**Actinomyces spp. gene expression in root caries lesions**

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**Background:** The studies of the distribution of *Actinomyces* spp. on carious and non-carious root surfaces have not been able to confirm the association of these bacteria with root caries, although they were extensively implicated as a prime suspect in root caries.

**Objective:** The aim of this study was to observe the gene expression of *Actinomyces* spp. in the microbiota of root surfaces with and without caries.

**Design:** The oral biofilms from exposed sound root surface (SRS; n = 10) and active root caries (RC; n = 30) samples were collected. The total bacterial RNA was extracted, and the mRNA was isolated. Samples with low RNA concentration were pooled, yielding a final sample size of SRS = 10 and RC = 9. Complementary DNA (cDNA) libraries were prepared and sequenced on an Illumina® HiSeq 2500 system. Sequence reads were mapped to eight *Actinomyces* genomes. Count data were normalized using DESeq2 to analyse differential gene expression applying the Benjamini-Hochberg correction (false discovery rate [FDR] < 0.001).

**Results:** *Actinomyces* spp. had similar numbers of reads (Mann-Whitney U-test; p > 0.05), except for *Actinomyces* OT178 (p = 0.001) and *Actinomyces gerencseriae* (p = 0.004), which had higher read counts in the SRS. Genes that code for stress proteins (clp, dnaK, and groEL), enzymes of glycolysis pathways (including enolase and phosphoenolpyruvate carboxykinase), adhesion (Type-2 fimbrial and collagen-binding protein), and cell growth (EF-Tu) were highly – but not differentially (p > 0.001) – expressed in both groups. Genes with the most significant upregulation in RC were those coding for hypothetical proteins and uracil DNA glycosylase (p = 2.61E-17). The gene with the most significant upregulation in SRS was a peptide ABC transporter substrate-binding protein (log2FC = -6.00, FDR = 2.37E-05).

**Conclusion:** There were similar levels of *Actinomyces* spp. gene expression in both sound and carious root biofilms. These bacteria can be commensals in root surface sites but may be cariogenic due to survival mechanisms that allow them to exist in acid environments and to metabolize sugars, saving energy.

**Keywords:** RNA-seq; *Actinomyces* spp.; root caries; transcriptome; differential expression

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Over the past decades, the world’s population has been experiencing a higher retention of teeth due to improvements in dental health care. This fact implies an increased number of exposed root surfaces that are susceptible to caries (1). Knowledge of root caries etiopathogeny, and especially the primary etiological factor – the microbial biofilm, can support new research for the development of potentially useful targets for therapeutics. However, the current understanding of biofilm dynamics in root caries remains limited.

The search for a pathogenic species that causes root caries has been the objective of much of the research in this field since the 1970s (2–5). *Actinomyces* spp. are non-acidophilus, gram-positive rods, and facultative anaerobic bacteria that are related to dental plaque aging (6). *Actinomyces viscosus* and *Actinomyces naeslundii* were implicated as the pathogen of root surface caries by animal models and culture-based studies (2, 3, 5, 7). However, studies of the distribution of *Actinomyces* spp. have revealed that biofilm from both sound and carious root...
surfaces contains high proportions of Actinomyces spp. (8–12). The role of this genus in root caries development or health maintenance may be potentially complex because they could be capable of surviving in both sites, adapting the metabolism to exploit the available substrate.

Many advances in the understanding of dental caries are currently related to the cultured-independent DNA-based methods. The most important advantage would be assessment of the underestimated non-cultivable microorganisms’ prevalence in oral biofilms. Studies using 16S rRNA gene cloning and sequencing confirmed that the predominant bacteria in both carious and sound root surfaces was Actinomyces spp., followed by Streptococcus mutans and lactobacilli (13). However, DNA-based approaches also have limitations; it is not possible to determine if the DNA is coming from a viable cell, and it is not feasible to determine if the bacteria are expressing virulence factors that contribute to caries development or if they are only present in the environment struggling to survive. Rapid evolution of molecular techniques has enabled understanding not only of the microbiota composition but also of the microbiota function. RNA-based methods have enabled the study of biofilm gene expression, and consequently, the assessment of its functions. Hence, the aim of this study is to compare the gene expression of Actinomyces spp. in root surfaces’ biofilms with and without caries using the RNA-seq approach.

