	0	1	0	1	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
819	7	0	1	0	2	6	0	2	14	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Limnobacter;s__
820	1	0	0	0	3	3	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__AT-s3-44;f__AT-s3-44;g__AT-s3-44;s__
821	2	0	2	0	4	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Spongiibacteraceae;g__BD1-7_clade;s__
822	6	0	3	0	2	1	0	1	4	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__DEV114;s__
823	0	0	0	0	0	0	0	0	4	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae
824	9	0	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__67-14;g__67-14;s__
825	1	0	4	0	0	0	0	1	6	1	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__
826	1	0	0	3	1	2	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia
827	2	0	0	0	3	5	0	1	1	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae
828	0	9	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Anaerostipes
829	6	0	0	0	1	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Rubritepida;s__
830	2	0	0	0	0	0	0	2	7	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__AKYH767;g__AKYH767;s__
831	9	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
832	0	0	8	0	0	1	0	3	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Pseudoalteromonadaceae;g__Pseudoalteromonas
833	0	0	1	0	0	1	0	16	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Luteimonas;s__Lysobacter_sp.
834	3	7	1	0	0	1	0	1	3	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__;s__Globicatella_sp.
835	1	7	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium
836	1	0	0	0	0	0	0	0	6	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
837	0	0	2	0	0	0	0	0	2	0	0	k__Bacteria;p__Myxococcota;c__Myxococcia;o__Myxococcales;f__Anaeromyxobacteraceae;g__Anaeromyxobacter
838	0	0	0	0	0	2	0	0	1	8	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Candidatus_Arthromitus;s__Candidatus_Arthromitus
839	0	6	0	0	0	0	0	0	0	0	0	Unknown
840	7	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micromonosporales;f__Micromonosporaceae;g__Actinoplanes
841	2	0	4	0	3	0	0	3	7	0	1	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae;g__LD29;s__
842	0	6	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Paenibacillales;f__Paenibacillaceae;g__Ammoniphilus;s__
843	1	0	0	0	0	1	0	0	5	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Pseudohongiellaceae;g__Pseudohongiella;s__
844	3	0	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
845	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria
846	0	0	0	0	0	0	0	0	3	1	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
847	0	5	1	0	0	0	0	3	1	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__67-14;g__67-14;s__
848	2	0	1	0	11	6	6	17	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Dermacoccaceae;g__Kytococcus
849	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
850	3	0	0	0	0	0	0	1	7	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Harryflintia;s__
851	3	0	0	0	0	2	0	8	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Iamiaceae;g__Iamia;s__
852	5	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Agrococcus
853	1	0	2	0	1	3	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Lautropia;s__

854	2	0	2	0	18	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
855	6	0	2	0	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
856	3	0	2	0	1	2	0	0	0	0	0	k__Bacteria;p__Latescibacterota;c__Latescibacterota;o__Latescibacterota;f__Latescibacterota;g__Latescibacterota;s__
857	1	6	0	0	0	2	0	2	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides
858	15	0	0	0	3	3	1	6	4	1	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix;s__
859	4	0	0	0	1	0	0	0	0	0	0	k__Bacteria
860	0	7	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides
861	0	0	0	0	0	1	0	29	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Acidibacter;s__
862	6	1	0	0	7	4	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
863	1	0	0	0	1	3	0	5	3	0	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__TRA3-20;g__TRA3-20;s__
864	1	0	1	1	2	2	0	1	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales;g__Peptoniphilus;s__Peptoniphilus_lacrimalis
865	0	4	0	0	0	0	0	0	0	0	0	Unknown
866	9	0	0	0	0	0	0	9	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__CCD24;f__CCD24;g__CCD24;s__
867	0	0	0	0	0	1	0	2	1	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
868	2	0	2	1	2	1	2	0	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
869	2	0	2	0	2	0	0	3	6	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
870	4	0	0	0	1	1	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Microtrichaceae;g__;s__
871	6	0	3	0	1	7	0	10	10	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
872	5	1	0	1	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__Clostridium_sp.
873	0	9	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Pseudomuriella_schumacherensis
874	2	0	2	0	2	2	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__KD4-96;o__KD4-96;f__KD4-96;g__KD4-96;s__
875	7	0	6	0	2	4	0	4	7	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Methylophilaceae;g__Methylotenera;s__
876	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Streptosporangiales;f__Streptosporangiaceae;g__Nonomuraea
877	1	0	0	0	0	7	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella;s__
878	5	0	0	0	0	0	0	9	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__Ellin6067;s__
879	0	0	7	0	1	1	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__SL56_marine_group;o__SL56_marine_group;f__SL56_marine_group;g__SL56_marine_group;s__
880	5	0	1	0	0	3	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Solibacillus
881	2	5	0	0	1	3	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
882	1	0	0	0	0	1	0	5	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Curvibacter;s__
883	4	9	0	0	0	3	1	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus_urinalis
884	0	0	0	0	0	0	0	9	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Rhodanobacteraceae;g__Dokdonella;s__
885	6	1	3	2	1	2	3	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
886	0	0	0	0	0	0	0	14	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Dongiales;f__Dongiaceae;g__Dongia
887	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Pseudonocardiales;f__Pseudonocardiaceae;g__Saccharopolyspora;s__
888	0	0	0	0	0	3	0	2	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Acidobacteriae;f__Acidobacteriae;g__Paludibaculum;s__
889	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__Solirubrobacter;s__
890	1	0	1	0	1	1	0	2	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Spirosomaceae;g__Pseudarcicella;s__
891	4	0	0	0	0	2	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
892	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__;g__;s__

893	2	0	5	0	3	3	0	15	8	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
894	1	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Bril41;g__Bril41;s__
895	3	1	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Frederiksenia;s__
896	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__PAUC26f;f__PAUC26f;g__PAUC26f;s__
897	0	0	1	0	3	1	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__Nitrosospira
898	0	0	0	0	0	0	0	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__mle1-7;s__
899	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__;g__;s__
900	0	0	0	0	0	0	0	4	0	0	0	Unknown
901	0	0	0	0	0	0	0	3	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__NS11-12_marine_group;g__NS11-12_marine_group;s__
902	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium
903	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae
904	0	0	0	0	0	0	0	14	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Dongiales;f__Dongiaceae;g__Dongia;s__
905	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria
906	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20
907	0	5	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Microtrichaceae;g__Sva0996_marine_group;s__
908	0	0	0	0	0	0	0	3	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
909	1	0	0	0	0	1	0	3	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Chitinibacteraceae;g__Chitinibacter;s__
910	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Leptotrichia;s__Leptotrichia_buccalis
911	4	0	1	0	0	4	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium;s__Flavobacterium_columnare
912	12	0	4	0	0	2	0	7	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__Nordella;s__
913	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria
914	0	0	0	0	0	0	0	1	2	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Bril41;g__Bril41;s__
915	0	0	3	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales
916	1	0	0	0	0	1	0	2	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__AKYH767;g__AKYH767;s__
917	3	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Azospirillales;f__Azospirillaceae;g__Azospirillum
918	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Edaphobacillum;s__Spumella-like_flagellate
919	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__;f__;g__;s__
920	0	0	2	0	2	1	0	0	0	0	1	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__Parvibacter
921	0	1	0	1	3	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_caecimuris
922	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__;f__;g__;s__
