ORCA – Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository:https://orca.cardiff.ac.uk/id/eprint/109372/

This is the author’s version of a work that was submitted to / accepted for publication.

Citation for final published version:

Nishio Ayre, Wayne , Melling, Genevieve , Cuveillier, Camille, Natarajan, Madhan, Roberts, Jessica L., Marsh, Lucy L., Lynch, Christopher D., Maillard, Jean-Yves , Denyer, Stephen P. and Sloan, Alastair J. 2018. Enterococcus faecalis demonstrates pathogenicity through increased attachment in an ex vivo polymicrobial pulpal infection. Infection and Immunity 86 (5) , e00871-17. 10.1128/IAI.00871-17

Publishers page: http://doi.org/10.1128/IAI.00871-17

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher’s version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.

Supplemental materials

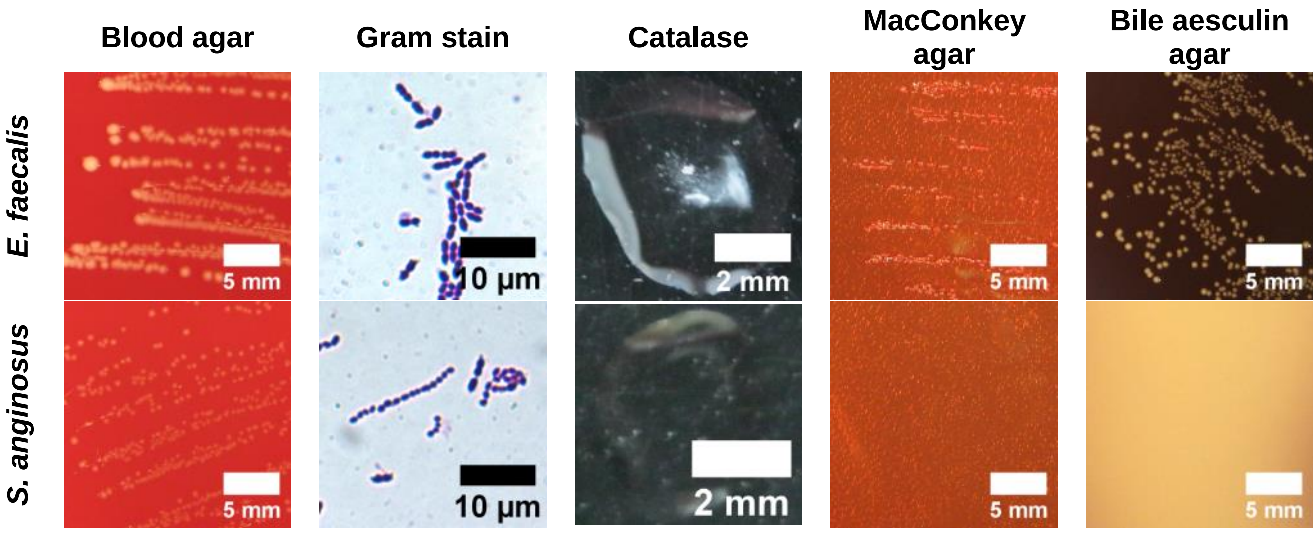


Fig. S1: Microbiology test results for the identification of *E. faecalis* and *S. anginosus*. The *E. faecalis* strain demonstrated large alpha haemolytic white colonies (1-2mm in size), chains of Gram-positive cocci approximately 1-2µm in diameter, was catalase negative, able to grow on MacConkey agar with a pink colony colour, able to grow on bile aesculin agar with a characteristic black agar colour change and belonged to the group D Lancefield grouping. The *S. anginosus* strain formed small alpha haemolytic colonies (<0.5mm) on blood agar, consisted of chains of Gram-positive cocci approximately 1-2µm in diameter, was catalase negative, unable to grow on MacConkey and bile aesculin agar and was positive for Group F Lancefield grouping. No growth of *S. anginosus* was detected after heat treatment and on bile aesculin agar supplemented with 6.5%w/w sodium chloride (data not shown).

A

E. faecalis clinical isolate D88 sequence showed a 97% sequence identity with *E. faecalis*

D88 (forward) sequence (query) alignment with *Enterococcus faecalis* strain CAU:180 16S ribosomal RNA gene, partial sequence (Accession number: MF369839.1; subject).

```
Query  12   GCGCT-CTATAATGCAGTCGACGCTTCTTTCCTCCCGAGTGCTTGCACTCAATTGGAAAG  70
            ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  8    GCGCTGCTATAATGCAGTCGACGCTTCTTTCCTCCCGAGTGCTTGCACTCAATTGGAAAG  67

Query  71   AGGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACT  130
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  68   AGGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACT  127

Query  131  TGGAAACAGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCG  190
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  128  TGGAAACAGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCG  187

Query  191  CTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGC  250
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  188  CTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGC  247

Query  251  TCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGA  310
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  248  TCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGA  307

Query  311  CACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCT  370
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  308  CACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCT  367

Query  371  GACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAG  430
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  368  GACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAG  427

Query  431  AAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGC  490
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  428  AAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGC  487

Query  491  TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGG  550
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  488  TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGG  547

Query  551  GCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGG  610
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  548  GCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGG  607

Query  611  GAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTA  670
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  608  GAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTA  667

Query  671  GCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAAGCGGCTCTCTGGTCTGT  730
            |||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||
Sbjct  668  GCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGT  727

Query  731  AACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA  790
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  728  AACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA  787

Query  791  CGCCGT-AACGATGAGTGCTAAGTGTTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAA  849
            |||||| |||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  788  CGCCGTAAACGATGAGTGCTAAGTG-TTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAA  846
```

```
Query  850  CGCATTAAGCACTCCGCCCTGGGGAGTACGACCGCAAG-T-GAACTTCAAAGGAA-TGAC  906
            |||||||||||||||| ||||||||||||||||||||| | |||  ||||||||| ||||
Sbjct  847  CGCATTAAGCACTCCG-CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGAC  905

Query  907  GGGGGCCCGCCACA-GCGTTG-AGCATGGTGGATTTATTCGA-GCAACGCGA-GA-C-T-  959
            |||||||||| ||| ||| || |||||| ||| || |||||| ||||||||| || | |
Sbjct  906  GGGGGCCCGC-ACAAGCGGTGGAGCATG-TGGTTTAATTCGAAGCAACGCGAAGAACCTT  963

Query  960  AC-AG-TCTTGACACTCTCTTTGA  981
            || || |||||||| || ||||||
Sbjct  964  ACCAGGTCTTGACA-TC-CTTTGA  985
```

