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1       **Comparison of Three Sourdough Postbiotics on High-Fat**  
2                               **Diet-Induced Intestinal Damage**

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## Abstract

There is significant interest in using postbiotics as an intervention strategy to address obesity. This study assesses the efficacy of postbiotics derived from different sourdough strains (*Lactiplantibacillus plantarum* LP1, LP25, and *Pediococcus pentosaceus* PP18) in mitigating intestinal injury in zebrafish fed on a high-fat diet. We screened postbiotics for their anti-colon cancer cell effects and compared various preparation methods applied to live bacterial strains, including heat-killing at different temperatures, pH adjustments, and ultraviolet radiation exposure. Heat-killing at 120°C proved to be the most effective preparation method. A marked variation health effects was observed in the heat-killed microbial cells, as evidenced by hydrophobicity and self-aggregation ability. A five-week high-fat dietary intervention study in zebrafish demonstrated that diets supplemented with 10<sup>8</sup> CFU/g K-LP25 significantly attenuated weight gain and body fat, along with reductions in *FASN*, *Leptin*, and *SREBF1* mRNA expression. However, diets supplemented with 10<sup>7</sup> CFU/g of K-PP18 only reduced *Leptin* and *SREBF1* mRNA expression. K-PP18 was more effective at mitigating gut barrier damage, promoting colonic *occludin*, *ZO-1*, and *claudin-1* levels. Additionally, K-LP25 supplementation markedly downregulated pro-inflammatory cytokines *TNF-α*, *IL-6*, and *IL-1β*, reducing intestinal inflammation. Supplementation with K-LP1 and K-PP18 increased the abundance of *Acinetobacter* spp., whereas K-LP25 increased the abundance of *Cetobacterium* and *Plesiomonas*. Collectively, these findings suggest that inactivated strains confer protective effects against high-fat diet-induced intestinal damage in zebrafish, with variation observed across different species. Studying the effects of sourdough-derived postbiotics on gut health may open new avenues for dietary interventions to manage gut-related diseases.

**Keywords:** Sourdough; Postbiotic; Heat-killed; Intestinal barrier; Gut microbiota

45 **1. Introduction**

46 Prolonged consumption of a high-fat diet (HFD) has been linked to many  
47 metabolic disorder, such as obesity, diabetes, hypertension, and cardiovascular  
48 diseases <sup>1</sup>. Consumption of a HFD can induce intestinal dysbiosis and affect gut  
49 permeability, leading to low-grade gut inflammation <sup>2</sup>. The resulting inflammatory  
50 intestinal micro-environment is associated with an increased risk of developing  
51 colorectal cancer, as chronic inflammation can promote genetic mutations and  
52 abnormal cell growth within the intestinal lining <sup>3</sup>. Long-term HFD-induced obesity  
53 has been also shown to influence intestinal stem and progenitor cell function and the  
54 cellular origins of intestinal dysplasia <sup>4</sup>.

55 Various strategies with gut microbiota modulation effects, including the use of  
56 probiotics, prebiotics, postbiotics, antibiotics, and fecal microbiota transplantation  
57 (FMT), have been employed for the prevention and treatment of colorectal cancer <sup>5</sup>.  
58 Postbiotics are involved in regulating intestinal microecology and improving the  
59 progression of colorectal cancer <sup>6, 7</sup>. The term postbiotic was defined by the  
60 International Scientific Association of Probiotics and Prebiotics (ISAPP) as “*a*  
61 *preparation of inanimate microorganisms and/or their components that confers a*  
62 *health benefit on the host.*” <sup>8</sup>. Recently, there has been a significant surge in the use of  
63 postbiotics due to the recognition that the beneficial effects of probiotics are not  
64 solely linked to their viable state, but also to their structural components and  
65 metabolites. Postbiotics offer several advantages over probiotics. Firstly, they  
66 eliminate the risk of introducing live microorganisms into the gastrointestinal tract,  
67 which may be a concern for certain individuals, such as those with compromised  
68 immune systems. Also, postbiotics are more stable and have a longer shelf life than  
69 probiotics, making them easier to store and transport. Furthermore, postbiotics can be  
70 standardized for consistent dosing, ensuring reliable efficacy <sup>9, 10</sup>. Postbiotics have  
71 now known to exhibit stronger gut microbiome modulatory effects compared with  
72 probiotics <sup>11, 12</sup>. Many studies have showed the postbiotics role in ameliorating  
73 obesity-related characteristics, including epididymal fat accumulation, inflammation

response and gut bacteria disorder<sup>13-15</sup>. A recent study aimed to compare the effectiveness of both live and heat-killed *Lactobacillus plantarum* Zhang-LL cells on dextran sulfate sodium (DSS)-induced ulcerative colitis in rats, suggesting that postbiotics demonstrate similar potential outcomes to probiotics in terms of exerting various health effects in individuals<sup>16</sup>. Extracellular polysaccharides (EPS) can improve intestinal barrier function and restore the integrity of the gut epithelium. Bacteriocins also play a role in promoting gut health at combatting specific intestinal pathogens, like *Latilactobacillus sakei*<sup>17, 18</sup>.

Sourdough is a complex fermentation system that comprises multiple strains of yeast, lactic acid bacteria (LAB), and molds. Fermentation is an important reservoir of postbiotics, the typical metabolic transformations carried out by LAB can become an important biotechnological process for the nutritional fortification and functionalization of sourdoughs due to the resulting postbiotics<sup>19</sup>. The postbiotic-like effect in sourdough may be influenced by factors such as temperature, pH, nutrient status, and the composition of LAB and yeasts<sup>20</sup>. *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus* are predominant LAB which have been frequently isolated from different sourdough samples. These bacterial species exhibit high adherence potential which is linked to their structural components and sets the base for their beneficial effects in the gut<sup>21, 22</sup>. *L. plantarum* has been reported to potentially alleviate nonalcoholic fatty liver disease by regulating fatty acid metabolism, combating oxidative stress effects, and modulating the gut microbiota in case of disease<sup>23, 24</sup>. It was also found that various strains of *P. pentosaceus* have been highlighted for probiotic use due to their probiotic potential attributed to their anti-inflammation, anticancer, antioxidant, detoxification, and lipid-lowering abilities<sup>25, 26</sup>. Moreover, postbiotics derived from certain strains of *L. plantarum* and *P. pentosaceus* are considered natural sources of antioxidants that enhance growth performance, anti-inflammatory responses, and immune function<sup>27-29</sup>.

Currently, the study of postbiotics prepared from *L. plantarum* is mainly focused on food preservation applications due to their strong antimicrobial and antioxidant

effects<sup>29</sup>. To date, no study has investigated the beneficial effects of postbiotics derived from sourdough strains. This work first screened 80 strains isolated from sourdough for postbiotic with low proliferation ability of SW620 colon cancer cells, and compares different methods to prepare effective postbiotics for the screened strains. Moreover, this work evaluates the effectiveness of postbiotics *L. plantarum* LP1, LP25, and *P. pentosaceus* PP18 strains on intestinal health. These finding provide theoretical support for further exploring the postbiotic effects and application of LAB.

**2. Methods and materials**

**2.1. Reagents and materials**

The 80 strains used in this trail were isolated from the Chinese sourdough and the information detailed Table S1. The source of zebrafish feed was from Tetra TetraBits Complete (#NO. T7002, Tecsa Co., Ltd., Germany) and ingredients contain: fish and fish derivatives, vegetable protein extract, cereals, derivatives of vegetable origin, molluscs and crustaceans, yeast, minerals, algae, oils and fats. Lard was purchased from Jinluo Flagship Store (Shandong, China). MRS medium (g/L) for LAB growth obtained from Beijing Aobox Biotechnology Co., Ltd. (Beijing, China, Peptone 10.0 g, beef meal 5.0 g, yeast 4.0 g, glucose 20.0g, sodium acetate 5.0 g, dipotassium hydrogen phosphate 2.0 g, magnesium sulfate 0.2 g, triammonium citrate 2.0 g, manganese sulfate 0.05 g, Tween 80 1 mL).

SW620 colon cancer cells were obtained from Chinese Type Culture Collection (Shanghai China). DMEM and fetal bovine serum (FBS) were from Biological Industries (Kibbutz Beit-Haemek, Israel). Phosphate buffer solution (PBS) purchased from Sevenbio (Beijing, China). Trypsin obtained from Saio Biotechnology Co., Ltd., Shanghai, China). Cell Counting Kit-8 was purchased from Sevenbio (Beijing, China), tumor necrosis factor  $\alpha$  (*TNF- $\alpha$* ), interleukins (IL)-1 $\beta$ , and *IL-6*, *Occludin*, *Claudin-1*, zonula occludens-1 (*ZO-1*), sterol regulatory element binding

transcription factor 1 (*SREBF1*), fatty acid synthase (*FASN*), and *Leptin* qPCR Kit were purchased from Sangon Biotech (Shanghai, China).

## 2.2. Determination of proliferation rate of metastatic colon cancer cells

The bacterial suspension was adjusted to an OD600 of 1.0-1.2 followed by complete thermal inactivation at 120 °C to prepare the inactivated bacteria. The SW620 cells were seeded into 96-well plates at a density of 5000 cells/well and cultured in DMEM medium containing 10% FBS and placed in an incubator at 37°C with 5% CO<sub>2</sub>. After the cells are fully apposed, the cells grow in a well containing 20 µL inactivated bacterial culture and 80 µL cell culture for 48 hours. Add 10 µL of CCK8 and continue to incubate for 2 h. The proliferation rate of the cells on 96-well plates was determined using Cell Counting Kit-8 (Sevenbio, Beijing, China). The absorbance value OD<sub>450</sub> was measured using a microplate reader and recorded as A<sub>2</sub>. an equal volume of culture medium was added as the control group, denoted as A<sub>1</sub>, and the Wells with culture medium only were used as the blank group, denoted as A<sub>0</sub>. The formula for calculating the inhibition rate of colon cancer cell proliferation is as follows:  $(1 - \frac{A_2 - A_0}{A_1 - A_0}) \times 100\%$ .