923	0	0	2	0	0	3	0	1	4	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__env.OPS_17;g__env.OPS_17;s__
924	0	0	0	0	1	4	0	0	0	1	0	k__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales;f__Gemmataceae;g__;s__bacterium_enrichment
925	5	0	0	0	0	1	0	1	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
926	0	1	0	2	0	1	1	3	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Christensenellales;f__Christensenellaceae;g__;s__
927	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
928	0	0	0	0	6	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia
929	1	0	0	0	0	3	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__;f__;g__;s__
930	0	0	0	0	0	0	0	10	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Steroidobacteraceae;g__Steroidobacter;s__
931	6	0	2	0	0	1	0	8	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__Ellin6067;s__

932	6	0	3	0	1	0	0	1	0	0	1	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae;g__Chthoniobacter;s__
933	0	0	0	0	0	2	1	0	3	0	3	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Butyricimonas
934	0	0	3	0	0	0	0	0	0	0	0	k__Bacteria
935	0	0	4	0	0	0	0	0	0	0	0	k__Bacteria;p__Sva0485;c__Sva0485;o__Sva0485;f__Sva0485;g__Sva0485;s__
936	0	0	4	0	0	1	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Vibrio
937	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Blastocatellales;f__Blastocatellaceae;g__;s__
938	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Rosea
939	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micromonosporales;f__Micromonosporaceae;g__Phytomonospora;s__Phytomonospora_endophytica
940	0	0	0	0	0	0	0	1	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
941	0	0	0	0	1	0	0	2	0	0	0	k__Bacteria
942	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria
943	12	7	0	0	0	2	2	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Massilia;s__
944	2	0	0	0	1	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales;f__Geminicoccaceae;g__Geminicoccus;s__
945	0	0	0	7	1	0	6	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
946	0	7	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Caproiciproducens;s__
947	0	0	0	0	0	0	0	8	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__IMCC26256;f__IMCC26256;g__IMCC26256;s__
948	5	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Myrmecia_israeliensis
949	0	0	0	0	0	1	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
950	0	4	0	0	0	0	0	0	0	0	0	Unknown
951	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas;s__Porphyromonas_endodontalis
952	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__
953	13	0	20	0	5	6	0	4	12	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Dinghuibacter;s__
954	3	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__SAR324_clade(Marine_group_B);c__SAR324_clade(Marine_group_B);o__SAR324_clade(Marine_group_B);f__SAR324_clade(Marine_group_B);g__SAR324_clade(Marine_group_B);s__bacterium_
955	3	0	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Dinghuibacter;s__
956	2	0	2	0	0	8	0	10	8	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Candidatus_Planktoluna;s__
957	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas
958	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria
959	1	2	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
960	2	1	0	2	3	2	1	0	8	0	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
961	0	0	0	0	2	1	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae
962	3	0	0	0	1	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Spirosomaceae;g__Huanghella;s__Huanghella_arctica
963	4	0	1	0	1	0	0	2	3	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Dinghuibacter;s__
964	0	0	1	0	0	1	0	2	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MND1;s__
965	5	0	1	0	1	2	0	1	6	1	1	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae
966	0	76	0	8	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_jensenii
967	0	0	0	0	0	3	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae;g__;s__
968	3	0	0	0	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia;s__
969	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Bin41;g__Bin41;s__
970	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Chitinophaga;s__Chitinophaga_japonensis

971	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__;g__;s__
972	0	0	0	0	0	0	0	2	1	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__;s__
973	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
974	6	2	12	0	32	26	2	8	35	0	2	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Colidextribacter;s__
975	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Veillonella;s__Veillonella_sp.
976	3	0	1	0	4	3	0	1	2	0	0	k__Bacteria
977	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__;s__
978	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria
979	0	0	1	0	0	3	0	1	1	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
980	0	0	0	0	1	0	0	0	3	0	0	k__Bacteria;p__Acidobacteriota
981	0	7	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__Oligoflexales;f__Oligoflexales;g__Oligoflexus;s__
982	0	0	1	0	0	2	0	2	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium
983	3	0	3	0	0	1	0	0	3	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae
984	0	0	0	4	0	0	1	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Psychrobacter
985	2	0	0	0	0	1	0	0	2	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae;g__;s__
986	1	0	0	0	1	0	1	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Monoglobales;f__Monoglobaceae;g__Monoglobus;s__
987	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Mobiluncus;s__Mobiluncus_curtisii
988	2	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bacteriovoracales;f__Bacteriovoracaceae;g__Peredibacter;s__microbial_mat
989	5	0	2	0	2	4	0	3	2	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
990	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium;s__
991	0	1	4	0	0	1	1	1	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__UBA1819;s__
992	0	0	2	5	0	1	0	0	1	0	3	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
993	0	0	0	0	0	0	0	0	4	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Roseiflexaceae;g__;s__
994	0	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Cutibacterium;s__Cutibacterium_acnes
995	2	0	1	0	1	0	0	1	1	0	0	k__Bacteria;p__Chloroflexi;c__Dehalococcoidia;o__SB05;f__SB05;g__SB05;s__
996	0	10	0	0	0	0	0	0	0	0	0	Unknown
997	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__KD4-96;o__KD4-96;f__KD4-96;g__KD4-96;s__
998	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
999	0	0	0	0	0	6	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;s__Acinetobacter_towneri
1000	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Gallionellaceae;g__Sideroxydans;s__
1001	0	0	0	0	1	4	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Monoglobales;f__Monoglobaceae;g__Monoglobus;s__
1002	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Roseisolibacter;s__
1003	1	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales;f__;g__;s__
1004	11	0	14	0	0	2	1	0	6	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas;s__Pseudomonas_fragi
1005	1	0	1	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Ga0077536;f__Ga0077536;g__Ga0077536;s__
1006	2	1	2	0	1	5	2	1	15	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
1007	23	44	42	1	0	11	47	6	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Cutibacterium;s__Cutibacterium_granulosum
1008	5	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Isosphaerales;f__Isosphaeraceae;g__Tundrisphaera;s__
1009	5	0	3	0	0	4	0	4	2	0	1	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__PeM15;f__PeM15;g__PeM15;s__

1010	0	0	3	0	0	0	0	0	3	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
1011	1	0	4	0	1	4	0	1	9	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae
1012	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Blastocatellales;f__Blastocatellaceae
1013	2	0	0	0	2	1	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales;f__Reyranellaceae;g__;s__
1014	2	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
1015	0	0	3	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1016	0	0	0	0	2	0	0	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
1017	1	0	5	0	1	0	0	2	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Opitutales;f__Opitutaceae;g__IMCC26134;s__
1018	6	0	0	0	0	0	1	13	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium
1019	3	0	0	0	1	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Thermomonas
1020	1	0	0	0	0	2	2	6	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales;f__Reyranellaceae;g__Reyranella;s__
1021	1	0	1	0	0	0	0	5	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Nocardioides
1022	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
1023	26	7	11	0	10	19	3	16	10	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1024	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria
1025	0	0	0	0	0	3	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Spirosomaceae;g__Pseudarcicella;s__
1026	0	0	1	0	0	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
1027	0	0	0	2	0	0	0	10	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Streptosporangiales;f__Thermomonosporaceae;g__Actinomadura;s__Actinomadura_sp.