B

E. faecalis clinical isolate E94 sequence showed a 97% sequence identity with *E. faecalis*

E94 (reverse) sequence (query) alignment with *Enterococcus faecalis partial 16S rRNA gene, isolate BS3-1 (Accession number HG798397.1; sbject).*

```
Query  16   CGACTTCACCCCA-TCATCTATTCCCACCTTTAGGCGGCTGGCTTCCAAAAAGGTTACCT  74
            ||||||||||||| ||||||| ||||||| |||||||||||||| |||||||||||||||
Sbjct  932  CGACTTCACCCCAATCATCTA-TCCCACC-TTAGGCGGCTGGCT-CCAAAAAGGTTACCT  876

Query  75   CACCGACTTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGG  134
            ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  875  CACCGACTT-CGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGG  817

Query  135  GAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCGGCTTCATGCAGGC  194
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  816  GAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCGGCTTCATGCAGGC  757

Query  195  GAGTTGCAGCCTGCAATCCCGAACTGAGAGAAGCTTTAAGAGATTTGCATGACCTCGCGG  254
            ||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  756  GAGTTGCAGCCTGCAATCC-GAACTGAGAGAAGCTTTAAGAGATTTGCATGACCTCGCGG  698

Query  255  TCTAGCGACTCGTTGTACTTCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCAT  314
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  697  TCTAGCGACTCGTTGTACTTCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCAT  638

Query  315  GATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCGCTAGAGTGC  374
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  637  GATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCGCTAGAGTGC  578

Query  375  CCAACTAAATGATGGCAACTAACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA  434
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  577  CCAACTAAATGATGGCAACTAACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA  518

Query  435  TCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTTTGTCCCCGAAGGGAAA  494
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  517  TCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTTTGTCCCCGAAGGGAAA  458

Query  495  GCTCTATCTCTAGAGTGGTCAAAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTC  554
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457  GCTCTATCTCTAGAGTGGTCAAAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTC  398

Query  555  GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAAC  614
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  397  GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAAC  338

Query  615  CTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGG  674
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  337  CTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGG  278

Query  675  AAACCCTCCAACACTTAGCACTCATCGTTTACG-CGTG-ACTACCA-GGTATCTAATCCT  731
            ||||||||||||||||||||||||||||||||| |||| ||||||| |||||||||||||
Sbjct  277  AAACCCTCCAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT  218
```

```
Query  732   GTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCGTCGCC  791
             |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  217   GTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCC  158

Query  792   ACTGGTGTTCCTCCATATATCTACGCATTTCAC-GCTACACATGGAGT-C-ACTCT-CTC  847
             ||||||||||||||||||||||||||||||||| |||||||||||| | | ||||| |||
Sbjct  157   ACTGGTGTTCCTCCATATATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTC  98

Query  848   TTCTGCACTCAAGTCTCC-AGTT-CCAATGAACCCTCCC-GGTGAAGCCGGGGCCTTTCT  904
             |||||||||||||||||| |||| ||||||| ||||||| |||  |||||||| |||||
Sbjct  97    TTCTGCACTCAAGTCTCCCAGTTTCCAATGA-CCCTCCCCGGTTGAGCCGGGGGCTTTCA  39

Query  905   CATCCCAACTTAA-AAACGCC-GCGCGTGCGCTT  936
             ||||  | ||||| ||||||| |||| | |||||
Sbjct  38    CATCAGA-CTTAAGAAACGCCTGCGC-T-CGCTT  8
```