Materials and methods

Volunteers with an exposed root surface on at least one tooth and no root caries lesions were included in the sound root surface (SRS) group (n = 10). Dental biofilms were collected from all available exposed root surfaces. The number of exposed root surfaces varied among individuals. All participants recruited for the root caries (RC) group (n = 30) had one primary cavitated root lesion in need of restorative treatment. All lesions showed characteristics of present activity (soft and yellow dentin). Biofilm and carious dentin samples (of soft and infected tissue) were collected from patients during the restorative treatment.

Ethical considerations

This study was approved by the ethics committee of the Federal University of Rio Grande do Sul (process n° 427.168) and by the National Research Ethics Service Committee Yorkshire & the Humber - Leeds West (protocol n° 2012002DD). Volunteers in the study were patients who attended clinics in two centres for any dental treatment: Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, and Leeds School of Dentistry, University of Leeds, Leeds, UK. All volunteers consented to participate by donating samples after receiving the information about the study.

RNA-seq

After clinical collection, samples were immediately placed in a nuclease-free microtube containing 1 mL of RNA-protect reagent (QIAGEN, Inc., Venlo, Netherlands), transferred to the laboratory, centrifuged at 10,000 × g for 30 s. Pellets were stored at −80°C until further processing.

The total RNA was extracted from all the samples using an UltraClean® Microbial RNA Isolation Kit (Mo-BIO Laboratories, Inc., San Diego, CA) with on-column DNase digestion (QIAGEN, Inc., Venlo, Netherlands). The extracted RNA samples were quantified using the Quant-iTTm RiboGreen® RNA Assay Kit (Invitrogen, Inc., Waltham, Massachusetts, USA), and samples with total RNA concentration <30 ng/RNA were pooled, leading to a final sample count of 10 SRS and 9 RC (Supplementary Table 1).

A Ribo-Zero™rRNA Removal Kit (Bacteria), (Epicentre, Illumina, Inc., San Diego, CA) was used for mRNA enrichment, and Illumina® TruSeq™ library prep protocols (Illumina, Inc.) were used for library preparation. Briefly, these steps included: RNA fragmentation, cDNA synthesis, DNA fragment repair, amplification by polymerase chain reaction and purification, and adapter ligation for multiplexing. The final quality of cDNA and library validation was carried out using an Agilent Bioanalyzer (Agilent Technologies). Pair-end sequencing was then performed on an Illumina® HiSeq2500 (Illumina, Inc., San Diego, CA) sequencer to obtain 2 × 100 bp sequence reads.

Selection of genomes in databases

RNA-seq sequencing data are available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive, under the accession numbers SRS779973 and SRS796739.

Genomes of n = 162 bacteria and their associated information were downloaded from the DNA Data Bank of Japan, NCBI, the Broad Institute, and the Human Oral Microbiome Database (HOMD); their data were combined and used as a single organism reference in the short read mapping, which was carried out within the CLC Genomics Workbench 7.5.1 software (CLC Bio, QIAGEN), as described by Do et al. (14). The genomes of eight Actinomyces strains were selected for further analysis; these included Actinomyces gengeresceriae DSM 6844, Actinomyces johnsonii F0542, A. naeslundii str. Howell 279, Actinomyces odontolyticus ATCC 17982, Actinomyces sp. oral taxon 170 str. F0386, Actinomyces sp. oral taxon 178 str. F0338, Actinomyces sp. oral taxon 448 str. F0400, and Actinomyces oris (formerly known as A. viscosus C505).

Bioinformatics and differential expression

Count tables were generated containing the read count for each oral Actinomyces spp. genome. Proportion of genes per
genome expressed (transcripts) was also observed (reads \(^* > 0\) in at least one sample per group). The cut-off for considering the putative presence of the organism in the sample was 2,000 mapped reads (approximately one read per gene).