1028	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Diplorickettsiales;f__Diplorickettsiaceae;g__Aquicella;s__
1029	1	0	0	0	0	1	0	3	2	0	0	k__Bacteria;p__Chloroflexi;c__KD4-96;o__KD4-96;f__KD4-96;g__KD4-96;s__
1030	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Pseudolabrys;s__
1031	2	0	1	0	0	1	1	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Pseudonocardiales;f__Pseudonocardiaceae;g__Actinomycetospora;s__
1032	2	0	0	0	1	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae
1033	0	0	0	0	0	0	0	4	3	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
1034	0	2	0	0	0	0	0	16	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium;s__
1035	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Devosiaceae;g__Devosia
1036	3	0	0	0	0	1	0	0	2	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20;s__
1037	0	0	0	0	0	0	0	19	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__;s__
1038	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Edaphobacillum;s__
1039	4	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio
1040	0	0	0	0	8	1	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1041	0	0	0	0	0	0	0	0	4	0	0	k__Bacteria
1042	4	0	1	0	0	1	0	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae
1043	3	0	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Hymenobacteraceae;g__Hymenobacter;s__Hymenobacter_nivis
1044	1	0	0	0	1	0	0	2	5	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadales;g__Saccharimonadales;s__
1045	0	0	0	0	0	1	0	0	6	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Eubacterium_brachy_group;s__
1046	2	0	0	0	1	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella;s__
1047	2	0	0	0	3	1	0	2	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
1048	18	2	7	15	10	1	1	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__GCA-900066575;s__
1049	2	0	0	0	3	0	0	0	2	0	0	k__Bacteria;p__MBNT15;c__MBNT15;o__MBNT15;f__MBNT15;g__MBNT15
1050	0	0	0	0	0	0	0	0	4	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Roseiflexaceae;g__;s__
1051	0	0	0	0	0	0	4	0	0	0	0	Unknown
1052	0	0	0	0	0	0	4	0	0	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadales;g__Saccharimonadales;s__
1053	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Acidobacteriota;c__AT-s3-28;o__AT-s3-28;f__AT-s3-28;g__AT-s3-28;s__
1054	8	0	0	0	0	1	3	0	4	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Methylobacterium-Methylorubrum
1055	9	0	0	2	0	5	0	1	0	1	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter;s__
1056	0	0	0	6	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Ferruginibacter;s__
1057	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Acidibacter;s__
1058	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio;s__
1059	0	0	0	0	6	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rs-E47_termite_group;g__Rs-E47_termite_group;s__
1060	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Acidobacteriota;c__Holophagae;o__Subgroup_7;f__Subgroup_7;g__Subgroup_7;s__
1061	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Kiloniellaceae;g__Tagaea;s__
1062	0	0	0	0	2	0	0	1	3	0	0	k__Bacteria;p__Nitrospirota;c__Thermodesulfovibrionia;o__;f__;g__;s__
1063	7	9	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Lautropia;s__
1064	2	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
1065	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria
1066	0	0	0	0	0	0	3	0	0	0	0	Unknown
1067	0	4	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Sporomusaceae;g__Pelosinus;s__
1068	1	0	0	0	1	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae
1069	206	26	39	23	9	19	3	7	13	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium
1070	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1071	1	0	0	0	0	1	0	2	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Mitochondria;g__Mitochondria;s__
1072	5	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__;g__
1073	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__;s__
1074	0	0	0	0	0	3	0	0	0	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfobacterales;f__Desulfosarcinaceae
1075	0	0	2	0	1	3	0	1	7	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Opitutales;f__Opitutaceae;g__Opitutus;s__
1076	2	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
1077	0	0	0	0	0	1	0	1	11	1	1	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae;g__Butyricimonas;s__
1078	0	3	3	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1079	0	0	0	0	2	0	0	0	2	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Bifidi19;f__Bifdi19;g__Bifdi19;s__
1080	5	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Nitrospirota;c__Nitrospiria;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__
1081	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria
1082	0	0	0	0	0	0	3	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Chloropicon_primus
1083	0	0	0	0	0	0	3	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Paludicola
1084	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Tenacibaculum
1085	0	0	0	0	0	0	0	1	3	0	0	k__Bacteria;p__Acidobacteriota;c__Aminicenantia;o__Aminicenantales;f__Aminicenantales;g__Aminicenantales;s__
1086	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Paenarthrobacter;s__Arthrobacter_sp.
1087	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae

1088	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Streptosporangiales;f__Thermomonosporaceae;g__Actinomadura
1089	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__B1-785;g__B1-785;s__
1090	0	5	0	0	0	0	10	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Nocardioidaceae;g__Marmoricola;s__
1091	2	0	4	0	0	1	0	2	3	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae;g__Chthoniobacter;s__
1092	0	0	1	0	4	4	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1093	1	0	2	0	0	0	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Holosporales;f__Holosporaceae;g__;s__
1094	0	0	0	8	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1095	5	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Acidiphilium;s__
1096	3	0	1	0	1	3	0	0	2	0	0	k__Bacteria;p__Acidobacteriota;c__Subgroup_22;o__Subgroup_22;f__Subgroup_22;g__Subgroup_22;s__
1097	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Chloroflexi;c__TK10;o__TK10;f__TK10;g__TK10;s__
1098	0	0	0	0	0	0	1	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Anaerotruncus;s__Anaerotruncus_colihominis
1099	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
1100	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Phormidesmiales;f__Nodosilineaceae;g__Nodosilinea_PCC-7104;s__Nodosilinea_sp.