### Preparation of bacteria postbiotics under different conditions

Two strains of *L. plantarum* (LP1 and LP25) and *P. pentosaceus* (PP18) stored in glycerol at -20 °C in our lab, were removed from storage. After thawing, the strains were inoculated onto MRS agar medium, and to further obtain single colony for observing the morphology and growth statement of strains. For preparation of a bacterial suspension, the fermentation broth was centrifuged at 8000 r/min for 10 min, the resulting precipitation was then washed twice with sterile phosphate buffer (PBS, pH=7.2), centrifuged and suspended in PBS buffer.

Heat-killed: After preparing the bacterial suspension, it was divided into 2 mL sterile centrifuge tubes, with 1.5 mL in each. The suspension was then inactivated at the temperature of 60, 80, 90, and 100 °C in a constant temperature water bath for 30,

60, 90 and 120 min, respectively, and the suspension was then inactivated at 120 °C in a sterilization pot for 10, 20 and 30 min, respectively. After the inactivation process, 1 mL of the bacterial solution was diluted with 0.85% saline, and the colony count was performed using the plate coating method to observe the effectiveness of the inactivation.

**pH Inactivation:** The bacterial suspension was prepared and divided into 2 mL sterile centrifuge tubes, with 1.5 mL per tube. After centrifuging at 8000 r/min for 10 min, the supernatant was discarded. Add 1mol/L HCl solution (pH=0.2), 0.1 mol/L HCl solution (pH=1), 0.01 mol/L HCl solution (pH=2), as well as 1 mol/L NaOH solution (pH=14), 0.1 mol/L NaOH solution (pH=13), 0.01 mol/L NaOH solution (pH=12) 1.5 mL, respectively. After the suspension, the bacteria were washed twice with PBS buffer and then resuspended in PBS buffer. 1 mL of the bacterial solution was diluted with 0.85% normal saline and plated for colony counting.

**UV Irradiation:** The UV lamp used in the test had a power of 15 watts, and emitted UV radiation at wavelength of 253.7 nm. Before the experiment, the UV light was turned on for 30 min. After the UV light stabilized, the UV intensity at 10, 20 and 30 cm perpendicular to the UV light was measured using a UV radiometer. The UV intensity at 10 cm was 1.2 mw/cm<sup>2</sup>, the UV intensity at 20 cm was 250 μW/cm<sup>2</sup>, and the UV intensity at 30 cm was 115 μW/cm<sup>2</sup>. After preparing the bacterial suspension, 20 mL of it was placed in a sterile Petri dish and irradiated at vertical distances of 10, 20, and 30 cm from the UV lamp. At the same time, the magnetic stirrer was used to stir at a constant speed to ensure that the bacterial suspension was uniformly irradiated. Samples were taken at 10, 20, 30, 60, 90, and 120 min, respectively. 1mL of the bacterial solution was diluted with 0.85% saline and plated for colony counting.

### 2.3. Scanning electron microscopy

The fermentation broth of LP1, LP25, and PP18 were centrifuged at 8000 r/min for 10 min, then washed twice with sterile PBS, centrifuged and suspended in PBS

buffer. The suspension was then inactivated at 120 °C for 10 min. The heat-killed bacteria were centrifuged at 8000 r/min for 10 minutes. 2.5% glutaraldehyde (pH 6.8) was added and mixed by blowing and suction. The samples were fixed by standing in the refrigerator for 4 h. Following fixation, the samples were washed three times with 0.2 mol/L PBS (pH=7.2). Then, the bacteria were dehydrated in 50%, 70%, 90% and 100% ethanol solutions for 10 min. Subsequently, they were treated with an ethanol-butyl alcohol mixture (1:1) and tert-butyl alcohol for 15 min each. After centrifuging at 5000 r/min for 10 min, the supernatant was discarded, and 0.5 mL tert-butyl alcohol was added. They were transferred to a Petri dish with filter paper and dried at 40 °C. They were sent to Jushang Scientific Research Service Company (Nanjing, China) for photographing the microscopic morphology of the bacteria surface using a scanning electron microscope (SEM) (Tescan Mira4, Nanjing Jushang Testing Technology Co. Ltd).

#### 2.4. Determination of hydrophobicity and self-aggregation

According to the method of Mohammed et al.<sup>30</sup>, 5mL of the suspension (OD<sub>600</sub> = 1.0-1.1) was taken in a test tube and inactivated at 120 °C to produce the inactivated bacterial suspension. The hydrophobicity of the live and the inactivated bacterial suspension was measured, and the initial OD<sub>600</sub> of the bacterial suspension was A<sub>1</sub>. The supernatant was collected with xylene solution to measure (OD<sub>600</sub>) after standing incubating at 37 °C for 3 h, recorded as A<sub>2</sub>. The sample hydrophobicity was calculated using the formula: hydrophobicity (%) =  $\left(\frac{A_1 - A_2}{A_1}\right) \times 100$ .

For self-aggregation, the bacterial suspension was adjusted to an OD<sub>600</sub> of 1.0-1.1 followed by complete thermal inactivation at 120 °C to prepare the inactivated bacteria suspension. The initial OD<sub>600</sub> of the bacterial suspension was A<sub>1</sub>. the bacterial suspension left at room temperature for 2, 4, 6, and 8 h, respectively, the result was recorded as A<sub>2</sub>. Calculate the sample self-condensation rate according to Equation<sup>30</sup>: self-aggregation rate (%) =  $\left(1 - \frac{A_2}{A_1}\right) \times 100$ .

**2.5. Animal experimental design**

Adult zebrafish, AB strain, imported from China Zebrafish Resource Center, was maintained at 28°C on a 14 h light/10 h dark cycle in Shanxi University zebrafish facility. After a one-week adaptation period, all zebrafish were randomly divided into 5 groups, each containing 10 fish: a control group (C), a high-fat diet group (H), a high-fat diet with 10<sup>8</sup> CFU/g of K-LP1 inactivated bacteria (HL1), a high-fat diet 10<sup>8</sup> CFU/g of K-LP25 inactivated bacteria (HL2), and high-fat diet 10<sup>7</sup> CFU/g of K-PP18 inactivated bacteria (HP) (Supplement materials, the maximum tolerated concentration test). C group were fed with a commercial diet (Tetra Bits Complete, Germany. Protein: ≥ 47.5%, Fat: ≥ 6.5%, Fiber: ≤ 2%), and the commercial diet was prepared in Milli-Q water (1 g in 100 mL water) and autoclaved. H group were fed with HFD, which consisted of a commercial diet supplemented with 25% (w/w) of lard. During the experiment, zebrafish were fed diets at 9:00 AM and 5:00 PM, respectively. The feeding ratio was 4% of the average body weight of zebrafish, and the water was changed daily after feeding. Zebrafish in each group were weighed weekly. After 5 weeks, zebrafish were anesthetized with an ice bath and sacrificed. The intestinal and liver tissue was removed after treatment for subsequent experiments.

This study was performed in strict accordance with the Laboratory Animal Guideline for Ethical Review of Animal Welfare (GB/T 35892-2018, China) and was approved by the Experimental Animal Welfare and Ethics Committee of the Shanxi University (SXULL2019004).

**2.6. Quantitative reverse transcription PCR (qRT-PCR)**

The tissues were collected by trizol after extraction, concentration determination, and organize all RNA concentration normalized processing, finally used for reverse transcription synthetic DNA to determine the subsequent qPCR. The RT-qPCR condition was as follows: 95 °C for 1 min, 1 cycle; 95 °C for 15 s, and 60 °C for 15 s, 72 °C for 60 s, 40 cycles. The *β-actin* was the internal reference gene. The mRNA

expression of *TNF- $\alpha$* , *IL-1 $\beta$* , *IL-6*, *Occludin*, *Claudin-1*, *ZO-1*, *SREBF1*, *FASN* and *Leptin* were detected. The primer sequences are displayed in Table 1.

## 2.7. High-throughput sequencing analysis of gut microbiota

Gut microbiota was analyzed using 16S rRNA gene sequencing. Samples of intestinal contents were collected 4 to 6 h after the last feeding. Each set of intestinal content samples was combined into one sample, which was stored on dry ice and sent to MegBio for amplicon sequencing analysis of the microbial community in the 16S V4 region. Total intestinal bacterial DNA was extracted using DNA extraction kit, and the V3-V4 region of 16S rRNA gene was amplified by bacterial universal primers. The PCR products were used to create a library and subjected to high-throughput sequencing. The sequences in this paper have been deposited in NCBI databank under the accession numbers PRJNA1134882. The contents include the analysis of characteristics, diversity, and correlation of intestinal flora, as well as the prediction of pathway functions.

## 2.8. Statistical analysis

The statistical differences in the cancer cell inhibition, intestinal adhesion tests, zebrafish's body weight, and genes expression between the groups were determined by a one-way ANOVA and a Tukey post-hoc tests, using the Statistical Package for the Social Sciences (SPSS) software (IBM SPSS Statistics for Windows, IBM Corp and Version 25.0.). The graphical visualization was achieved by Graph Pad Prism 8.0.2 software. All data were expressed as mean  $\pm$  standard error of mean. Clustering correlation heatmap with signs was performed using the OmicStudio tools at <https://www.omicstudio.cn>.