C

S. anginosus clinical isolate D88 sequence showed 98% sequence identity with *S. anginosus*

D88 (forward) sequence (query) alignment with Streptococcus anginosus strain ChDC B407-I 16S ribosomal RNA gene, partial sequence (Accession number KC569583.1; sbject).

```
Query  8     GGCG-GGCT-ATA-ATGCA-GTAGGACGCACAGTTTATACCGTAGCTTGCTACACCATAG  63
             |||| | || ||| ||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  22    GGCGTGCCTAATACATGCAGGTAGGACGCACAGTTTATACCGTAGCTTGCTACACCATAG  81

Query  64    ACTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTATTAGAGGGGGATAAC  123
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  82    ACTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTATTAGAGGGGGATAAC  141

Query  124   TATTGGAAACGATAGCTAATACCGCATAACAGTATGTAACACATGTTAGATGCTTGAAAG  183
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142   TATTGGAAACGATAGCTAATACCGCATAACAGTATGTAACACATGTTAGATGCTTGAAAG  201

Query  184   ATGCAATTGCATCGCTAGTAGATGGACCTGCGTTGTATTAGCTAGTAGGTAGGGTAATGG  243
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202   ATGCAATTGCATCGCTAGTAGATGGACCTGCGTTGTATTAGCTAGTAGGTAGGGTAATGG  261

Query  244   CCTACCTAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG  303
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262   CCTACCTAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG  321

Query  304   ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGGAACCC  363
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322   ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGGAACCC  381

Query  364   TGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAAG  423
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  382   TGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAAG  441

Query  424   GAAGAACGAGTGTGAGAATGGAAAGTTCATACTGTGACGGTACTTAACCAGAAAGGGACG  483
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  442   GAAGAACGAGTGTGAGAATGGAAAGTTCATACTGTGACGGTACTTAACCAGAAAGGGACG  501

Query  484   GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATT  543
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  502   GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATT  561

Query  544   GGGCGTAAAGCGAGCGCAGGCGGTTAGAAAAGTCTGAAGTGAAAGGCAGTGGCTCAACCA  603
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  562   GGGCGTAAAGCGAGCGCAGGCGGTTAGAAAAGTCTGAAGTGAAAGGCAGTGGCTCAACCA  621
```

```
Query  604  TTGTAGGCTTTGGAAACTGTTTAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGT  663
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  622  TTGTAGGCTTTGGAAACTGTTTAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGT  681

Query  664  AGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTG  723
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  682  AGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTG  741

Query  724  TAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC  783
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  742  TAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC  801

Query  784  ACGCCGT-AACGATGAGTGCTAGGTGTTGGGTCCTTTCC-GGACTCAGTGCCGCAGCTA-  840
            ||||||| |||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct  802  ACGCCGTAAACGATGAGTGCTAGGTGTTGGGTCCTTTCCGGACTCAGTGCCGCAGCTAA  861

Query  841  CGCA-TAAGCACTCCGCCCTGGGGGAGTACGACCGCAAGGTTTGTAACTTCAAAGGAATT  899
            |||| ||||||||||||| | ||||||||||||||||||||| | |||| ||||||||||
Sbjct  862  CGCATTAAGCACTCCGCC-T-GGGGAGTACGACCGCAAGGTT-GAAACT-CAAAGGAATT  917

Query  900  GTACCGGGGGGCCCGC  915
            | || ||||| |||||
Sbjct  918  G-AC-GGGGG-CCCGC  930
```

D

S. anginosus clinical isolate E94 sequence showed a 96% sequence identity with *S. anginosus*

E94 (reverse) sequence (query) alignment with Streptococcus anginosus strain SK52 16S ribosomal RNA, complete sequence (Accession number NR_041722.2; sbject).

```
Query  19    CGACTTCACCCCA-TCATCTATCCCACCTTAGGCGGCTGGCTCCTTACGGTTACCTCACC  77
             ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1511  CGACTTCACCCCAATCATCTATCCCACCTTAGGCGGCTGGCTCCTTACGGTTACCTCACC  1452

Query  78    GACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACG  137
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1451  GACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACG  1392

Query  138   TATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTT  197
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1391  TATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTT  1332

Query  198   GCAGCCTACAATCCGAACTGAGACTGGCTTTCAGAGATTAGCTTGCCGTCACCGGCTTGC  257
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1331  GCAGCCTACAATCCGAACTGAGACTGGCTTTCAGAGATTAGCTTGCCGTCACCGGCTTGC  1272

Query  258   GACTCGTTGTACCAGCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGAT  317
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1271  GACTCGTTGTACCAGCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGAT  1212

Query  318   TTGACGTCATCCCCACCTTCCTCCGGTTTATTACCGGCAGTCTCGCTAGAGTGCCCAACT  377
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1211  TTGACGTCATCCCCACCTTCCTCCGGTTTATTACCGGCAGTCTCGCTAGAGTGCCCAACT  1152

Query  378   CAATGATGGCAACTAACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCAC  437
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1151  TAATGATGGCAACTAACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCAC  1092

Query  438   GACACGAGCTGACGACAACCATGCACCACCTGTCACCGATGTTCCGAAGAAACTTCCTAT  497
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1091  GACACGAGCTGACGACAACCATGCACCACCTGTCACCGATGTTCCGAAGAAACTTCCTAT  1032
```

```
Query  498   CTCTAGAAATAGCATCGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTA  557
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1031  CTCTAGAAATAGCATCGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTA  972

Query  558   AACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCG  617
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  971   AACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCG  912

Query  618   GTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCGGCACTGAGTCCCGGAAAGGA  677
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  911   GTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCGGCACTGAGTCCCGGAAAGGA  852

Query  678   CCCAACACCTAGCACTCATCGTTTACGGCGTGTACTACCAGGGTATCTAATCCTGTTCGC  737
             |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  851   CCCAACACCTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGC  792

Query  738   TCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCTTTCGCCACCGGT  797
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  791   TCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCTTTCGCCACCGGT  732

Query  798   GTTCCTCCATATATCTACGCATTTCAC-GCTACACATGGAAT-CCACTCTCCCC-TCTGC  854
             ||||||||||||||||||||||||||| |||||||||||||| ||||||||||| |||||
Sbjct  731   GTTCCTCCATATATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCCCTTCTGC  672

Query  855   ACTCAAGT-AA-CAGTT-CCAA-GCCTACATGGGTTGAGGCACTGCGTT-CAATTCGAAA  909
             |||||||| || ||||| |||| |||||||  ||||||| |||||| || || |||   |
Sbjct  671   ACTCAAGTTAAACAGTTTCCAAAGCCTACAATGGTTGAGCCACTGCCTTTCACTTCAGAC  612

Query  910   TTTTCTAAC-GCCTGGGGCT-GCTTTTATAGTCC-ATAATTCCGGAAAA-GCACTGGGAC  965
             ||||||||| ||||| | || ||||| |  | || |||| |||||| || || | |||||
Sbjct  611   TTTTCTAACCGCCTGCG-CTCGCTTT-AC-GCCCAATAAATCCGGACAACGCTC-GGGAC  556

Query  966   C-ACG  969
             | |||
Sbjct  555   CTACG  551
```