1101	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__Sphingobacteriaceae
1102	3	0	1	0	0	1	0	0	1	0	2	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Microcystaceae;g__Microcystis_PCC-7914;s__Microcystis_aeruginosa
1103	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Myxococcota;c__Myxococcia;o__Myxococcales;f__Anaeromyxobacteraceae;g__Anaeromyxobacter
1104	1	0	0	0	0	2	0	3	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
1105	2	0	0	0	1	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__PLTA13;f__PLTA13;g__PLTA13;s__
1106	0	33	1	27	0	4	3	2	25	1	5	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
1107	1	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
1108	0	0	1	0	0	2	0	0	6	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
1109	1	0	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__;s__
1110	2	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Bacteroidota;c__Kapabacteria;o__Kapabacteriales;f__Kapabacteriales;g__Kapabacteriales;s__
1111	0	0	0	0	0	2	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1112	0	0	0	0	0	1	0	1	4	0	0	k__Bacteria;p__MBNT15;c__MBNT15;o__MBNT15;f__MBNT15;g__MBNT15;s__
1113	1	0	1	0	3	0	0	0	4	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Nostocaceae;g__Cuspidothrix_LMECYA_163;s__Cuspidothrix_issatschenkoi
1114	0	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Obscuribacterales;f__Obscuribacteraceae;g__Obscuribacteraceae;s__
1115	5	0	2	0	1	1	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae
1116	0	83	1	2	1	0	3	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Dermabacteraceae;g__Brachybacterium
1117	1	1	1	1	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
1118	0	0	0	0	0	0	0	0	0	3	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales
1119	1	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Nitrospirota;c__Thermodesulfovibrionia;o__;f__;g__;s__
1120	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae
1121	0	0	0	0	1	1	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella;s__
1122	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Streptosporangiales;f__Streptosporangiaceae;g__Thermopolyspora;s__Streptosporangiaceae_str.
1123	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__TX1A-33;c__TX1A-33;o__TX1A-33;f__TX1A-33;g__TX1A-33;s__
1124	3	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Aureimonas;s__Aureimonas_sp.
1125	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Staphylococcaceae;g__Nosocomiicoccus;s__
1126	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1127	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria
1128	1	0	2	0	0	1	0	4	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Hyphomonadaceae;g__SWB02;s__
1129	0	0	0	0	0	3	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
1130	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria
1131	0	1	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Pseudanabaenales;f__Pseudanabaenaceae;g__Pseudanabaena_PCC-7429
1132	0	0	0	0	0	0	0	3	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella;s__
1133	0	0	2	0	0	0	0	0	2	0	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylococcaceae;s__
1134	65	0	12	0	5	19	0	9	180	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas
1135	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Dependentiae;c__Babeliae;o__Babeliales;f__UBA12409;g__UBA12409;s__
1136	0	0	1	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Holosporales;f__Holosporaceae;g__Candidatus_Berleisia;s__
1137	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae;g__Bryobacter;s__
1138	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__Thauera
1139	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae
1140	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium;s__
1141	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Leptotrichia;s__Leptotrichia_sp.
1142	0	0	0	0	1	1	0	2	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_farciminis
1143	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__mle1-7;s__
1144	0	0	0	0	0	1	0	3	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Ferrovibrionales;f__Ferrovibrionales;g__Tsuneella;s__
1145	0	0	0	0	0	1	0	0	7	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1146	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Gemmatimonadota;c__BD2-11_terrestrial_group;o__BD2-11_terrestrial_group;f__BD2-11_terrestrial_group;g__BD2-11_terrestrial_group;s__
1147	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Kallotenuales;f__AKIW781;g__AKIW781;s__
1148	0	0	1	0	0	0	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1149	0	0	0	0	1	0	0	0	0	0	1	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__;s__
1150	0	0	0	0	1	1	0	0	0	0	0	k__Bacteria
1151	0	0	0	0	4	2	0	2	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1152	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1153	10	0	0	0	0	0	0	24	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Streptomycetales;f__Streptomycetaceae;g__Streptomyces
1154	1	0	0	0	0	2	0	3	3	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidetes_VC2.1_Bac22;f__Bacteroidetes_VC2.1_Bac22;g__Bacteroidetes_VC2.1_Bac22;s__
1155	69	27	2	0	0	1	5	11	12	5	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus;s__Enterococcus_faecalis
1156	5	0	4	0	6	3	0	2	7	3	1	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
1157	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Diplorickettsiales;f__Diplorickettsiaceae;g__Aquicella;s__
1158	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria
1159	1	5	2	1	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Filifactor
1160	0	0	0	0	0	3	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Methylophilaceae;g__Candidatus_Methylopumilus;s__
1161	0	0	0	0	0	3	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Clostridium_methylpentosum_group;g__Clostridium_methylpentosum_group;s__
1162	7	0	0	0	0	0	0	0	0	0	0	Unknown
1163	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
1164	0	0	0	0	0	3	0	6	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__;g__;s__
1165	2	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__

1166	0	0	0	0	0	1	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Flavonifractor;s__
1167	2	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Desulfuromonadia;f__Geothermobacteraceae;g__Geothermobacter;s__
1168	2	0	0	0	1	1	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Unknown_Family;s__
1169	0	0	0	0	0	0	0	1	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Steroidobacteraceae;g__;s__
1170	1	0	2	0	0	0	0	0	1	0	0	k__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales;f__Gemmataceae;g__;s__
1171	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1172	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__Gitt-GS-136;f__Gitt-GS-136;g__Gitt-GS-136;s__
1173	0	0	0	0	0	0	2	0	0	0	0	Unknown
1174	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
1175	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria
1176	3	0	0	0	0	4	0	0	5	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__TRA3-20;g__TRA3-20;s__
1177	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Elsterales;f__;g__;s__
1178	0	0	5	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Dialister;s__Dialister_micraerophilus
1179	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria
1180	5	0	0	0	0	2	0	0	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__AKYH767;g__AKYH767;s__
1181	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__A4b;g__A4b;s__
1182	1	0	0	0	2	0	0	2	1	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae;g__Bryobacter;s__
1183	0	0	1	0	0	2	0	1	0	0	0	k__Bacteria
1184	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Deferrisomatota;c__Deferrisomatia;o__Deferrisomatales;f__Deferrisomataceae;g__Deferrisoma;s__
1185	4	0	3	0	0	1	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium
1186	3	0	0	0	1	0	0	1	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__
1187	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1188	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia;s__
1189	1	0	2	0	2	1	0	0	1	0	0	k__Bacteria
1190	0	0	0	0	0	0	0	0	0	2	0	Unknown
1191	0	0	0	0	0	2	0	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1192	0	0	0	0	1	0	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__TRA3-20;g__TRA3-20;s__
1193	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Bria41;g__Bria41;s__
1194	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
1195	3	1	20	8	3	4	1	10	5	0	2	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes;s__
1196	0	0	3	0	0	1	0	0	0	0	0	k__Bacteria;p__Gemmatimonadota;c__BD2-11_terrestrial_group;o__BD2-11_terrestrial_group;f__BD2-11_terrestrial_group;g__BD2-11_terrestrial_group;s__
1197	0	2	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
1198	0	0	2	0	0	0	0	1	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium
1199	2	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__Neisseria;s__Neisseria_elongata
1200	0	0	3	0	0	0	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__;s__
1201	0	0	0	0	2	0	0	0	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Cytophagaceae;g__Cytophaga;s__
1202	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__IS-44;s__
1203	1	0	0	0	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
1204	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Chloroflexi;c__JG30-KF-CM66;o__JG30-KF-CM66;f__JG30-KF-CM66;g__JG30-KF-CM66;s__
1205	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales
1206	2	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Porticoccaceae;g__Porticoccus;s__
1207	0	0	3	0	0	2	0	3	6	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnohabitans;s__
1208	154	0	23	2	11	23	0	42	255	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas
1209	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria
1210	0	0	0	0	5	1	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella;s__
1211	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__NB1-j;c__NB1-j;o__NB1-j;f__NB1-j;g__NB1-j;s__
1212	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micromonosporales;f__Micromonosporaceae;g__Salinispora;s__
1213	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade;s__bacterium_enrichment
1214	0	0	0	0	1	1	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Paracaedibacterales;f__Paracaedibacteraceae;g__;s__Candidatus_Intestinusbacter
1215	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Rhodanobacteraceae;g__Rhodanobacter;s__Rhodanobacter_sp.