**3. Results**

**3.1. Characteristics of strains**

As depicted in Fig. 1, LP1 (Fig. 1A, B) and LP25 (Fig. 1D, E) exhibited a rod-shaped morphology, characterized by round, milky white, shiny colonies with well-defined edges. These colonies appeared opaque and non-motile. LP1 reached the stationary phase after 6 h. At 18-24 h, OD<sub>600</sub> began to decline, indicating that the growth of the strain had entered a death phase (Fig. 1C). LP25 strain entered a stationary phase at 6 h, and the colony density increased slightly from 9 h to 15 h (Fig. 1F). PP18 was spherical and arranged in sheets (Fig. 1G). Single colonies were round, milky white, with smooth shiny surface, neat edges, opaque, and non-motile (Fig. 1H). By the sixth hour, its logarithmic phase is over, and then entered the stationary phase (Fig. 1I).

**3.2. Screening of the anti-colon cancer cell for postbiotics**

In this study, the proliferation rate of colon cancer cells SW620 was used as an indicator to screen for postbiotic with pro-gut health potential from 80 inactivated LAB isolated from sourdough, the strains basic information of 80 depicted in Table S2 and results are shown in Fig. 2. Among them, the proliferation ability of SW620 cells was the lowest after the addition of K-PP18, which was only 27.6%, followed by K-LP25 and K-LP1, which were 33.6% and 34.8%, respectively. The MTT activity of cell extracts, cell-free supernatant against Caco-2 showed the highest cell-free supernatant of 77.8% (as cell viability 22.2%) in *L. brevis* 0912<sup>31</sup>. It is similar to the proliferation rate of the colon cancer cells screened in this study, which can be used as research subjects for further research.

**3.3. Screening of the preparation conditions for postbiotics**

**3.3.1 Heat-killed**

Different combinations of temperature and time gradients were used to inactivate the strains (Fig. 3A-C). LP1 was found to be completely inactivated when heated for 90 min at 60 °C, for 60 min at 80 °C and 90 °C, for 30 min at 100 °C, and at 10 min at 120 °C. LP25 was found to be completely inactivated at 60 °C and 80 °C for 90 min, at 90 °C for 60 min, at 100 °C for 30 min, and at 10 min at 120 °C. PP18 was completely inactivated for 90 min at 60 °C and 80 °C, and for 60 min at 90 °C and 100 °C. When the temperature was raised to 120 °C, the activity of the PP18 strain was completely lost within 10 min. The results showed that at the one temperature, the activity of the strains decreased, and the number of colonies decreased over time. With increasing temperature, the time required for strain inactivation also decreased. Heat inactivation conditions varied among different strains, but all three strains were completely inactivated at 10 min at 120 °C.

### 3.3.2 pH adjustment Inactivation

Each strain was resuspended in HCl solution with pH values of 0.2, 1, and 2, and in NaOH solution with pH values of 12, 13, and 14, respectively. After mixing, the strains were left for  $10^1$ ,  $10^2$ ,  $10^{2.5}$ ,  $10^3$ ,  $10^{3.5}$ , and  $10^4$  min, respectively, to explore the inactivation effect of pH on the strains. The results are depicted in Fig 3D-F. The target strains were not completely inactivated after being resuspended in pH 2 solution and pH 12 solution for  $10^4$  min. At pH 2, LP1 inactivated 4.1 log CFU/mL and LP25 inactivated 5.9 log CFU/mL, respectively. PP18 inactivated 3.4 log CFU/mL. At pH 12, 5.4, 5.3, and 4.9 log CFU/mL cells of LP1, LP25, and PP18, respectively were inactivated. LP1 and PP18 were completely inactivated by resuspension for  $10^4$  min at pH1, while LP25 was completely inactivated by resuspension for  $10^{3.5}$  min. Resuspending in NaOH for  $10^4$  min at pH 13 completely inactivated all three target strains. At pH 14, it took  $10^{3.5}$  min to inactivate LP1 and LP25 strains, while it took  $10^3$  min to inactivate PP18. At pH value of 0.2. the inactivation time of LP1, LP25 and PP18 were  $10^{3.5}$  min,  $10^3$  min and  $10^{2.5}$  min, respectively.

In general, as the concentration of HCl and NaOH solutions increased, the time

to inactivate of the strains was shorted. Among them, the HCl solution with a pH of 0.2 had the most effective inactivation effect as it completely inactivated all the target strains in  $10^{3.5}$  min. The time required for heat inactivation at 120 °C was much shorter (10 minutes) than that required for acid/base inactivation, making the effect of heat inactivation better than that of acid/base inactivation.

**3.3.3 UV irradiation inactivation**

The distance between the Petri dish containing a 20 mL bacterial suspension and the UV irradiation was systematically varied to 10, 20, and 30 cm. Exposure durations of 30, 60, 90, and 120 minutes were implemented to assess the impact of UV inactivation on the activity of the target strains. As depicted in Fig 3G-I, when the UV lamp was positioned at the distance of 30 cm, the UV intensity measured 115  $\mu\text{W}/\text{cm}^2$ . After 10 min of irradiation, PP18 was inactivated 4.8 log CFU/mL, LP1 5.6 log CFU/mL, and LP25 6.5 log CFU/mL. This was followed by a tailing period. After 120 min of irradiation, the three strains were not completely inactivated, whereas, the inactivation rates were 2.73 log CFU/mL, 3.36 log CFU/mL, and 4.12 log CFU/mL, respectively. At the distance of 20 cm from the UV lamp, where the UV intensity was 250  $\mu\text{W}/\text{cm}^2$ , PP18, LP1, and LP25 were inactivated 4.61 log CFU/mL, 6.61 log CFU/mL, and 6.57 log CFU/mL, respectively, before entering the tailing phase. After 120 min of irradiation, the inactivation rates for the three strains were 2.23 log CFU/mL, 2.59 log CFU/mL, and 3.46 log CFU/mL, respectively. PP18 was completely inactivated after 90 min of exposure to a UV intensity of 1.2  $\text{mW}/\text{cm}^2$  at the distance of 10 cm from the UV lamp, while LP1 and LP25 were required 60 min of exposure for complete inactivation.

The results indicated a reduction in the number of strain inactivation with prolonged irradiation time. At a specific irradiation duration an increase in irradiation intensity resulted in more significant inactivation effects. Among the tested methods, positioning the bacterial suspension 10 cm from the UV lamp proved to be the most effective method which resulted in inactivation in 90 min. This duration exceeded that of heat inactivation at 120 °C but shorter than that required for pH inactivation.

Consequently, heat inactivation demonstrates greater efficiency compared to both UV and pH inactivation methods.

### 3.4. Effect of heat inactivation on microscopic morphology of strains

After heat inactivation, changes in cell morphology of the non-viable microorganism cells were observed compared to their morphology (e.g. cell integrity), in viable form as depicted in Fig. 4. Heat-killing LP1 (K-LP1) preserved the cell morphology but compromised the cell surface integrity, increasing surface roughness and creating noticeable depressions (Fig. 4A, D). LP25 underwent significantly damaged following heat inactivation (K-LP25). Changes in cell morphology were evident, with increased surface roughness and unevenness. A considerable number of lysed cells and cell debris were observed, indicating leakage of intracellular contents (Fig. 4B, E). For PP18, heat inactivation (K-PP18) resulted in enhanced surface roughness marked depressions, and evidence of cell lysis and cell debris, which led to the leakage of intracellular contents (Fig. 4C, F).

### 3.5. Hydrophobicity and self-aggregation ability of the postbiotics

As shown in Fig. 5A. The hydrophobicity levels of PP18 were the lowest among the strains, measuring less than 2% both before and after inactivation. For LP1 and LP25, the hydrophobicity was 78.5% and 76.9%, respectively, but the K-LP1 and K-LP25 decreased to 46.6% and 44.6%, respectively. Fig. 5B shows that the self-aggregation capability of the bacterial strains increased over time, and there was a significant difference between different strains, and the self-aggregation capability of the viable cells of all three strains was higher than that of the non-viable cells. Self-aggregation was the strongest at 8h for all cells, which could reach more than 80% for LP1 and LP25, and 45.67% for PP18 viable cells. However, the self-aggregation capability of inactivated bacterial cells decreased compared with that of bacterial cells, which was 42.97% for K-LP1, and 37.29% and 33.06% for K-LP25 and K-PP18, respectively. The self-aggregation of inactivated bacterial cells is lower

compared to that of live bacterial cells, possibly due to the impact of temperature on adhesion capability. Higher temperatures tend to decrease adhesive ability<sup>32</sup>. In the Caggia study<sup>33</sup>, hydrophobic and self-aggregation capability of the *L. rhamnosus* GG are all about 70%, and the K-LP1 and K-LP25 were higher than *L. rhamnosus* GG. Another study finding that the self-aggregation capability at 24 hour was about 25% to 50% after heat inactivation<sup>34</sup>. In contrast, the self-aggregation capability and hydrophobicity of the selected K-LP1 and KK-LP25 were still at a high level. Therefore, K-PP18 has low self-aggregation capability and hydrophobicity, which has poor adhesion, while K-LP1 and K-LP25 have strong self-aggregation capability and hydrophobicity before and after inactivation, which have some adhesion to the intestine.