Fig. S2: Sequence alignments for *E. faecalis* and *S. anginosus.* Bacterial 16S rRNA DNA sequence reactions were performed using DNA from clinical isolates of *E. faecalis* (A and B) and *S. anginosus* (C and D) with universal primers D88 (A and C) and E94 (B and D). The 16S rRNA sequencing revealed that the clinical isolates used in this study had an average of 97% sequence identity with *E. faecalis* and *S. anginosus* species respectively. The microbiological tests and the sequencing results confirmed that the clinically isolated species of bacteria used in this study were *E. faecalis* and *S. anginosus*

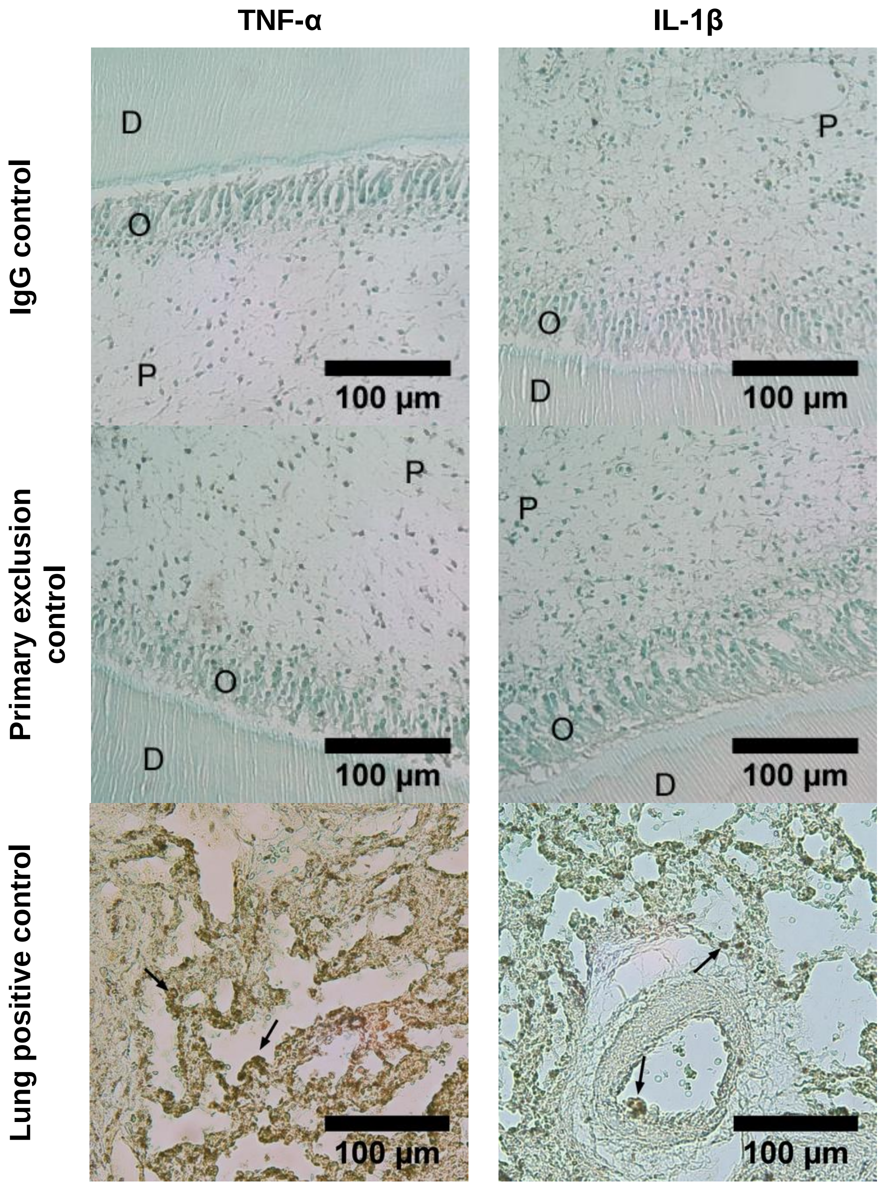


Fig. S3: Immunohistochemistry controls for TNF-α and IL-1β for the mixed species infection experiment. P represents the dental pulp, O the odontoblast region and D the dentine. Representative images from three experimental repeats shown.