1216	1	0	5	0	2	1	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__GCA-900066575;s__
1217	1	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Blastocatellales;f__Blastocatellaceae
1218	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Defluviicoccales;f__Defluviicoccaceae;g__Defluviicoccus;s__
1219	1	0	5	0	2	0	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Erysipelatoclostridium;s__
1220	0	0	1	0	0	0	2	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
1221	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1222	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Chloroflexi;c__Dehalococcoidia;o__SAR202_clade;f__SAR202_clade;g__SAR202_clade;s__
1223	1	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Woeseiaceae;g__Woeseia;s__
1224	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria
1225	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Hydrogenophilaceae;g__;s__
1226	0	0	0	0	0	2	0	0	5	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae
1227	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Sandaracinaceae;g__;s__
1228	3	1	3	0	0	0	3	9	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__Ilumatobacter;s__
1229	5	0	0	0	2	1	0	1	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Rubrivivax;s__
1230	5	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli
1231	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Cavicella;s__
1232	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Thermomicrobiales;f__AKYG1722;g__AKYG1722;s__
1233	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Diplorickettsiales;f__Diplorickettsiaceae;g__;s__
1234	6	0	4	0	0	28	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium
1235	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
1236	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes
1237	0	0	1	0	0	0	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1238	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Elusimicrobiota;c__Lineage_IIa;o__Lineage_IIa;f__Lineage_IIa;g__Lineage_IIa;s__
1239	0	0	0	0	0	2	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium;s__
1240	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__A4b;g__A4b;s__
1241	0	0	2	0	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Anaerovorax;s__
1242	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas;s__
1243	0	0	1	0	3	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eubacterium_ventriosum_group;s__

1244	2	0	1	0	0	1	0	0	3	0	0	k__Bacteria;p__Myxococcota;c__Myxococcia;o__Myxococcales;f__Myxococcaceae;g__P3OB-42;s__
1245	0	0	1	0	0	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Gallionellaceae;g__Candidatus_Nitrotoga;s__
1246	1	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Sphaerotilus
1247	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium;s__
1248	1	0	2	0	2	2	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae
1249	1	0	1	0	1	1	0	1	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknown_Family;g__Addibacter;s__
1250	2	0	0	0	0	0	0	0	0	0	0	Unknown
1251	0	3	0	0	0	0	0	0	0	0	0	Unknown
1252	0	0	1	0	0	35	0	2	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
1253	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__Chryseolinea;s__
1254	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadales;g__Saccharimonadales;s__
1255	0	0	0	2	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1256	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Elev-165-1166;f__Elev-165-1166;g__Elev-165-1166;s__
1257	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae;g__hgcI_clade
1258	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__mle1-27;f__mle1-27;g__mle1-27;s__Myxococcales_bacterium
1259	1	0	0	0	1	0	0	0	1	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Nostocaceae;g__Sphaerospermopsis_BCCUSP55
1260	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__;s__
1261	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__;g__;s__
1262	0	0	2	0	0	0	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;g__;s__
1263	9	0	0	0	2	2	0	4	8	0	2	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Synechococcales;f__Cyanobiaceae;g__Cyanobium_PCC-6307;s__
1264	6	2	1	0	0	0	0	0	0	0	1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1265	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Bril41;g__Bril41;s__
1266	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales;f__Haliangiaceae;g__Haliangium;s__
1267	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae
1268	1	0	0	0	1	2	0	0	1	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__denitrifying_bacterium
1269	0	2	1	0	0	0	1	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1270	5	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__Prevotella_melaninogenica
1271	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Fluviicoccus
1272	0	0	0	0	0	0	0	2	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__PeM15;f__PeM15;g__PeM15;s__
1273	0	6	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella
1274	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
1275	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria
1276	2	0	0	0	0	0	0	0	0	0	1	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Acidobacteriales
1277	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Halieaceae;g__Halioglobus;s__
1278	0	0	0	0	0	0	0	9	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae
1279	0	0	0	0	7	0	0	0	1	0	0	k__Bacteria;p__Acidobacteriota;c__Subgroup_22;o__Subgroup_22;f__Subgroup_22;g__Subgroup_22;s__
1280	13	4	1	0	0	0	0	3	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
1281	2	0	1	0	0	0	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Roseomonas;s__Acetobacteraceae_bacterium
1282	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae
1283	0	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1284	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__;g__;s__
1285	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales
1286	0	0	0	0	2	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Paenibacillales;f__Paenibacillaceae;g__Paenibacillus
1287	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast;s__Pseudochlorella_signiensis
1288	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia
1289	0	0	0	0	0	0	0	0	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Lautropia;s__
1290	1	0	0	0	3	2	0	0	4	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae
1291	0	2	0	0	0	0	0	0	0	0	0	Unknown
1292	4	0	0	0	1	1	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Methylophilaceae;g__MM2;s__
1293	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1294	14	0	2	0	4	7	1	7	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__MWH-Ta3;s__
1295	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__DNF00809;s__
1296	0	0	0	0	4	1	0	0	1	0	0	k__Bacteria;p__Latescibacterota;c__Latescibacterota;o__Latescibacterota;f__Latescibacterota;g__Latescibacterota;s__
1297	0	2	9	1	0	153	2	2	0	0	3	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
1298	14	0	5	0	0	5	0	5	17	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnohabitans;s__Limnohabitans_sp.