### 3.6. Effects of postbiotics on high-fat induced intestinal injury in zebrafish

#### 3.6.1 Effect of postbiotics on the health index

Body length (mm) was recorded and body mass index (BMI, body weight/body length<sup>2</sup>) was calculated. As shown in Fig. 6A, the HL2 group (6.6%) showed a significant reduction in the body weight gain rate compared with the H group (14.3%,  $P < 0.05$ ), and there was no significant difference in HL1 (11.2%) and the HP groups (14.4%). Similar trends were observed for body mass index (Fig. 6B). However, the HL1 group (33.6%) was significantly higher than that of the control group. The results indicate that the introduction of K-LP25 contributed to a decrease in the body weight and body mass index of the high-fat zebrafish.

To further assess the impact of postbiotics on hepatic lipid metabolism, we qualified the relative mRNA expression levels of *FASN*, *Leptin*, and *SREBF1* with results presented in Figures 6C-E. Compared to the control (C) group, expression levels of *FASN*, *Leptin* and *SREBF1* were significantly higher in H group. Notably, the HL2 group exhibited a substantial reduction in the relative mRNA expression of *FASN*, *Leptin* and *SREBF1* when compared to the H group, indicating a potential effect of postbiotic intervention on hepatic dysregulation. The expression levels of the

*Leptin* and *SREBF1* genes were significantly reduced in the HP group, although *FASN* expression did not differ significantly from the control. Within the HL1 group, *FASN* and *Leptin* expression levels saw a significant decline, but there was no significant difference for expression of *SREBF1* genes compare to the H group.

These findings suggest distinct roles of postbiotics derived from K-LP1, K-LP25, and K-PP18 in regulation of genes associated with hepatic lipid synthesis.

### 3.6.2 Impact of postbiotics on tight junction protein and inflammatory gene expression in colon

We assessed the relative mRNA expression levels of the tight junction (TJ) protein genes *Occludin*, *Claudin-1*, and *ZO-1*, with results illustrated in Fig. 7A-C, in the high-fat diet (H) group, expression levels of *Occludin*, *Claudin-1*, and *ZO-1* were significantly reduced compared to control (C) group ( $P < 0.05$ ). In contrast, the HL1, HL2 and HP groups exhibited a significant upregulation in the expression of the *Occludin*, *Claudin-1* relative expression genes relative to the H group. Only the change in *ZO-1* did not reach statistical significance both the HL1 and HL2 groups.

Further investigation into the effects of postbiotics on intestinal inflammation induced by a high-fat diet involved evaluating the expression of pro-inflammatory genes *TNF- $\alpha$* , *IL-1 $\beta$* , and *IL-6*, as depicted in Fig. 7D-F. The H group exhibited significantly elevated levels of *TNF- $\alpha$* , *IL-1 $\beta$* , and *IL-6* compared to the control group. In comparasion to the H group, the HP group showed an increase in *TNF- $\alpha$*  and *IL-1 $\beta$*  expression levels, with no significant change in *IL-6* expression. The HL1 group demonstrated a significant decrease in *TNF- $\alpha$*  and *IL-1 $\beta$*  expression levels, while *IL-6* levels were significantly increased. Conversely, in the HL2 group, both *TNF- $\alpha$*  and *IL-6* expressions were significantly reduced. These findings indicate that a high-fat diet triggers intestinal inflammation in zebrafish, and supplementation with inactivated LP25 bacteria can effectively modulate the expression of inflammatory genes and mitigate intestinal inflammation.

3.6.3 Effect of postbiotics on gut microbiota in high-fat zebrafish

The overlap and similarity of OTUs makeup of samples were depicted visually in the Venn diagram (Fig. 8A). The total number of shared OTUs was 17, indicating that there was a certain degree of similarity between the groups and that there were some common core species in the microbial community. The number of unique OTUs in group C was 31, the number of unique OTUs in groups H and HL2 was 3, the number of unique OTUs in group HL1 was 5, and the number of unique OTUs in group HP was 8. This indicates that a HFD and the intake of postbiotics changed the structure of the gut microbial community. OTU67, OTU88, OTU87, and OTU117 are the top four species with the highest sharing. OTU67 had the highest abundance in the HP group, accounting for 42.65%, while it also accounted for 36.87% in the HL1 group and 11.17% in the C group, indicating that OTU67 was a major core species with a high abundance in all three samples (Fig. 8B, C).

Alpha Diversity analysis evaluates the richness and diversity of microbial communities in samples using the Shannon index as a metric. The Shannon curve, depicted in Fig. S1, exhibits a plateauing trend with increasing sequencing depth and sample size, indicating stabilization in species richness estimates. The community structure, composition, and proportion of each sample were then examined at the phylum and genus levels. At the phylum level, *Proteobacteria*, *Firmicutes* and *Fusobacteria* were the predominant phyla in the gut of zebrafish. In comparison to the C group, the H group showed a decrease in the relative abundance of *Proteobacteria* and an increase in *Firmicutes*. The HL1, HL2, and HP groups all contributed to an increase the relative abundance of *Proteobacteria* and decrease that of *Firmicutes*. Additionally, the HL2 group led to an increase in the relative abundance of *Clostridia* (Fig. 9A). At the genus level (Fig. 9B), the predominant genera included *Acinetobacter*, *Cetacobacterium*, and *Plesiomonas*. The *Acinetobacter* genus decreased in the H group, whereas HL1 and HL2 groups increased the abundance of *Acinetobacter* sp. The abundance of *Cetacobacterium* and *Plesiomonas* increased in HP group, which are linked to increasing the efficiency of

457 dietary carbohydrate utilization and maintain the intestinal health of zebrafish by  
458 regulating glucose metabolism<sup>35</sup>.

459 The OTU analysis was summarized using PICRUST 2 to illustrate the microbial  
460 diversity Fig. 9. As a result of the use of inactivated *Lactobacillus*, it was observed  
461 that the abundance of glutathione metabolism and fatty acid degradation increased in  
462 comparison to the high-fat diet group, while fatty acid biosynthesis remained  
463 unaltered. LP25 up-regulated the abundance of quorum sense system (Fig. 10A). It  
464 has been reported that common diseases including obesity can be mediated by the gut  
465 microbiota through quorum sense<sup>36, 37</sup>. Resident microbiota via quorum sensing  
466 system to restore their population in response to anti-inflammatory substances<sup>38</sup>.  
467 Additionally, HL1 and HP groups enhanced the abundance of 2.3.1.9 acetyl-CoA C-  
468 acetyltransferase compared to the H group (Fig. 10B). These findings provide insight  
469 into the partial inhibition of lipid production.

470 Intestinal microbiota has formed an impressive repertoire of mechanisms for  
471 monitoring host physiological activity and signaling by secreting effector substances  
472<sup>39</sup>. Previous studies reported that inflammatory response partly depends on the  
473 composition of intestinal microbiota, as bacterium-derived products or pathogen that  
474 leak into the blood from the gut might induce inflammatory response<sup>40</sup>. Therefore,  
475 the potential correlation between the key gut microbial genus level and genes  
476 expression level related inflammatory response and intestinal barrier significantly  
477 regulated by the heat-killing strains were also revealed through Spearman's  
478 correlation analysis and visualized by a heatmap. As shown in Figure 11. *ZOR006*  
479 was found to be positively related to *TNF- $\alpha$* , *IL-6*, and *IL-1 $\beta$* , but negatively related  
480 to *ZO-1*, *Occludin*, and *Claudin-1*. *Acinetobacter* was found to be positively  
481 associated with *TNF- $\alpha$*  and *IL-6*, but negatively associated with *SREBF1*, *Occludin*,  
482 and *Claudin-1*. These results suggest that K-LP1, K-LP25, and K-PP18 intervention  
483 may alter the intestinal function by changing the composition of intestinal microbiota.  
484 However, the relationship between specific microbial and the intestinal function  
485 associated with high-fat diet needs to be validated by germ-free mice or fecal

bacterial transplantation.

**4. Discussion**

The presented results shed light on the ameliorative effects of postbiotics on high-fat diet-induced intestinal damage. This expands our understanding of how sourdough-derived postbiotics can improve intestinal homeostasis and broadens the industrial applications of postbiotics.

Postbiotic compounds (metabolites and/or microbial cells fractions) are usually produced by microorganisms during fermentation, and they have valuable functional and health promoting effects <sup>41</sup>. In this study, we found that postbiotics derived from sourdough, namely K-LP1, K-LP25, and K-PP18, have a beneficial effect on the gut by reducing lipid accumulation, strengthening gut barrier, reducing inflammation response, as well as modulating the gut microbiota. The postbiotics from the mentioned above strains showed to have a variable effect on other bacterial species at the genus and strain levels. This shows that there is not generic mechanism behind this induced growth through postbiotic exposure, but rather there are genus- and sometimes strain-specific interaction mechanisms.

Postbiotics outperform probiotics in ease of transportation, storage, and longer shelf life due to their stable nature. They benefit from advanced production techniques, allowing for precise dosing and enhanced protection. This stability and control make postbiotics a reliable and efficient option for health promotion <sup>42, 43</sup>. Different inactivated methods may affect structural components of the cell differently, and influence postbiotic biological activities <sup>44</sup>. The heat treatment is the most commonly used technique for removal of non-viable cells, and the heat inactivation procedure can successfully preserve the health-promoting properties of beneficial microorganisms during postbiotics production <sup>45</sup>. The heat inactivation conditions vary depending on the strain, necessitating distinct temperature and exposure time requirements for effective inactivation. The three strains used in our trial were shown to be completely inactivated within 10 min at 120 °C. Heat inactivation proved to be

514 better than UV and pH adjustments to attain complete inactivation. However, the  
515 assessment of postbiotics cell viability has been largely based on plating in this  
516 research, some disadvantages can be pointed such as the impossibility to provide  
517 results in real time, and the lack of information regarding cell integrity and metabolic  
518 activity. The information on cell integrity is of foremost importance because damaged  
519 and injured cells can still maintain some metabolic activity that could contribute to  
520 promotion of health benefits <sup>32</sup>. It can be combined with techniques such as  
521 fluorescent staining to assess the viability of inactivated strains and provide detailed  
522 information on cell integrity and damage, including metabolic activity.