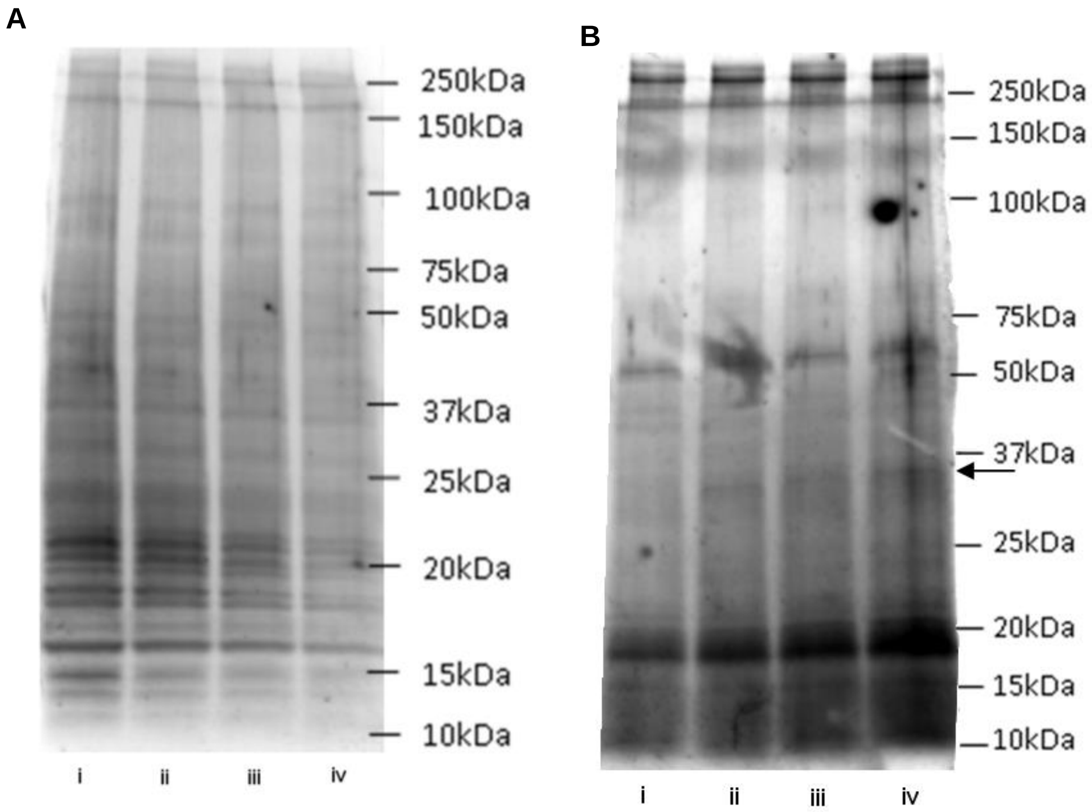


Fig. S4: SDS-PAGE and silver stain of bacterial (A) water soluble cell wall proteins and (B) culture supernatants: (i) *S. anginosus,* (ii) *E. faecalis,* (iii) 50:50 *S. anginosus : E. faecalis* and (iv) 90:10 *S. anginosus : E. faecalis*. Arrow highlights a difference in supernatant proteins at approximately 35kDa.

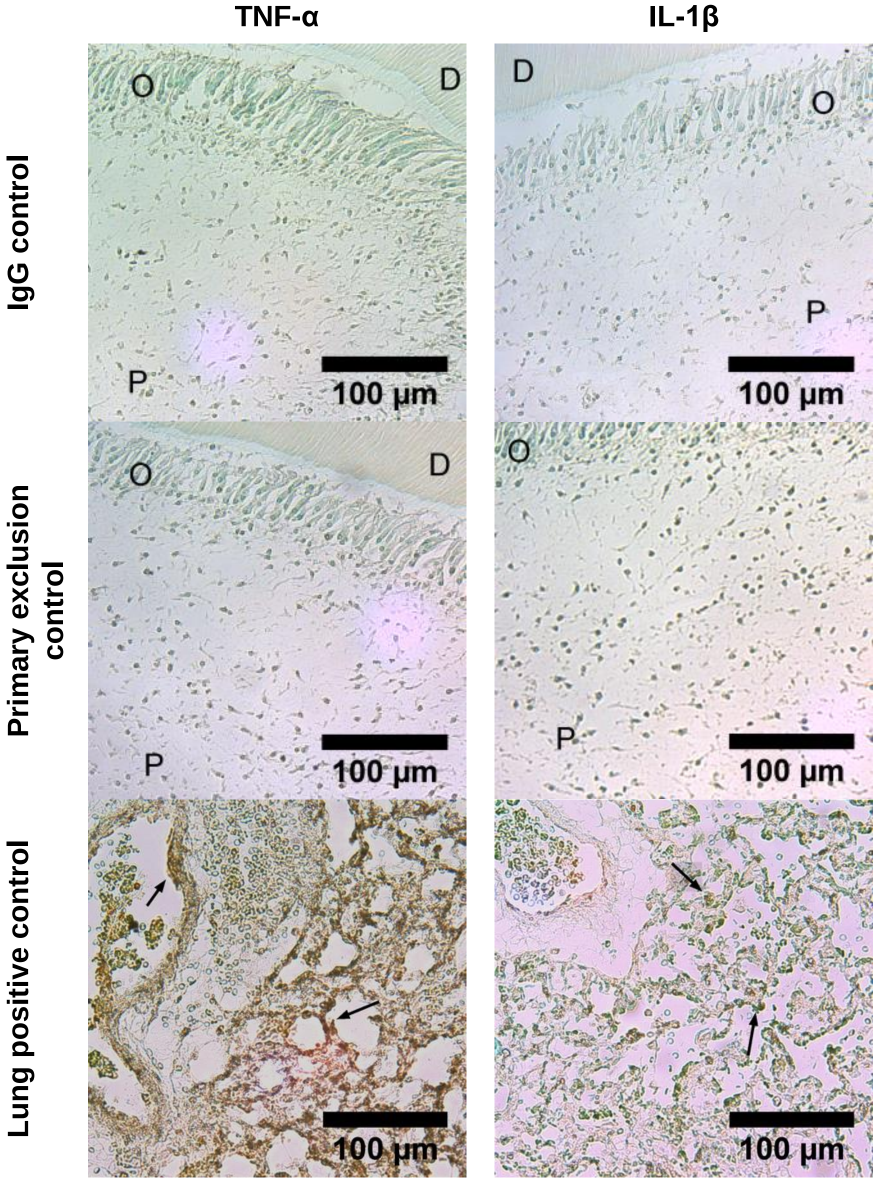


Fig. S5: Immunohistochemistry controls for TNF-α and IL-1β for the *E. faecalis* heat killed and supernatant experiment. P represents the dental pulp, O the odontoblast region and D the dentine. Representative images from three experimental repeats shown.