1299	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfomicrobiaceae;g__Desulfomicrobium;s__Desulfomicrobium_orale
1300	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
1301	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Dubosiella;s__
1302	2	0	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria
1303	1	0	0	0	0	1	0	2	1	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
1304	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia
1305	0	0	1	0	0	0	0	2	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__Clostridia_vadinBB60_group;g__Clostridia_vadinBB60_group;s__
1306	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Family_XIII_AD3011_group;s__
1307	2	0	3	0	1	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__FFCH16263;f__FFCH16263;g__FFCH16263;s__
1308	0	0	0	0	0	1	0	1	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__PLTA13;f__PLTA13;g__PLTA13;s__
1309	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1310	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__Gitt-GS-136;f__Gitt-GS-136;g__Gitt-GS-136;s__
1311	0	0	1	0	1	0	0	2	0	0	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__;s__
1312	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria
1313	0	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales
1314	3	0	1	0	0	1	0	2	4	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria
1315	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1316	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Dongiales;f__Dongiaceae;g__Dongia
1317	0	8	0	2	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1318	8	0	1	0	2	2	0	3	8	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Ilumatobacteraceae;g__CL500-29_marine_group;s__
1319	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales;f__Sporichthyaceae
1320	0	0	2	0	0	1	0	1	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Mitochondria;g__Mitochondria;s__
1321	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria

1322	0	0	0	0	0	3	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1323	0	2	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Rhodanobacteraceae;g__;s__
1324	2	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__UCG-010;g__UCG-010;s__
1325	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__Pedosphaeraceae
1326	1	0	0	0	0	1	0	2	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium;s__
1327	1	0	0	0	1	0	0	0	0	3	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus
1328	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__Vicinamibacteraceae;g__Vicinamibacteraceae;s__
1329	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Opitutales;f__Opitutaceae;g__Lacunisphaera
1330	0	0	0	1	3	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Brevibacteriaceae;g__Brevibacterium
1331	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Roseiflexaceae;g__
1332	0	0	0	0	0	0	0	0	0	2	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Hungateiclostridiaceae
1333	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio
1334	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__WPS-2;c__WPS-2;o__WPS-2;f__WPS-2;g__WPS-2;s__hydrothermal_vent
1335	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Bacteroidota;c__SJA-28;o__SJA-28;f__SJA-28;g__SJA-28;s__
1336	1	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadales;g__Saccharimonadales;s__
1337	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria
1338	4	1	0	105	0	0	0	1	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Brevundimonas
1339	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Frankiales
1340	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__TM7a;s__
1341	2	0	1	0	2	5	0	1	1	0	0	k__Bacteria;p__Gemmatimonadota;c__BD2-11_terrestrial_group;o__BD2-11_terrestrial_group;f__BD2-11_terrestrial_group;g__BD2-11_terrestrial_group;s__
1342	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Selenomonadaceae;g__Megamonas;s__
1343	1	0	1	0	0	0	0	1	2	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__Nordella;s__
1344	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
1345	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Cyanobacteriales;f__Chroococcidiopsaceae;g__Chroococcidiopsis_SAG_2023
1346	0	0	0	0	0	1	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__JG36-GS-52;f__JG36-GS-52;g__JG36-GS-52;s__
1347	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1348	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae
1349	2	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__;g__;s__
1350	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Steroidobacteraceae;g__;s__
1351	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales;f__67-14;g__67-14;s__
1352	0	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Gastranaerophilales;f__Gastranaerophilales;g__Gastranaerophilales;s__
1353	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae;g__Bryobacter;s__
1354	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales
1355	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Coxiellales;f__Coxiellaceae;g__Coxiella;s__
1356	2	0	1	0	0	1	0	0	1	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__053A03-B-DI-P58;f__053A03-B-DI-P58;g__053A03-B-DI-P58;s__
1357	2	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Nitrospirota;c__Thermodesulfovibrionia;o__;f__;g__;s__
1358	0	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__ITB23;f__ITB23;g__ITB23;s__
1359	5	0	0	0	0	0	1	0	0	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__TM7a;s__
1360	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Yersiniaceae;g__Serratia;s__Serratia_liquefaciens
1361	2	0	0	0	0	0	0	0	0	0	0	Unknown
1362	0	0	0	0	0	0	0	8	0	0	0	k__Bacteria;p__Gemmatimonadota;c__S0134_terrestrial_group;o__S0134_terrestrial_group;f__S0134_terrestrial_group;g__S0134_terrestrial_group;s__
1363	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Actinobacteriota
1364	1	0	0	0	2	0	0	0	3	0	0	k__Bacteria
1365	0	0	0	0	1	0	0	1	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Spongiibacteraceae;g__BD1-7_clade;s__
1366	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas;s__Porphyromonas_circumdentaria
1367	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20;s__
1368	0	0	0	0	0	2	1	0	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Erysipelotrichaceae;s__
1369	0	0	0	2	0	0	0	0	0	0	0	Unknown
1370	1	0	3	0	1	0	0	0	6	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Aurantimicrobium;s__
1371	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
1372	0	0	0	0	0	0	0	6	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MND1;s__
1373	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__B1-7BS;g__B1-7BS;s__
1374	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales
1375	0	0	3	0	0	0	0	0	4	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1376	1	0	2	0	1	2	0	3	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__SH3-11;s__
1377	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Pseudohongiellaceae;g__Bly10;s__
1378	0	1	0	0	0	0	0	9	0	0	0	k__Bacteria;p__Acidobacteriota;c__Holophagae;o__Subgroup_7;f__Subgroup_7;g__Subgroup_7;s__
1379	1	0	0	0	0	1	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__A0839;g__A0839;s__
1380	108	16	4	0	3	1	0	7	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter
1381	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1382	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales;f__;g__;s__
1383	0	0	0	0	0	0	0	3	0	0	0	Unknown
1384	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Bacteroidota;c__Rhodothermia;o__Rhodothermales;f__Rhodothermaceae;g__;s__
1385	2	0	1	0	1	0	0	0	1	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Roseiflexaceae;g__;s__
1386	0	0	0	0	0	0	0	7	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiales_Incertae_Sedis;g__Bauldia;s__
1387	0	0	1	0	0	0	0	2	0	0	0	k__Bacteria;p__Elusimicrobiota;c__Elusimicrobia;o__Lineage_IV;f__Lineage_IV;g__Lineage_IV;s__
1388	0	0	1	0	0	0	0	3	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Thermomicrobiales;f__JG30-KF-CM45;g__JG30-KF-CM45;s__
1389	0	0	1	0	0	1	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Prosthecobacter;s__
1390	0	4	0	1	0	4	1	0	1	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
1391	6	0	16	0	7	134	0	2	32	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
1392	3	2	0	0	1	2	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1393	0	0	0	0	2	0	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae;g__Halomonas;s__Candidatus_Halomonas
1394	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Candidatus_Stoquefichus;s__
1395	4	0	2	0	1	4	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__AKYH767;g__AKYH767;s__
1396	1	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__MND1;s__
1397	0	0	1	0	3	1	0	2	2	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria
1398	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1399	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria

1400	0	0	0	0	0	1	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylomonadaceae;g__Methylomonas
1401	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1402	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Methylomirabilota;c__Methylomirabilia;o__Rokubacteriales;f__WX65;g__WX65;s__
1403	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1404	0	0	1	0	2	0	0	0	2	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Bifidi19;f__Bifdi19;g__Bifdi19;s__
1405	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae
1406	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
1407	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia
1408	0	0	0	0	0	0	2	0	0	0	0	Unknown
1409	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Steroidobacteraceae;g__;s__
1410	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
1411	2	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Pedobacter;s__Pedobacter_sp.