523 K-LP25 demonstrated a more pronounced effect in reducing body weight and  
524 body fat gain induced by a high-fat diet compared to K-LP1 and K-PP18.  
525 Additionally, it was also successful in decreasing the relative expression of *FASN*,  
526 *Leptin*, and *SREBF1* mRNA. In line with findings from <sup>46</sup>, postbiotics demonstrate a  
527 lipid-lowering function, attributed to their interaction with prebiotic. This combined  
528 effect triggers the expression of markers associated with thermogenic capacity in  
529 white adipose tissue, particularly SREBP-1c and *FASN*. *FASN* is critical in  
530 promoting de novo lipogenesis (DNL) in the liver, a process that leads to triglyceride  
531 accumulation via a series of biochemical transformations. *FASN* is markedly  
532 overexpressed in cancer cells characterized by altered lipid metabolism, including  
533 those found in breast, colorectal, and hepatocellular carcinomas. Furthermore,  
534 *SREBF1* acts as an essential transcriptional regulator of lipid synthesis in the liver,  
535 playing a pivotal role in *de novo* DNL. Additionally, *Leptin*, an adipokine, is crucial in  
536 the regulation of body weight and metabolism <sup>47</sup>. Obesity increases the risk of  
537 colorectal cancer, and these risk factors have been linked, at least in part, to  
538 abnormalities in lipid metabolism <sup>48</sup>. The ability of *FASN* to synthesize lipids makes it  
539 a promising target for the treatment of metabolic diseases <sup>49</sup>. The *SREBF1/FASN*  
540 pathway has been identified as a key target for colorectal therapy <sup>50</sup>. The postbiotics  
541 K-LP25 exerted anti-adipogenic activity in HFD-fed zebrafish through regulating  
542 adipogenesis-related genes.

Inactivated preparations are more effective in terms of epithelial penetration to exert positive effects due to smaller sizes of active components<sup>51</sup>. The composition of culture supernatant of *Cetobacterium somerae* and *Lactococcus lactis* exhibited a positive effect on skin mucus, liver, gut health, the structure and function of gut microbiota fed with high-fat diet<sup>52</sup>. *B. bifidum* postbiotic supplementation alleviates DSS-induced colitis by maintaining gut barrier and suppressing nuclear factor kappa B activation signaling molecules<sup>53</sup>. In addition, *Lactobacillus casei* and *Bifidobacterium bifidum* were found to regulate intestinal microbial diversity after being subjected to heat treatment (121 °C, 15 min)<sup>54</sup>. Heat-killed bacteria providing disrupted cells and released bacterial components, could better reproduce *in vivo* the physiological conditions in the gut lumen and outer mucus layer, with key components reaching eukaryotic cells and enhancing the mucosal integrity<sup>55</sup>. This is supported by the localization of gut microbiota in the colon in the absence of mucosal damage. The intestinal mucosal mechanical barrier, which serves as the primary defense against foreign invasion in the intestinal tract, is primarily composed of the tight junction protein (TJ). This protein complex comprises three transmembrane proteins: *ZO-1*, *Occludin*, and *Claudin-1*. The integrity of the cell membrane is crucial for preventing harmful substances from invading the intestinal lumen and plays a vital role in modulating cell permeability. A high consumption of fat-rich foods poses a potential risk for increased intestinal permeability and the onset of intestinal damage. Our research demonstrated a marked decrease in the mRNA expression of the TJ proteins in the zebrafish intestine following a diet in high fat, leading to heightened barrier permeability. Corresponding results were observed at the gene expression level, with a reduction in the levels of *ZO-1*, *Occludin*, and *Claudin* in high-fat diet mice<sup>56</sup>. When the intestinal permeability increased, bacterial translocation facilitates the entry of various toxins, such as LPS, which induces local inflammation and triggers systemic inflammation by rapidly elevating cytokine levels<sup>57</sup>. In the present study, the relative expressions of intestinal *Claudin-1*, *ZO-1* and *Occludin* were significantly increased in the K-PP18 group compared with the

model control. These results suggest that K-PP18 has a pivotal role on improving the intestinal barrier.

The gene levels of intestinal inflammatory factors in high-fat diet-induced zebrafish were significantly upregulated, while these gene levels were notably downregulated when supplemented with postbiotics diets, particularly K-LP25. Lipoteichoic acid and EPS from *L. plantarum* have been proposed to contribute to in anti-inflammatory responses, demonstrating the properties in preparations containing heat-killed bacteria<sup>58, 59</sup>. EPS biosynthesis is related to glucose metabolism<sup>60</sup>. It was reported that Lactobacillus-derived EPSs with high fructose content had excellent anti-inflammatory activity *in vivo* and revealed the anti-inflammatory activity had interspecific variability, and the mechanism of anti-inflammatory action was different<sup>61</sup>. This may also be the reason for the inconsistent anti-inflammatory effects of K-LP1 and K-LP25.

Although the three inactivated strains did not differ significantly in their regulation of  $\alpha$ -diversity, they exhibited a relatively strong ability to regulate microbial composition. Postbiotic supplementation helps reduced the relative abundance of *Proteobacteri*, suggesting improved gut microbial homeostasis<sup>62</sup>. *Proteobacteria* is commonly considered as source of pathogens and their higher abundances may be harmful to fish health<sup>63</sup>. According to our results, heat-killed strains K-LP1 (82%) and K-PP18 (69%) supplementation reversed the HFD-induced reduction (15%) of *Proteobacteria*. Increased *Fusobacterium* abundance has been reported to be associated with a variety of intestinal diseases<sup>64</sup>. It has been observed to correlate positively with obesity following supplementation with ultrasound-inactivated postbiotics<sup>65</sup>. Thus, postbiotics derived from K-LP1, K-LP25, and K-PP18 may help maintain the integrity of the intestinal mucosal barrier, prevent the growth of pathogens, and enhance host-microbiome homeostasis. These mechanisms collectively contribute to the maintenance of gut eubiosis<sup>66, 67</sup>.

In conclusion, the postbiotics K-LP25 investigated in our study displayed significant anti-inflammatory effects. Notably, K-LP25 was distinguished by its lipid-

lowering capabilities and K-PP18 significantly strengthened the intestinal barrier. The potential of K-LP25 in mitigating inflammation triggered by a high-fat diet, particularly through reducing intestinal harm and rebalancing the gut microbiota, signifies its role in nutrition. Future research should focus on pinpointing the active metabolic components of K-LP25 and K-PP18, along with sequencing and characterizing the genes of these three strains to explore the mechanisms through which they mitigate intestinal damage induced by a high-fat diet. There is a potential for K-LP25 and K-PP18 to be utilized in functional foods aimed at enhancing human intestinal health.

**CRedit authorship contribution statement**

**Yujuan Yu:** Methodology, Formal analysis, Visualization, Writing-original draft. **Min Zhou** Data curation, Methodology, Investigation, Visualization. **Faizan A Sadiq:** Validation of the concept, scientific advises and support in writing. **Pengli Hu:** Data curation, Software. **Feng GAO:** Methodology, Validation. **Juanxia Wang:** Software, Investigation. **Aowen Liu:** Software, Investigation. **Yue Liu:** Validation, Writing editing. **Haili Wu:** Methodology, Validation. **Guohua Zhang:** Conceptualization, Writing-review & editing, Supervision.

**Declaration of interest statement**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Abbreviations

**HFD:** High-fat diet. **LAB:** Lactic acid bacteria. **TNF- $\alpha$ :** Tumor necrosis factor  $\alpha$ . **IL- $1\beta$ :** Interleukins-1 $\beta$ . **IL-6:** Interleukins-6. **ZO-1:** Zonula occludens-1. **SREBF1:** Sterol regulatory element binding transcription factor 1. **FASN:** fatty acid synthase.