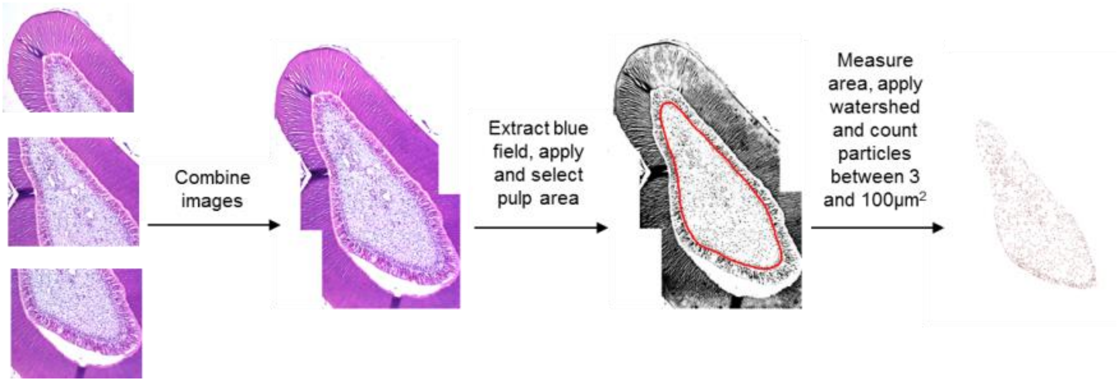
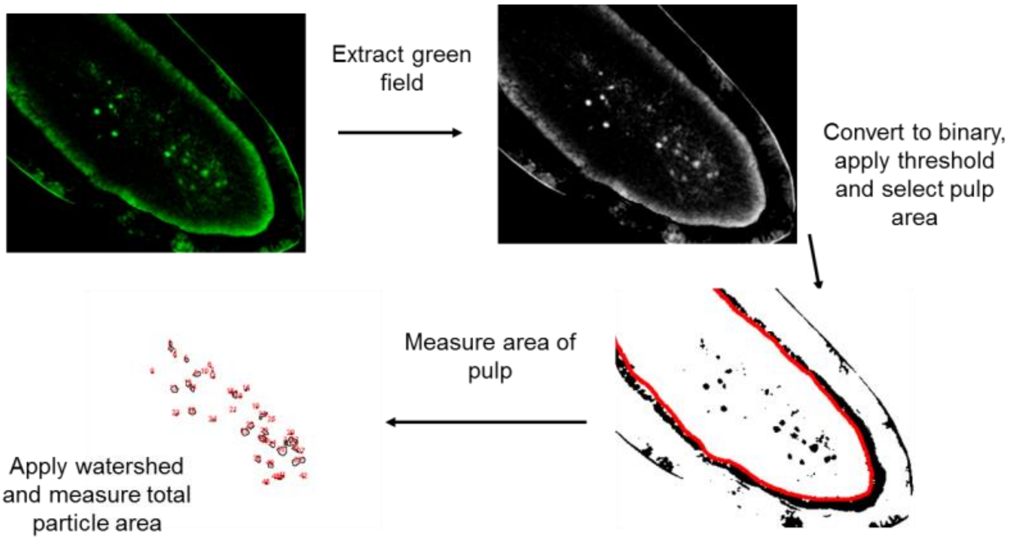


Fig. S6: Method of counting pulpal cells using ImageJ software.



Percentage of pulp covered by bacteria = (measured area/area of pulp) x 100

Fig. S7: Method of fluorescence quantification using ImageJ software.

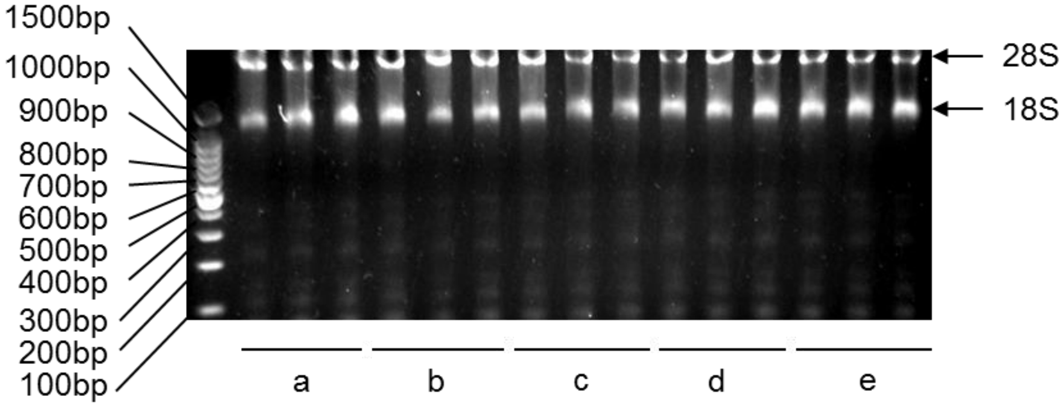


Fig. S8: RNA integrity for (a) Control, (b) *E. faecalis*, (c) *S. anginosus*, (d) 50:50 *S. anginosus:E. faecalis* and (e) 90:10 *S. anginosus:E. faecalis* samples showing 28S and 18S RNA bands.