1412	1	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__Oligoflexales;f__;g__;s__
1413	0	5	0	0	0	0	0	0	0	0	0	k__Bacteria
1414	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnohabitans
1415	0	5	0	0	0	0	0	0	0	0	0	Unknown
1416	2	0	0	0	0	0	0	0	0	0	0	Unknown
1417	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Pseudonocardiales;f__Pseudonocardiaceae;g__Pseudonocardia;s__Pseudonocardia_dioxanivorans
1418	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Acidobacteriota;c__Thermoanaerobaculia;o__Thermoanaerobaculales;f__Thermoanaerobaculaceae;g__Subgroup_10
1419	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Reyranellales;f__Reyranellaceae;g__;s__
1420	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Pirellulales;f__Pirellulaceae;g__Pirellula;s__
1421	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella
1422	0	4	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__Microtrichaceae;g__;s__
1423	0	0	0	0	0	0	0	1	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Taibaiella
1424	0	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1425	0	2	0	0	0	0	0	0	0	0	0	Unknown
1426	0	0	7	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Vibrio
1427	0	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Methylomirabilota;c__Methylomirabilia;o__Methylomirabilales;f__Methylomirabilaceae;g__Sh765B-TzT-35;s__
1428	0	0	9	0	0	0	0	0	95	0	0	k__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__;s__
1429	0	0	4	0	0	1	0	0	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Ramlibacter;s__
1430	4	4	0	0	0	0	0	9	3	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Intrasporangiaceae;g__Terrabacter;s__Terrabacter_sp.
1431	0	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__A2
1432	35	0	16	0	8	19	0	20	38	3	1	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1433	1	0	0	0	1	3	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Steroidobacteraceae;g__
1434	1	0	0	0	1	0	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Cavicella
1435	0	0	0	0	0	2	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Brachymonas;s__Brachymonas_denitrificans
1436	0	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Roseiflexaceae;g__;s__
1437	0	0	1	0	0	0	0	0	0	1	0	k__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__
1438	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__Muribaculaceae;s__
1439	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae;g__Bryobacter
1440	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Chloroflexi;c__OLB14;o__OLB14;f__OLB14;g__OLB14;s__
1441	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Nitrospirota;c__Thermodesulfovibrionia;o__;f__;g__;s__
1442	0	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1443	3	0	15	0	11	72	4	2	42	1	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eubacterium_xylanophilum_group;s__
1444	0	0	1	0	0	2	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__RBG-13-54-9;f__RBG-13-54-9;g__RBG-13-54-9;s__
1445	39	44	17	53	8	13	210	28	24	1	1	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Staphylococcaceae;g__Staphylococcus
1446	2	0	0	0	0	1	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae
1447	3	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__;s__
1448	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Acidimicrobiia;o__Microtrichales;f__;g__;s__
1449	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Staphylococcaceae;g__Staphylococcus
1450	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria
1451	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__A4b;g__A4b;s__
1452	4	0	1	0	0	2	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
1453	0	2	0	1	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Acetitalfactor;s__
1454	0	0	0	0	0	0	0	2	1	0	0	k__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Bryobacterales;f__Bryobacteraceae;g__Bryobacter;s__
1455	2	0	0	0	0	2	0	1	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Crocinitomicaceae;g__Fluviicola;s__
1456	2	0	0	0	1	1	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__JG36-GS-52;f__JG36-GS-52;g__JG36-GS-52;s__
1457	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae
1458	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae
1459	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Crocinitomicaceae;g__Fluviicola;s__
1460	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__;s__
1461	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Chryseobacterium;s__Chryseobacterium_indologenes
1462	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1463	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio;s__Bdellovibrio_exovorus
1464	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Bifdi19;f__Bifdi19;g__Bifdi19;s__
1465	0	0	0	0	2	2	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1466	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__;s__
1467	0	0	0	0	1	0	0	2	0	2	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Eubacterium_coprostanoligenes_group;g__Eubacterium_coprostanoligenes_group;s__
1468	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Nitrosomonadaceae;g__WND1;s__
1469	0	0	3	0	0	2	0	1	1	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__
1470	0	2	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales;f__Halieaceae;g__OM60(NOR5)_clade;s__
1471	0	0	3	0	0	0	0	0	0	0	0	Unknown
1472	0	0	1	0	0	0	0	0	0	2	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1473	1	0	0	0	1	1	0	1	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus_lentus
1474	13	0	0	0	2	6	0	0	2	0	3	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia;s__Lachnospiraceae_bacterium
1475	0	0	1	0	1	0	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
1476	12	0	5	0	3	5	0	9	13	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__C39;s__
1477	3	0	0	0	0	0	0	0	1	0	0	k__Bacteria;p__Planctomycetota;c__Phycisphaerae;o__Phycisphaerales;f__Phycisphaeraceae;g__CL500-3;s__

1478	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Subgroup_11;o__Subgroup_11;f__Subgroup_11;g__Subgroup_11;s__
1479	3	0	2	0	0	1	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Paenibacillales;f__Paenibacillaceae;g__Paenibacillus
1480	0	5	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
1481	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria
1482	0	0	4	0	1	5	0	1	4	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Aquabacterium;s__
1483	1	0	1	0	2	0	0	1	0	0	0	k__Bacteria;p__Chloroflexi;c__OLB14;o__OLB14;f__OLB14;g__OLB14;s__
1484	0	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Saprospiraceae;g__;s__
1485	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Acidobacteriota;c__Subgroup_5;o__Subgroup_5;f__Subgroup_5;g__Subgroup_5;s__
1486	5	0	1	0	0	0	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Micrococcaceae;g__Arthrobacter;s__Arthrobacter_agilis
1487	0	0	2	0	0	1	0	2	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__GKS98_freshwater_group
1488	1	8	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium
1489	0	0	0	0	0	2	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
1490	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Armatimonadota;c__Fimbriimonadia;o__Fimbriimonadales;f__Fimbriimonadaceae;g__Fimbriimonadaceae;s__
1491	0	0	0	0	0	1	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Hydrogenophilaceae;g__Thiobacillus;s__
1492	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae
1493	0	0	0	0	0	1	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__UBA1819;s__
1494	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli
1495	0	0	2	0	2	0	0	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Sandaracinaceae;g__
1496	0	0	0	0	0	0	0	0	0	3	0	Unknown
1497	0	0	0	2	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014;g__Clostridia_UCG-014;s__
1498	3	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Moryella;s__Moryella_sp.