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- 859

860 **Tables**

861 **Table 1**

862 Table 1 Primer sequences used for quantitative analysis of genes related to intestinal  
863 inflammation, tight junction proteins, and lipid metabolism.

| Genes            | Forward primers (5'~3') | Reverse primers (3'~5')   |
|------------------|-------------------------|---------------------------|
| <i>TNF-α</i>     | AGGCAATTTCACTTCCAAGG    | AGGTCTTTGATTCAGAGTTGTATCC |
| <i>IL-1β</i>     | ATCCAAACGGATACGACCAG    | TCGGTGTCTTTCTGTCCAT       |
| <i>IL-6</i>      | TCAACTTCTCCAGCGTGATG    | TCTTTCCTCTTTTCCTCCTG      |
| <i>Occludin</i>  | AGATGTGGAGGACTGGGTCA    | ATTACGGACGGGCAGAATC       |
| <i>Claudin-1</i> | CTGCTGTATCTGTGGGAGTGAA  | TAATCAGGAGAACAGGCGAAG     |
| <i>ZO-1</i>      | ACAAGAACAGGGCGGAACAGT   | ACCTCCAGAAATCAGCACGA      |
| <i>SREBF1</i>    | CATCCACATGGCTCTGAGTG    | CTCATCCACAAAGAAGCGGT      |
| <i>FASN</i>      | GAGAAAGCTTGCCAAACAGG    | GAGGGTCTTGCAGGAGACAG      |
| <i>Leptin</i>    | AGCTCTCCGCTCAACCTGTA    | CAGCGGGAATCTCTGGATAA      |
| <i>β-Actin</i>   | ATGAAGATCCTGACCGAG      | TAGCTCTTCTCCAGGGAG        |

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## Figures

## Figure 1

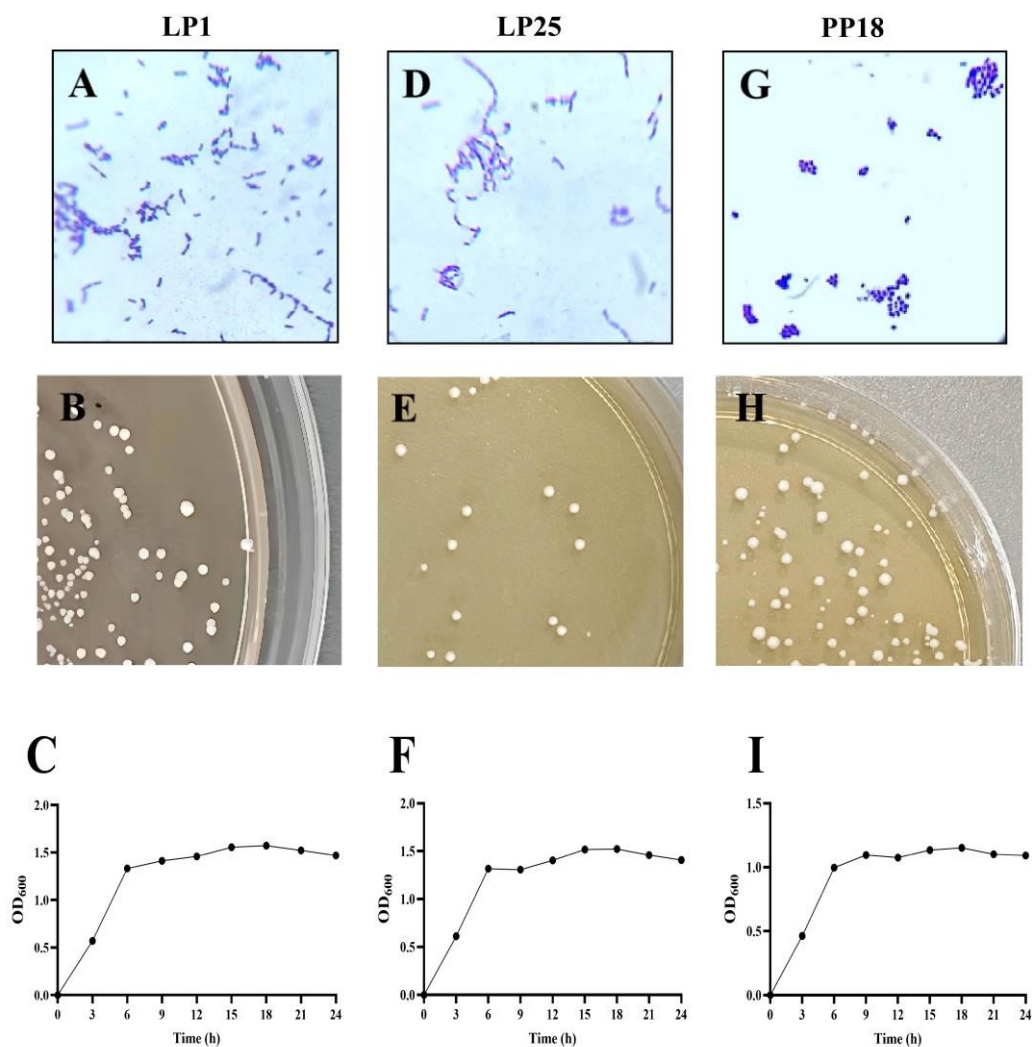


Figure 1. The microscopic characteristics (A, D, G): 100x magnification, colony morphology (B, E, H), and growth curves (C, F, I) of the strains LP1 and LP25, as well as PP18.

Figure 2

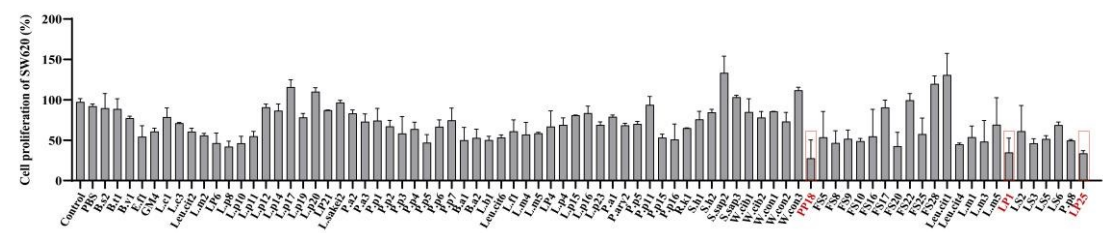


Figure 2. Screening of postbiotics for inhibition of colon cancer cell proliferation.

870 **Figure 3**

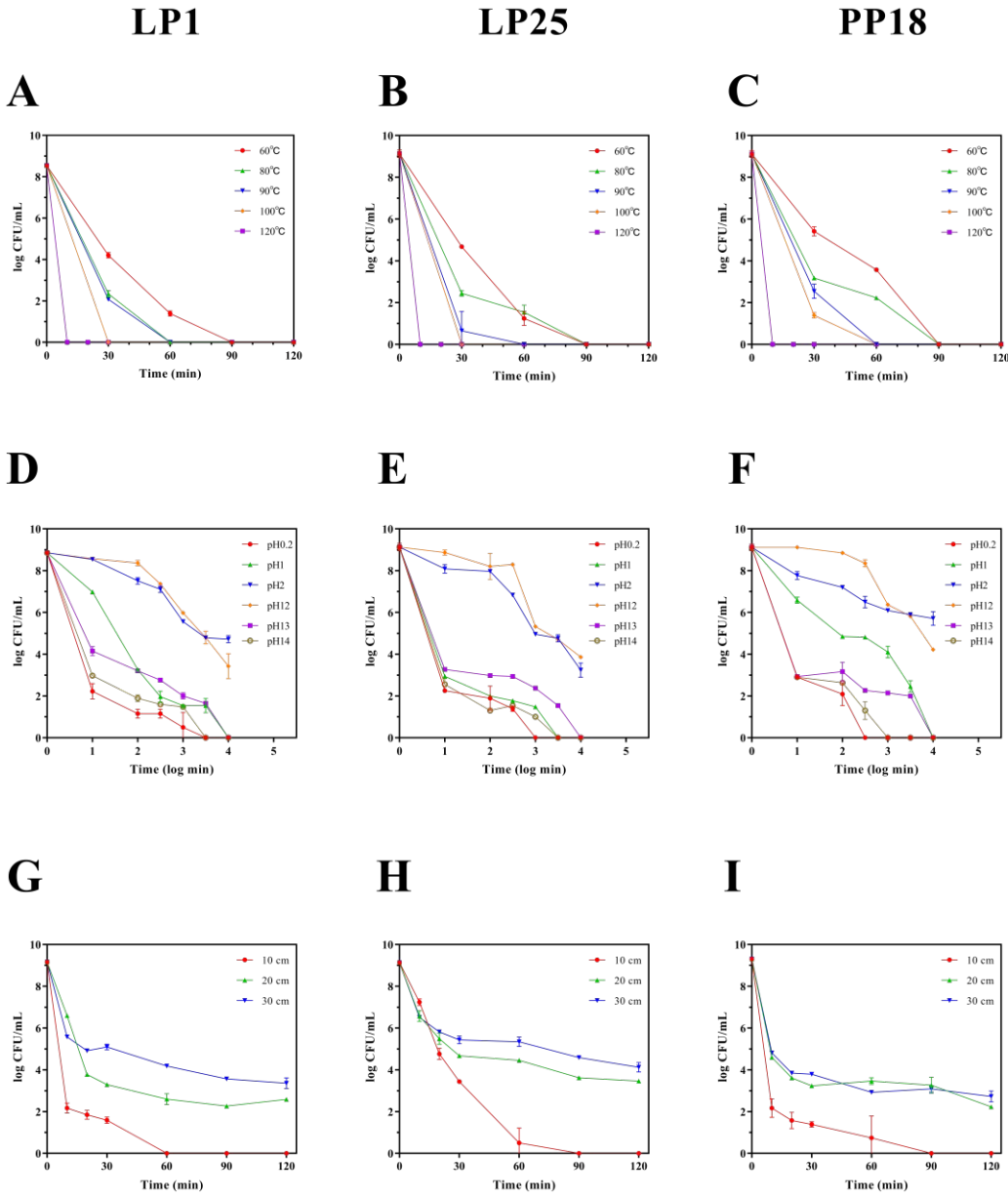


Figure 3. The impact of various methods of inactivation on the activity of the strain. (A-C) Heat inactivation, (D-F) pH adjustment inactivation, (G-I) Ultraviolet inactivation.