The relative mean expression (RME) level for *Actinomyces* spp. was calculated for each of the sample groups (SRS and RC) by species. The median of the relative expression values, obtained from the library size estimation carried out within the R package DESeq, was then calculated for each gene. The RME values in all genomes analysed here were added and ranked to observe and compare the most highly expressed *Actinomyces* spp. transcripts in the SRS and RC samples.

Differential gene expression among sample groups was carried out using the R package DESeq2 for each species (15). The cut-off for designating a gene as being differentially expressed was a change in transcript levels of at least 1-log2 fold (a two-fold difference) (positive values = upregulated in RC and downregulated in SRS, negative values = downregulated in RC and upregulated in SRS) and a Benjamini-Hochberg adjusted \(P\)-value (FDR) of less than \(10^{-3}\) (16). Principal component analysis (PCA) plots were constructed within the DESeq2 package to display the gene expression similarity among samples. The number of overexpressed genes by *Actinomyces* strains was determined per condition (health or disease metabolism).

**Results**

Eight strains of *Actinomyces* were mapped and evaluated in this study, comprising a total of 21,337 analysed genes. The total number of reads per sample per organism ranged from SRS = 6,250–883,308 and RC = 2,461–2,015,578 (Table 1). Proportion of expressed genes per genome ranged from 49.3–75.9 (SRS) and 28.0–89.8% (RC). All *Actinomyces* spp. had a similar number of reads and transcripts (Mann-Whitney U-test; \(p > 0.05\)), except for *Actinomyces* OT178 (median of reads = 39,174 in SRS and 4,816 in RC; \(p = 0.001\)) and *A. gerencseriae* (median of reads = 85,639 in SRS and 47,150 in RC; \(p = 0.004\)), which had higher read counts for the SRS samples (Table 1). *Actinomyces* sp. OT448 presented the higher number of reads – 883,308 (sample SRS_12) and 2,015,578 (sample RC_H), representing around 60% of total *Actinomyces* gene expression in all samples, RC and SRS, and achieving 86% of total *Actinomyces* gene expression in RC_A (Supplementary Fig. 1). *Actinomyces* sp. OT170 and *A. johnsonii* F0542 had the lowest number of genes per genome expressed in SRS (49.3 and 49.5%), while *Actinomyces* sp. OT178 had the lowest number of genes per genome expressed in RC (only 28% of their total number of genes). The other *Actinomyces* spp. expressed an average of 65% of their genes.

**Most expressed genes**

The log scale of RME of the 30 highest values at gene level sorted in ascending order for the RC samples are displayed in Fig. 1. Also, the 10 highest values at species level were sorted in ascending order for the RC samples and are displayed in Supplementary Fig. 2. The corresponding values for SRS samples for the same genes were plotted as well. Certain common features could be observed in the expression levels of most genes independent of whether the biofilms came from (RC or SRS).

A subset of genes that code for stress proteins and enzymes of glycolysis pathways was highly, but not differentially (\(p > 0.001\)), expressed in both groups. These genes code for key proteins involved in the maintenance of cell viability and metabolism in oral biofilms, meaning

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**Table 1.** Descriptive analysis of proportion of transcripts and number of significantly upregulated genes (upreg) by species of *Actinomyces* spp.