1499	1	0	1	0	0	0	0	0	1	0	0	k__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__;g__;s__
1500	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Chloroflexi;c__Chloroflexia;o__Chloroflexales;f__Chloroflexaceae;g__Oscillochloris;s__
1501	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
1502	2	0	0	0	1	0	0	0	1	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella;s__
1503	0	1	1	0	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
1504	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Polyangiaceae;g__Polyangium;s__
1505	0	0	1	0	0	2	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales;f__Pedosphaeraceae;g__DEV114;s__
1506	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia
1507	0	2	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Ralstonia;s__Ralstonia_insidiosa
1508	0	0	1	0	2	2	0	0	0	0	0	k__Bacteria;p__MBNT15;c__MBNT15;o__MBNT15;f__MBNT15;g__MBNT15
1509	1	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Sporolactobacillaceae;g__Tuberibacillus;s__Tuberibacillus_calidus
1510	2	0	3	0	0	0	0	0	0	0	0	k__Bacteria;p__Verrucomicrobiota;c__Chlamydiae;o__Chlamydiales
1511	4	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae
1512	0	2	0	0	0	0	0	0	0	0	0	Unknown
1513	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales
1514	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Marinomonadaceae;g__Marinomonas;s__
1515	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__RF39;f__RF39;g__RF39;s__
1516	0	0	0	0	0	0	0	0	3	0	0	k__Bacteria;p__NB1-j;c__NB1-j;o__NB1-j;f__NB1-j;g__NB1-j;s__

1517	2	0	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20;f__0319-6G20;g__0319-6G20;s__
1518	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031
1519	0	0	0	0	0	0	0	5	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Lysobacter
1520	0	0	1	0	0	0	0	0	1	0	0	k__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Gemmatales;f__Gemmataceae;g__;s__
1521	1	0	0	0	3	0	0	0	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Dinghuibacter;s__
1522	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Cytophagaceae;g__Sporocytophaga;s__
1523	1	0	2	0	3	3	0	2	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Cytophagales;f__Microscillaceae;g__OLB12;s__
1524	0	0	0	0	0	0	0	1	3	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales
1525	4	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Methylomirabiliota;c__Methylomirabilia;o__Rokubacteriales;f__Rokubacteriales;g__Rokubacteriales;s__
1526	1	0	0	0	0	0	0	1	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Candidatus_Hepatincolaceae;g__Candidatus_Hepatincola;s__
1527	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Paenibacillales;f__Paenibacillaceae;g__Paenibacillus;s__Paenibacillus_alginolyticus
1528	1	0	1	0	0	3	0	2	2	0	0	k__Bacteria;p__Nitrospirota;c__Nitrospiria;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__Nitrospira_defluvii
1529	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bacteriovoracales;f__Bacteriovoracaceae;g__Peredibacter
1530	6	1	20	1	19	6	0	8	64	5	10	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
1531	0	0	0	0	3	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Paludicola;s__
1532	9	28	1	2	0	1	3	0	1	0	3	k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Cutibacterium
1533	2	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__OM27_clade;s__
1534	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Diplorickettsiales;f__Diplorickettsiaceae;g__
1535	19	0	0	3	4	2	1	0	3	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group;s__Lachnospiraceae_bacterium
1536	0	1	1	0	1	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Harryflintia;s__
1537	0	0	0	0	0	0	0	5	1	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__KF-JG30-B3;g__KF-JG30-B3;s__
1538	0	0	0	0	0	0	1	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales;f__Geminicoccaceae;g__Candidatus_Alysiosphaera;s__
1539	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__R7C24;f__R7C24;g__R7C24;s__
1540	1	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Mucilaginibacter;s__Mucilaginibacter_sp.
1541	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Chloroflexi;c__JG30-KF-CM66;o__JG30-KF-CM66;f__JG30-KF-CM66;g__JG30-KF-CM66;s__
1542	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria
1543	0	0	0	0	0	0	2	0	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Polyangiaceae;g__Labilithrix;s__Labilithrix_luteola
1544	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Marivita;s__
1545	0	0	0	0	0	0	0	3	0	0	0	k__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales;f__Bril41;g__Bril41;s__
1546	0	0	0	0	0	0	0	4	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__;s__Terrimonas_sp.
1547	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Crocinitomicaceae;g__Fluviicola;s__
1548	2	0	0	0	3	1	0	1	1	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia
1549	6	0	1	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Ferruginibacter;s__
1550	0	0	2	0	0	0	0	0	0	0	0	k__Bacteria;p__Dependentiae;c__Babeliae;o__Babeliales;f__Babeliales;g__Babeliales;s__
1551	0	0	0	0	2	0	0	0	0	0	0	k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__UCG-004
1552	10	8	0	0	1	2	0	0	4	1	0	k__Bacteria;p__Cyanobacteria;c__Cyanobacteriia;o__Chloroplast;f__Chloroplast;g__Chloroplast
1553	0	0	3	0	0	0	0	0	2	0	2	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus
1554	0	0	0	0	0	0	0	0	2	0	0	k__Bacteria
1555	1	0	0	0	2	0	0	0	2	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium;s__Flavobacterium_resistens

1556	1	0	1	0	0	0	0	0	2	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
1557	0	0	2	0	0	0	0	1	1	0	0	k__Bacteria;p__Elusimicrobiota;c__Elusimicrobia;o__Lineage_IV;f__Lineage_IV;g__Lineage_IV;s__
1558	3	0	0	0	0	1	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Chryseobacterium
1559	1	0	1	0	1	0	0	0	0	0	0	k__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio;s__
1560	0	0	0	0	0	0	0	0	37	0	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Paenalcaligenes;s__Paenalcaligenes_hominis
1561	0	3	0	0	0	0	0	0	0	0	0	k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__;g__;s__
1562	0	0	0	0	0	0	0	2	0	0	0	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__;s__
1563	1	0	0	0	0	0	0	4	0	1	0	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales;f__Woeseiaceae;g__JTB255_marine_benthic_group;s__