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**Figure 4**

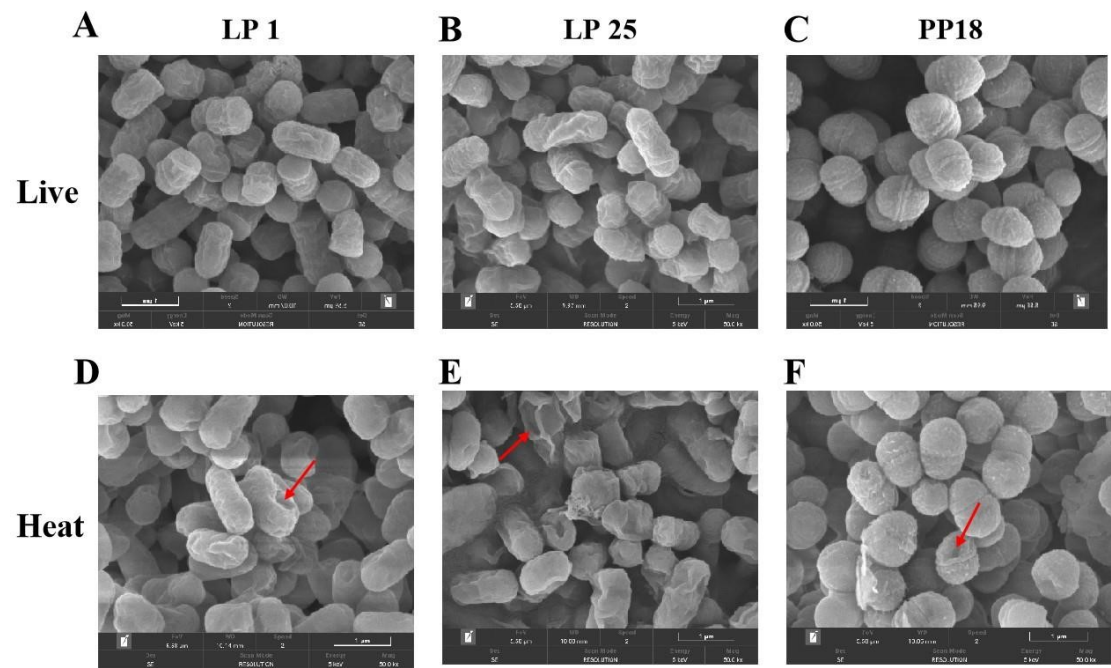


Figure 4. The impact of heat inactivation on the microscopic structure of various bacterial strains was examined. Electron micrographs of live bacteria of LP1 (A), LP25 (B), and PP18 (C), respectively. Inactivated bacteria of LP1 (D), LP25 (E), and PP18 (F), respectively.

Damaged cells are highlighted by red arrows.

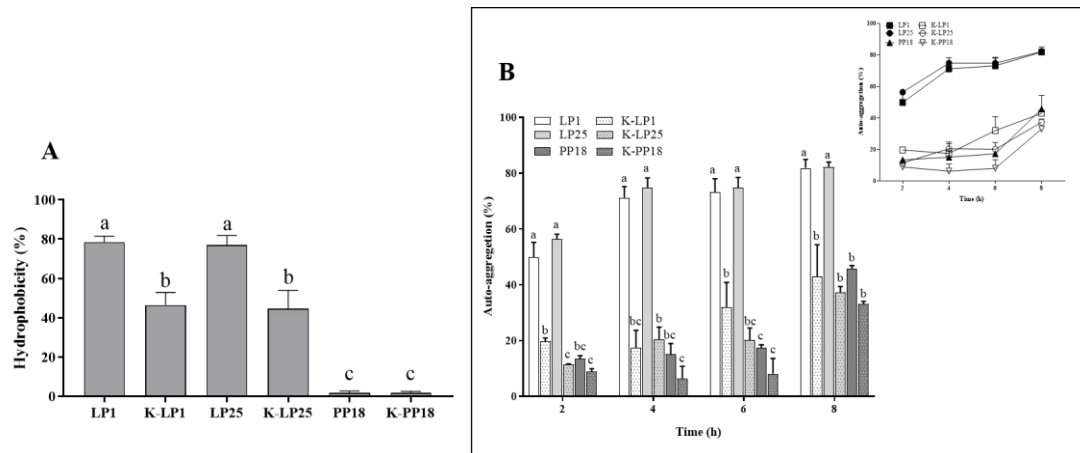
874 **Figure 5**

Figure 5. (A): Hydrophobicity; (B): Self-aggregation ability, the upper right shows the curve of self-aggregating force with time. Different letters indicate differences. (n=3).

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876 **Figure 6**

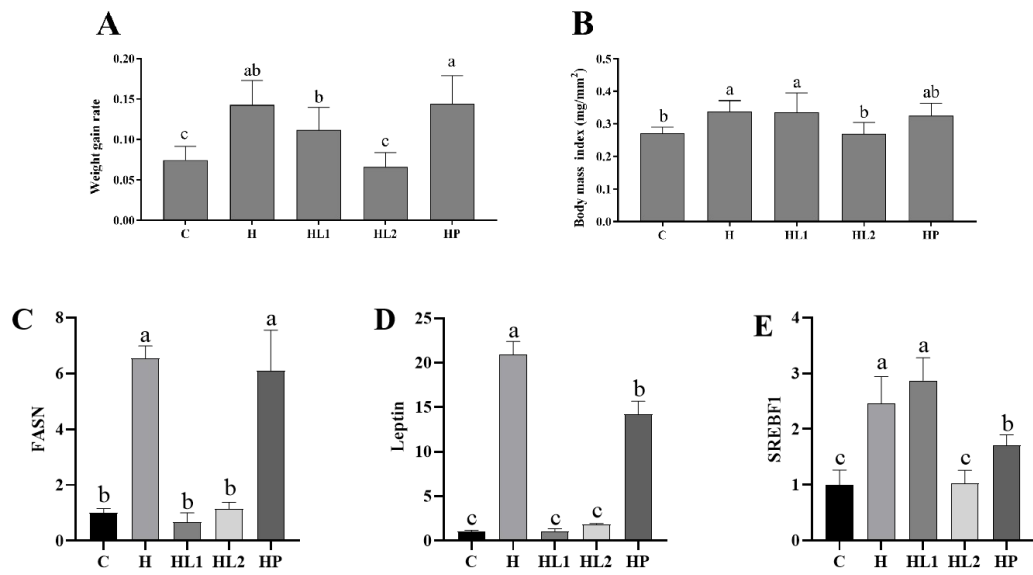


Figure 6. Effect of postbiotics on lipid metabolism and body index. (A) Weight growth rate, (B) body mass index, (C) relative expression of *FASN* mRNA, (D) relative expression of *Leptin* mRNA and (E) relative expression of *SREBF1* mRNA. Different letters indicate differences. (n=3).

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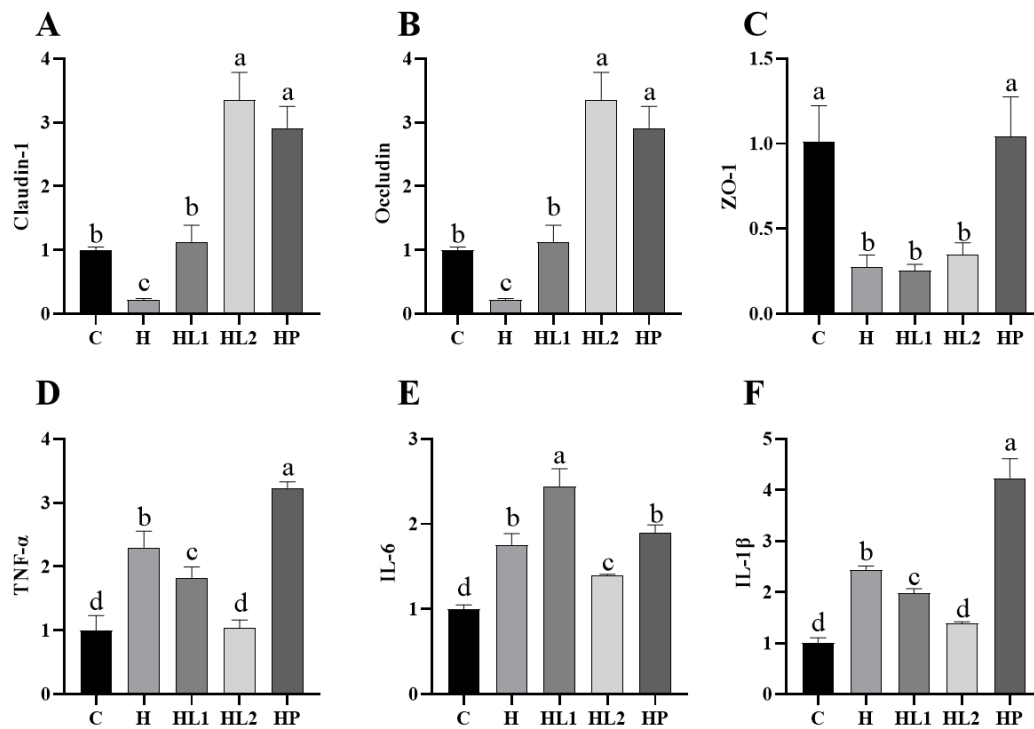
878 **Figure 7**

Figure 7. Impact of postbiotics on tight junction protein and inflammatory gene expression in colon of HFD zebrafish. Relative mRNA expression of TJ proteins in the intestinal barrier (A) *Claudin-1*, (B) *Occludin* and (C) *ZO-1* mRNA. Relative expression of intestinal inflammatory mRNA (D) *TNF- $\alpha$* , (E) *IL-6* and (F) *IL-1 $\beta$* . Different letters indicate differences. (n=3).