| Strain               | Total number of genes | Number of reads (Median (range)) | Number of upreg\(^a\) (%) | SRS | Number of reads (Median (range)) | Number of upreg\(^a\) (%) | RC  |
|----------------------|-----------------------|----------------------------------|----------------------------|-----|----------------------------------|---------------------------|-----|
| *A. gerencseriae* DSM 6844 | 2,875                 | 85,639* (28,147–306,920)         | 17 (0.6)                   |     | 47,150 (23,687–385,450)         | 80 (2.8)                   |     |
| *A. johnsonii* F0542   | 3,324                 | 28,930 (8,453–50,070)            | 21 (0.6)                   |     | 8,697 (3,020–85,250)            | 105 (3.2)                  |     |
| *A. naeslundii* str. Howell 279 | 2,930                 | 29,657 (9,990–117,608)           | 19 (0.6)                   |     | 23,143 (12,438–96,996)          | 88 (3.0)                   |     |
| *A. odontolyticus* ATCC 17982 | 1,982                 | 12,153 (6,250–145,837)           | 4 (0.2)                    |     | 5,806 (2,461–55,096)            | 40 (2.0)                   |     |
| *A. sp. oral taxon 170* str. F0386 | 3,042                 | 25,728 (7,865–53,541)           | 24 (0.8)                   |     | 12,577 (3,263–76,652)           | 89 (2.9)                   |     |
| *A. sp. oral taxon 178* str. F0338 | 2,308                 | 39,174* (15,871–74,3480)         | 35 (1.4)                   |     | 4,816 (2,451–43,108)           | 54 (2.3)                   |     |
| *A. sp. oral taxon 448* str. F0400 | 2,342                 | 45,822 (10,116–883,308)          | 9 (0.4)                    |     | 237,720 (11,327–2,015,578)      | 88 (3.8)                   |     |
| *A. oris* C505          | 2,534                 | 58,122 (22,440–619,447)          | 35 (1.4)                   |     | 74,763 (22,486–682,971)         | 226 (8.9)                  |     |
| **TOTAL**              | **21,337**            | **484 (2.0)**                    |                            |     | **770 (4.0)**                   |                           |     |

*\(p < 0.05\); Mann-Whitney U-test. 
\(^a\)Upreg = upregulated genes; FDR < 0.001; calculated by DESeq2.
Actinomyces are metabolically active in both caries and sound root surfaces biofilms. Stress-related genes clp, dnaK, and groEL were highly expressed for Actinomyces OT448, A. naeslundii, and A. gerencseriae. The enzymes with functions in glycolysis pathways enolase, formate-acetyltransferase, phosphoenolpyruvate carboxykinase (GTP) (pckA) (gluconeogenesis), and glyceraldehyde-3-phosphate dehydrogenase (gap) had higher gene expression for Actinomyces. Also, among those highly expressed genes, there were genes coding for proteins related to adhesion (such as Type-2 fimbrial and collagen-binding protein) and cell growth (EF-Tu).