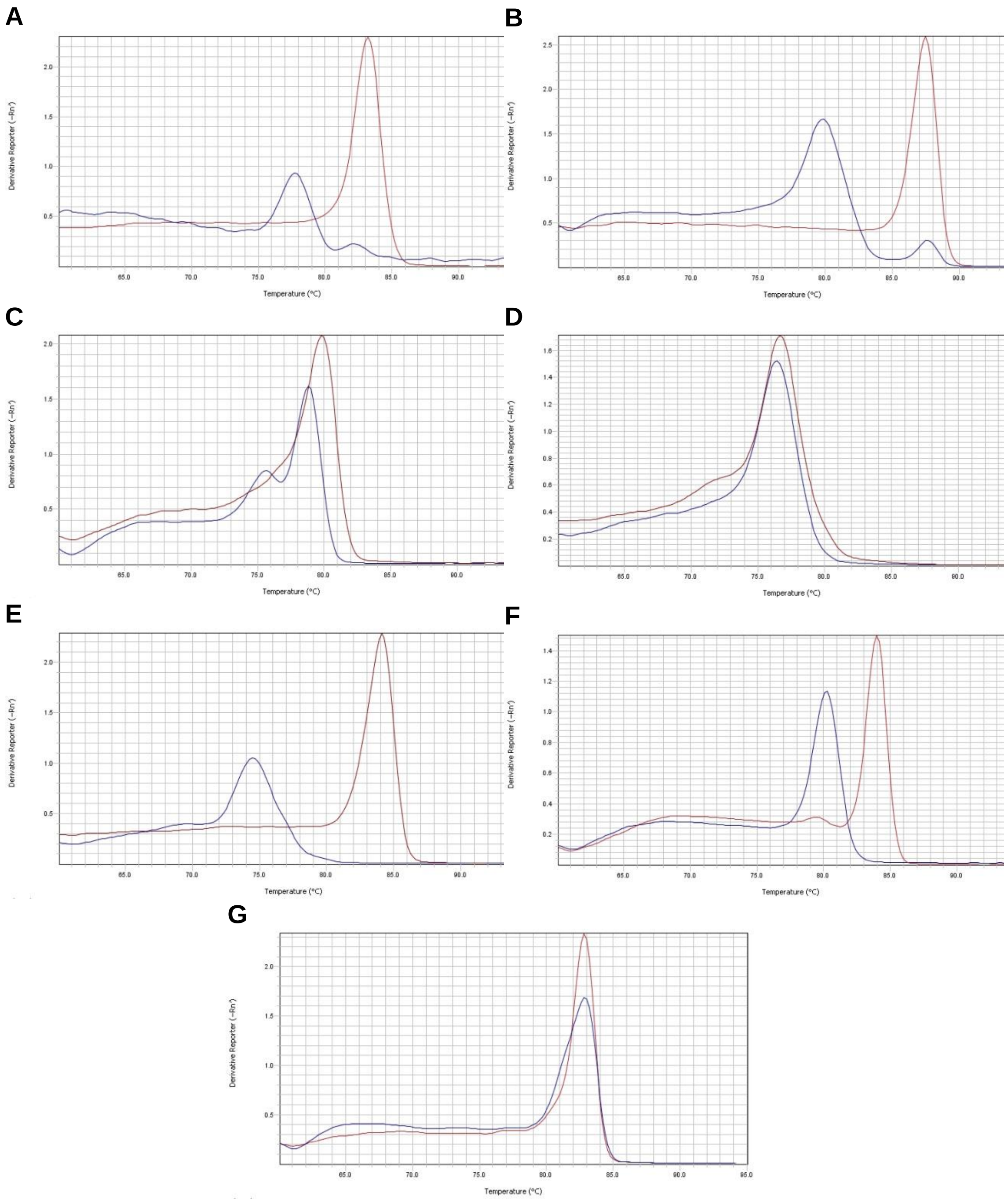


Fig. S9: Representative melt curve peaks for (A) GAPDH, (B) β-actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF-α and (G) IL-1β (red line represents a samples whilst the blue line represents a no template control).

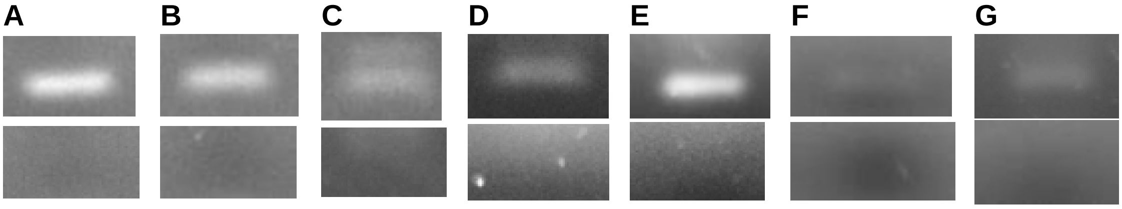


Fig. S10: Representative qPCR products run on gel for one *E. faecalis* infected tooth sample (top row) and the no template control (bottow row): (A) GAPDH, (B) β-actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF-α and (G) IL-1β.