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Figure 8

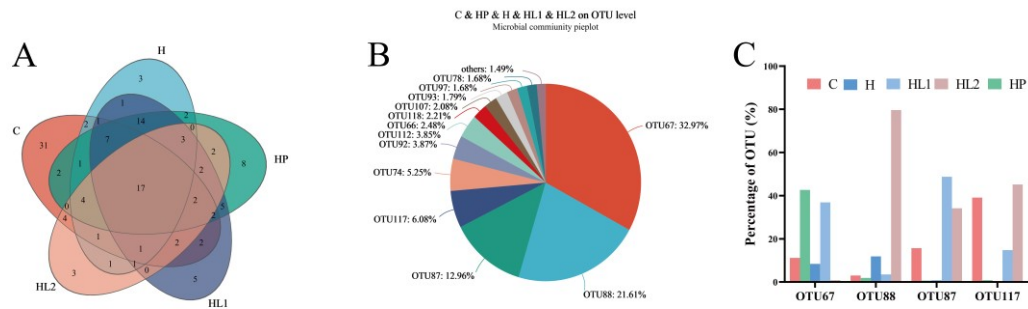


Figure 8. Venn analysis. (A) species shared and unique in different group. Different colors represent different group, the numbers in the overlapping parts represent the number of species shared by multiple groups, and the numbers in the non-overlapping parts represent the number of species unique to the corresponding group. (B) Species distribution pie chart. Displays the distribution of common species in different groups. Different colors represent different species, and the pie chart area represents the percentage of the species in the total number of species. (C) Percentage of top 4 species in each group.

Figure 9

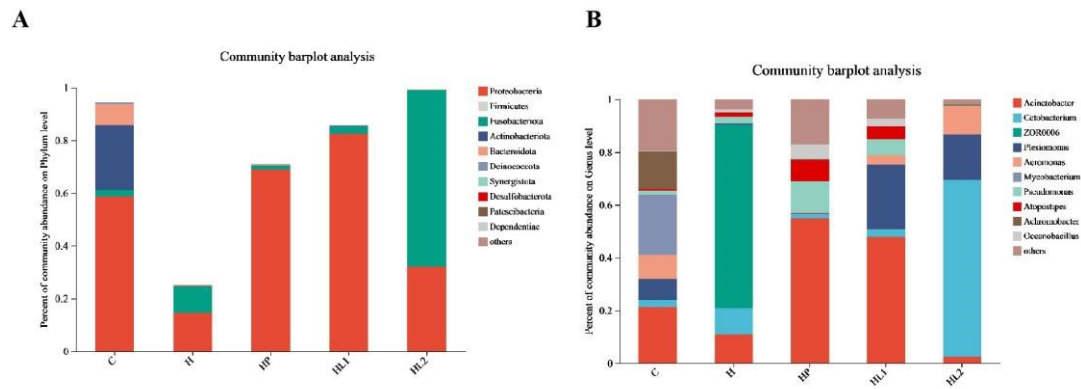


Figure 9. Effect of postbiotics on gut microbiota composition in HFD zebrafish. (A) The relative abundance of bacterial community at the phylum level and (B) the genus level.

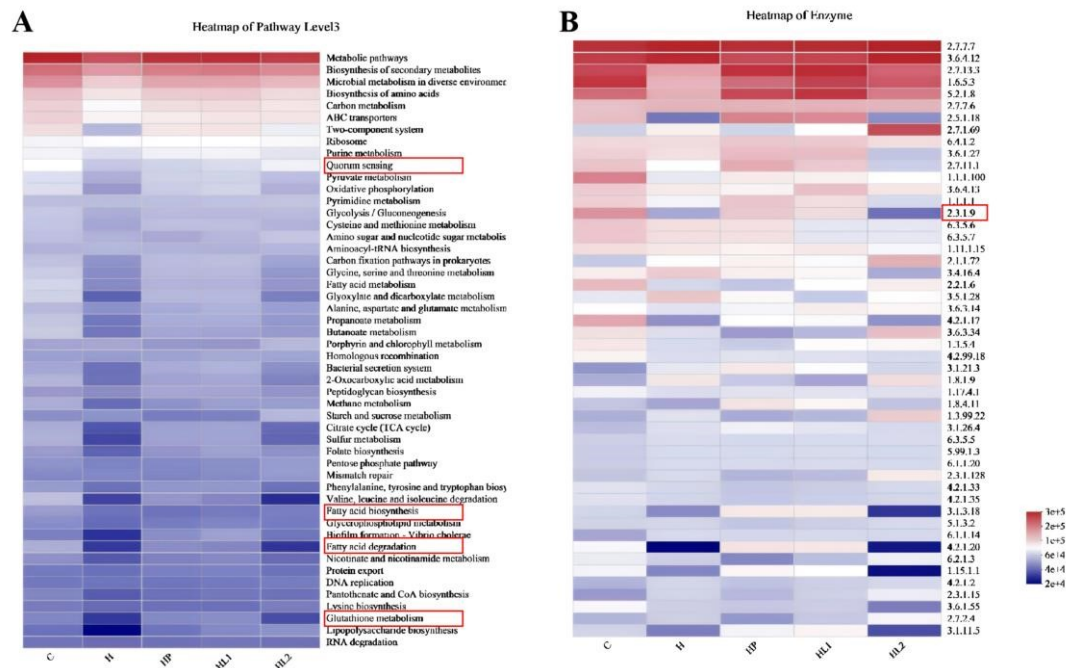
886 **Figure 10**View Article Online  
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Figure 10. Heatmap of pathway level 3 (A) and enzyme (B) through PICRUSt 2 analysis based on the KEGG database.

887 **Figure 11**

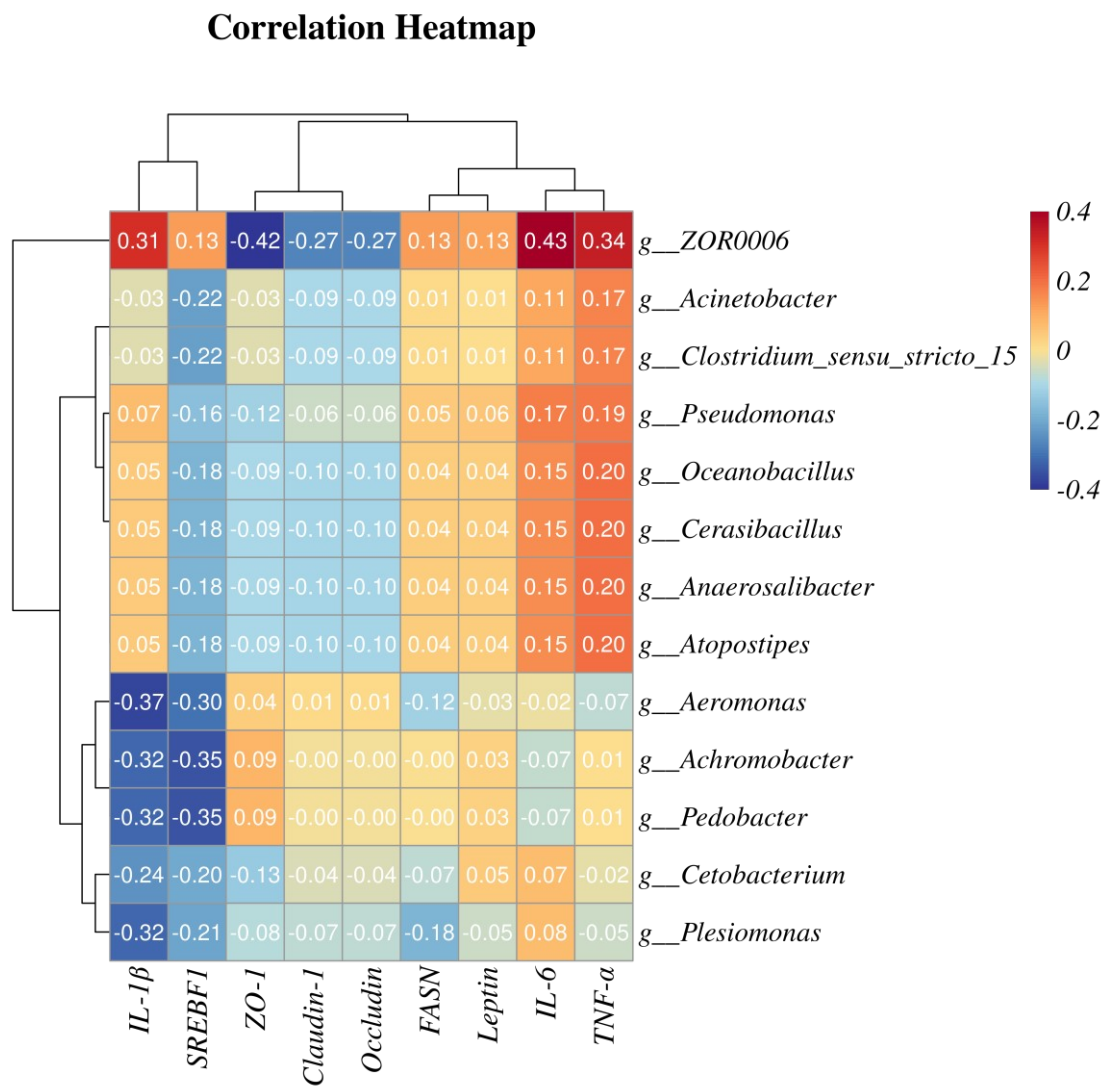


Figure 11. Heatmap according to Spearman's correlation. The number in the box indicates the *p*-value.

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## Data availability statements

Data for this article, including [description of data types] are available at [name of repository] at [URL – format <https://doi.org/DOI>].