Differential expression analysis
Using a conservative approach and considering up- or downregulated genes with more than a two-fold difference and FDR <10^-3, it was shown that only 5.9% (21,337 genes, 1,254 with significant differential expression) of Actinomyces genes had significant differential expression. There were quite a few genes up- and downregulated in
| Actinomyces spp. gene expression in root caries lesions and in the sound root surfaces samples | Root caries | Sound root surfaces |
|---|---|---|
| | baseMean | log2FC | FDR | baseMean | log2FC | FDR |
| Hypothetical protein | 4.54 | 5.98 | 5.84E-05 | LSU ribosomal protein L18p | 71.56 | -2.78 | 9.81E-07 |
| Mobile element protein 14 | 162.40 | 4.86 | 5.07E-10 | CRISPR-associated protein Cse4 | 213.40 | -2.66 | 3.85E-05 |
| ABC transport protein, ATP-binding subunit | 48.37 | 4.67 | 5.07E-10 | LSU ribosomal protein L9p | 46.62 | -2.33 | 4.13E-04 |
| Hypothetical protein | 126.87 | 4.62 | 5.14E-09 | Aspartate carbamoyltransferase | 48.35 | -2.30 | 7.92E-04 |
| Hypothetical protein | 13.85 | 4.55 | 1.30E-06 | SSU ribosomal protein S19p (S15e) | 224.61 | -2.30 | 3.73E-04 |
| Mobile element protein 13 | 113.90 | 4.48 | 3.39E-11 | Glycerol-3-phosphate ABC transporter | 599.22 | -2.26 | 5.92E-04 |
| ATP-dependent DNA helicase | 33.01 | 4.42 | 1.16E-06 | Phosphoenolpyruvate protein phosphotransferase (PTS) | 261.52 | -2.24 | 3.04E-04 |
| Narrowly conserved hypothetical protein | 201.96 | 4.35 | 5.83E-06 | LSU ribosomal protein L4p (L1e) | 109.98 | -2.23 | 3.96E-04 |
| Transcriptional regulator, MerR family | 16.91 | 4.22 | 5.14E-09 | LSU ribosomal protein L5p (L11e) | 292.01 | -2.18 | 1.58E-04 |
| Hypothetical protein | 8.95 | 4.19 | 5.14E-09 | Fructokinase | 47.45 | -2.17 | 1.96E-04 |
| Hypothetical protein | 145.15 | 6.85 | 3.61E-15 | Cell wall-binding repeat protein | 17.08 | -4.04 | 8.35E-04 |
| Hypothetical protein | 4.83 | 6.67 | 7.14E-05 | L-lactate dehydrogenase | 12.05 | -3.53 | 1.97E-04 |
| Multidrug resistance protein, putative | 11.23 | 6.28 | 1.42E-06 | FIG00545076: hypothetical protein | 48.27 | -3.28 | 4.75E-05 |
| Hypothetical protein | 2.69 | 6.17 | 2.86E-04 | Fructose-bisphosphate aldolase class II | 57.29 | -3.24 | 1.88E-08 |
| 5-keto-2-deoxy-D-glucanate-6 phosphate aldolase [form 2] | 2.23 | 6.16 | 2.14E-04 | Hypothetical protein | 171.66 | -3.13 | 1.53E-04 |
| HMPREF1979_01399 | 2.63 | 6.14 | 3.34E-04 | Heat shock protein 60 family chaperone GroEL | 119.35 | -2.97 | 1.47E-04 |
| Hypothetical protein | 2.77 | 6.14 | 3.52E-04 | HMPREF1979_01533 | 14.96 | -2.91 | 3.30E-04 |
| Predicted L-rhamnose permease RhaY | 9.00 | 6.12 | 1.62E-05 | HMPREF1979_01229 | 15.90 | -2.85 | 8.37E-05 |
| Octaprenyl diphosphate synthase/dimethylallyl transferase/(2E,6E)-farnesyl diphosphate synthase/geranylgeranyl diphosphate synthase | 94.25 | 6.11 | 8.97E-12 | HMPREF1979_01249 | 3.50 | -2.84 | 6.89E-04 |
| Hypothetical protein | 4.50 | 6.03 | 4.32E-05 | HMPREF1979_01215 | 5.30 | -2.82 | 9.53E-04 |
| ABC transporter related | 70.12 | 5.82 | 1.94E-11 | Fructose-bisphosphate aldolase class II | 268.02 | -3.82 | 2.69E-10 |
| Hypothetical protein | 53.83 | 5.69 | 2.18E-11 | Phosphoenolpyruvate carboxykinase [GTP] | 17.86 | -3.34 | 1.10E-04 |
| Sialic acid transporter (permease) NanT | 10.33 | 5.43 | 5.57E-06 | rplN_2_ALJK01000095 | 38.27 | -3.20 | 1.06E-05 |
| Inosose dehydratase (EC 4.2.1.44) | 12.35 | 5.26 | 2.36E-06 | SSU ribosomal protein S14p (S29e) | 9.53 | -3.07 | 5.14E-04 |
| Hypothetical protein | 2.59 | 5.18 | 2.62E-04 | NAD-dependent glyceraldehyde-3-phosphate dehydrogenase | 287.60 | -3.02 | 1.10E-04 |
| Hydroxymethylpyrimidine phosphate kinase ThiD (EC 2.7.4.7) | 13.71 | 5.12 | 2.56E-06 | Hypothetical protein | 42.89 | -2.99 | 9.03E-04 |
| A. naeslundii | Inosose isomerase (EC 5.3.99.-) | 44.85 | 5.01 | 4.76E-14 | Ferric enterobactin-binding periplasmic protein FeP | 17.70 | -2.94 | 2.57E-05 |