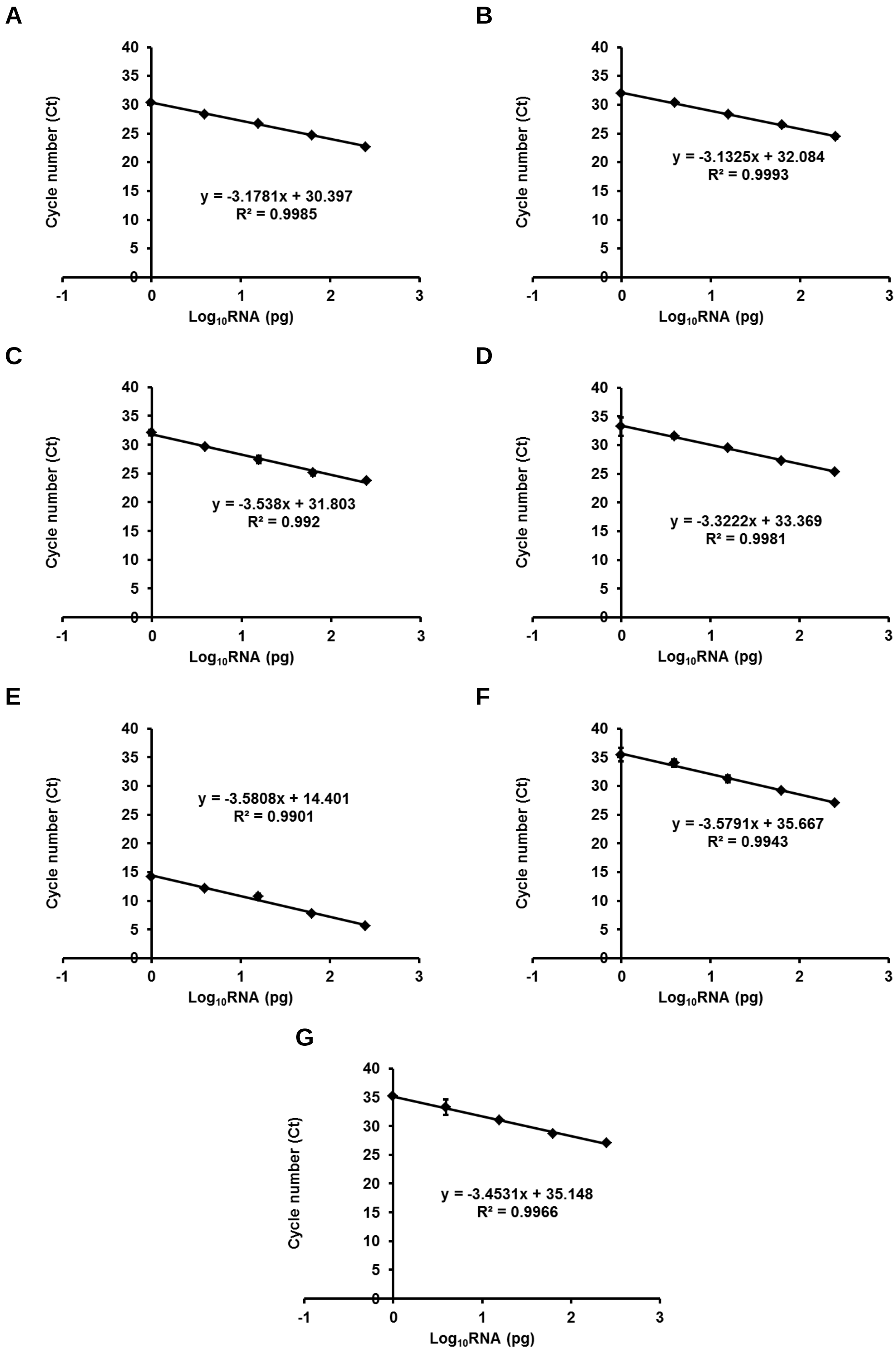


Fig. S11: Primer efficiency curves for (A) GAPDH, (B) β-actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF-α and (G) IL-1β.

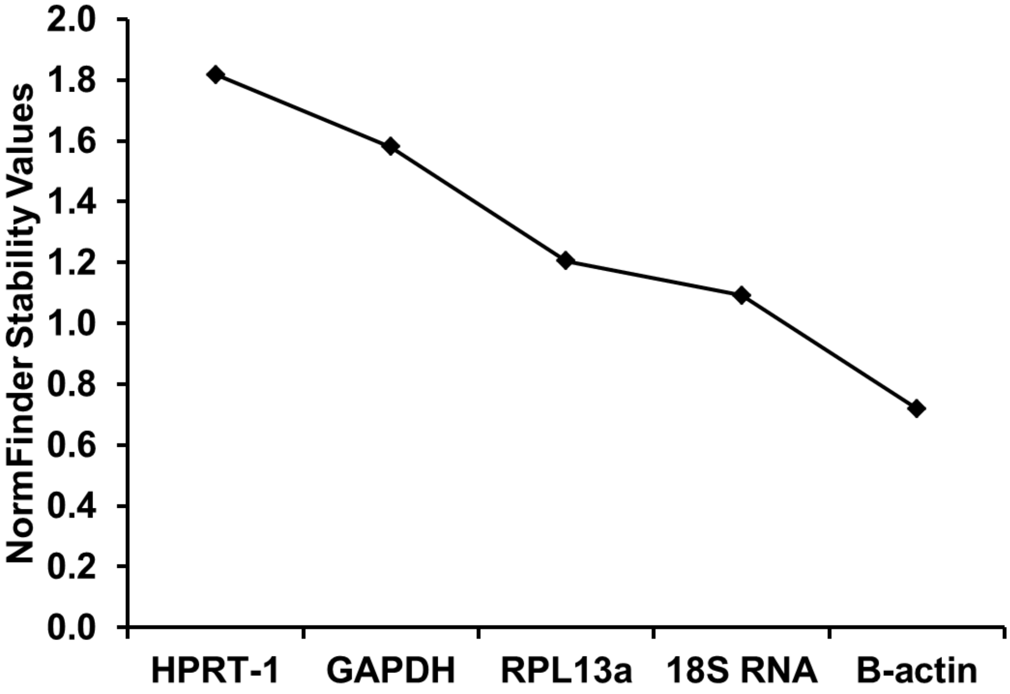


Fig. S12: NormFinder results for qPCR reference gene selection.