**Table 2.** Upregulated *Actinomyces* spp. genes in the root caries and in the sound root surfaces samples.
| Protein Description | baseMean | log2FC | FDR     | Protein Description | baseMean | log2FC | FDR     |
|---------------------|----------|--------|---------|---------------------|----------|--------|---------|
| Hypothetical protein | 2.49     | 5.00   | 2.85E-04| FIG00545076: hypothetical protein | 100.64   | -2.88  | 2.37E-07|
| Carotenoid cis-trans isomerase (EC 5.2.-.-) | 7.86     | 4.89   | 1.10E-04| HMPREF1129_0405_ALJK01000057 | 30.18    | -2.84  | 3.33E-06|
| Hypothetical protein | 2.95     | 4.84   | 3.83E-04| L-lactate dehydrogenase | 68.53    | -2.65  | 8.82E-05|
| Uracil DNA glycosylase | 28.23    | 6.60   | 2.61E-17| Hypothetical protein | 6.51     | -2.92  | 2.37E-04|
| Para-aminobenzoate synthase | 42.91    | 5.85   | 3.71E-12| pgk_3_NZ_DS264 | 74.76    | -2.23  | 2.49E-07|
| Fructose-bisphosphate aldolase | 12.63    | 5.65   | 4.70E-04| 50S ribosomal protein L6 | 66.95    | -1.81  | 2.18E-04|
| CDP-alcohol phosphatidyltransferase | 20.84    | 5.62 | 1.07E-08| 30S ribosomal protein S8 | 53.44    | -1.79  | 4.75E-05|
| Membrane protein | 1.86     | 5.55   | 3.41E-04| DNA helicase | 21.23    | 2.07   | 4.60E-04|
| LysR family transcriptional regulator | 1.72     | 5.34   | 6.94E-04| Sodium:proton antiporter | 21.21    | 2.58   | 3.32E-04|
| MFS transporter | 19.96    | 5.32   | 1.34E-08| Lipoate–protein ligase A | 9.86     | 2.59   | 5.03E-04|
| Two-component system response regulator | 3.69     | 4.99   | 2.17E-04| Uracil–xanthine permease | 60.81    | 2.69   | 2.18E-04|
| Hypothetical protein | 2.51     | 4.95   | 7.01E-04| ABC transporter ATP-binding protein | 2.82     | 2.95   | 3.16E-04|
| Glycosyltransferase family 1 | 2.00     | 4.87   | 5.03E-04| Sialic acid transporter | 3.80     | 2.99   | 6.27E-04|
| Transcriptional regulator, AraC family | 5.41     | 6.58   | 5.10E-05| Fructose-bisphosphate aldolase class II | 133.34   | -4.27  | 5.68E-10|
| HMPREF9056_00703_AFBL01000016 | 2.42     | 5.86   | 5.17E-04| Ferric enterobactin-binding periplasmic protein FepB | 10.32    | -3.89  | 1.22E-04|
| Probable transposase for insertion sequence | 33.82    | 5.82   | 2.50E-09| SSU ribosomal protein S14p (S29e) | 5.46     | -3.60  | 9.23E-04|
| L-xylulose 5-phosphate 3-epimerase | 12.25    | 5.78   | 4.33E-06| Sucrose-6-phosphate hydrolase | 20.86    | -3.22  | 1.94E-04|
| HMPREF9056_00704_AFBL01000016 | 2.18     | 5.70   | 8.18E-04| LSU ribosomal protein L6p (L9e) | 26.16    | -3.22  | 9.65E-05|
| Hypothetical protein | 2.05     | 5.65   | 9.47E-04| FIG00545076: hypothetical protein | 108.49   | -3.01  | 1.69E-09|
| Hypothetical protein | 7.31     | 5.58   | 3.01E-05| Multiple sugar ABC transporter, membrane-spanning permease protein MsMF | 47.15    | -2.98  | 5.71E-05|
| FIG00448805: hypothetical protein | 8.66     | 5.55   | 3.34E-06| Glycerol-3-phosphate ABC transporter, ATP-binding protein UgpC (TC 3.A.1.1.3) | 202.36   | -2.91  | 2.44E-05|
| Hypothetical protein | 35.13    | 5.49   | 2.46E-07| Pyruvate formate-lyase (EC 2.3.1.54) | 23.67    | -2.90  | 5.97E-04|
| Putative ABC transporter ATP-binding protein | 16.21    | 5.49   | 5.82E-06| SSU ribosomal protein S7p (S5e) | 12.59    | -2.88  | 6.09E-06|
| Hypothetical protein | 4.04     | 5.53   | 1.36E-04| Peptide ABC transporter substrate-binding protein | 4.82     | -6.00  | 2.37E-05|
| Polysaccharide biosynthesis protein | 26.83    | 5.31   | 7.22E-12| Hypothetical protein, partial | 3.99     | -5.89  | 3.51E-05|
| Inosine-uridine nucleoside N-ribohydrolase | 7.84     | 4.88   | 2.36E-05| Co-chaperone GrpE, partial | 4.16     | -5.80  | 5.74E-05|
| ATP-dependent Lon protease | 20.64    | 4.61   | 2.25E-06| Lipase | 3.45     | -5.64  | 9.37E-05|
| Plasmid replication-like protein | 3.02     | 4.56   | 6.84E-04| Membrane protein | 4.34     | -5.59  | 1.59E-04|
| Molybdopterin biosynthesis protein MoeB | 5.70     | 4.43   | 1.33E-05| glmZ(sRNA)-inactivating NTPase | 3.11     | -5.51  | 1.51E-04|
### Table 2 (Continued)

| Gene Description                          | Root caries baseMean | log2FC | FDR    | Sound root surfaces baseMean | log2FC | FDR    |
|-------------------------------------------|----------------------|--------|--------|-------------------------------|--------|--------|
| CDP-alcohol phosphatidytransferase        | 13.53                | 4.42   | 1.91E-10 | Hypothetical protein          | 3.05   | -5.50  | 1.59E-04 |
| Two-component system response regulator   | 13.77                | 4.39   | 1.10E-14 | Thioredoxin                   | 3.02   | -5.43  | 2.04E-04 |
| Hypothetical protein                      | 12.16                | 4.36   | 4.31E-06 | Sugar-binding protein         | 6.15   | -5.40  | 2.98E-06 |
| 50S ribosomal protein L33                 | 9.87                 | 4.30   | 1.32E-13 | Dihydroxyacetone kinase       | 3.04   | -5.36  | 3.04E-04 |
| MFS transporter                           | 25.71                | 5.20   | 8.72E-06 | ABC transporter substrate-binding protein | 998.79 | -2.47  | 9.57E-05 |
| Hypothetical protein                      | 2.88                 | 5.02   | 6.14E-04 | Elongation factor Ts          | 365.12 | -2.27  | 1.25E-04 |
| Transposase                               | 5.77                 | 4.86   | 2.24E-04 | 50S ribosomal protein L23     | 314.22 | -2.14  | 5.23E-04 |
| Hypothetical protein                      | 2.53                 | 4.79   | 2.63E-04 | HMPREF9062_RS03960            | 40.36  | -1.89  | 9.57E-05 |
| Hypothetical protein                      | 2.42                 | 4.76   | 6.85E-04 | 30S ribosomal protein S3      | 384.20 | -1.88  | 1.58E-04 |
| Major facilitator superfamily protein     | 3.40                 | 4.63   | 4.97E-04 | 50S ribosomal protein L2      | 385.80 | -1.75  | 8.83E-05 |
| Fumarate hydrolyase                       | 6.90                 | 4.15   | 7.19E-05 | FOF1 ATP synthase subunit gamma | 97.09  | -1.56  | 1.90E-05 |
| Type II secretion system protein          | 17.26                | 4.08   | 9.28E-07 | 50S ribosomal protein L22     | 173.31 | -1.51  | 8.43E-04 |
| Hypothetical protein                      | 37.88                | 4.03   | 1.07E-06 | 50S ribosomal protein L1      | 129.96 | -1.33  | 9.77E-04 |
| Hypothetical protein                      | 3.63                 | 3.95   | 8.10E-04 | Hypothetical protein HMPREF9062_RS10410 | 37.18  | 1.36   | 9.20E-04 |
| Hypothetical protein XRE family transcriptional regulator | 3.88                 | 5.78   | 7.68E-06 | CRISPR-associated protein Cse1  | 5.17   | -3.94  | 3.34E-05 |
| XRE family transcriptional regulator      | 9.19                 | 5.10   | 5.29E-07 | groEL                         | 1441.54 | -2.76  | 7.42E-05 |
| Hypothetical protein                      | 3.79                 | 4.65   | 2.52E-05 | Fructose-bisphosphate aldolase class II | 4512.01 | -2.68  | 3.68E-04 |
| CAAX amino protease                       | 6.94                 | 4.52   | 2.82E-06 | 1,4-alpha-glucan-branching protein | 463.60  | -2.67  | 8.26E-06 |
| Hypothetical protein                      | 1.27                 | 4.52   | 7.48E-04 | 50S ribosomal protein L9      | 74.66  | -2.51  | 5.23E-07 |
| Cro/Ci family transcriptional regulator   | 3.05                 | 4.36   | 3.96E-04 | Universal stress protein      | 18.39  | -2.51  | 6.15E-04 |
| ABC transporter ATP-binding protein       | 1.93                 | 4.33   | 7.83E-04 | Sugar transporter             | 36.13  | -2.50  | 5.29E-07 |
| HMPREF0059_RS05590                        | 3.14                 | 4.26   | 4.21E-05 | 30S ribosomal protein S11     | 126.46 | -2.39  | 9.65E-05 |
| Aminoglycoside phosphotransferase         | 3.18                 | 4.24   | 5.54E-05 | Elongation factor Ts          | 197.32 | -2.34  | 2.17E-05 |
| WXG100 family type VII secretion target   | 8.53                 | 4.23   | 3.86E-06 | Sugar ABC transporter permease | 198.18 | -2.30  | 2.50E-04 |

The list of genes is ranked in descending order to the log2FoldChange values. Positive log2FoldChange represents the upregulated genes in RC (or downregulated in SRS); Negative log2FoldChange represents upregulated genes in SRS (or downregulated in RC). baseMean is the average of the normalized count values (divided by size factors). log2FC, log2FoldChange: corresponds to the effect size estimate indicating the change in gene expression between both sample groups; FDR, false discovery rate; Benjamini and Hochberg adjusted p-values.
the samples independent of whether they belonged to a sound or a caries biofilm. RC presented a higher number of overexpressed genes ($n = 484$ genes in SRS and $n = 770$ genes in RC) (Table 1). *A. oris* C505 had the highest differential expression (8.9% of genes upregulated in RC). Only *Actinomyces* OT178 presented a large number of overexpressed genes in SRS (355 upregulated and 54 downregulated genes in SRS).

Table 2 displays the most significantly differentially expressed genes for each condition. The bioinformatics tool DESeq2 suggested that genes with the most significant upregulation in RC were those coding for many

![Fig. 2. Principal component analysis (PCA) plots displaying sample-to-sample distances for root caries' (blue) and sound root surfaces' (green) biofilms based on the differential expression by *Actinomyces* spp.](image-url)
hypothetical proteins and uracil DNA glycosylase (log2FC = 6.60; FDR = 2.61E-17), which is an important protein for transcription and mutagenesis prevention.

Two systems responsible for alkalinization of the biofilm had upregulated genes in RC: arginine biosynthesis and urea catabolism. In arginine biosynthesis, some genes had significant upregulation in RC (none upregulated in SRS). *A. oris* C505 was coding for arginine deiminase (log2FC = 3.2, FDR = 0.00003); *Actinomyces* OT178 was coding for arginine ABC transporter ATP-binding protein (log2FC = 3.77; FDR = 2.09E-10); and *A. oris* C505 was coding for arginine ABC transporter ATP-binding protein (log2FC = 1.75; FDR = 0.0009). In the urea metabolism, one gene showed significant upregulation in RC: urea transporter (log2FC = 2.8; FDR = 0.0006) expressed by *A. oris* C505.

In the case of significant downregulated genes in RC, the most significant one was a peptide ABC transporter substrate-binding protein (log2FC = 6.00, FDR = 2.37E-05). Several hypothetical proteins were identified as well as proteins such as lipase (log2FC = −6.00, FDR = 9.37E-05), membrane proteins (log2FC = −5.64, FDR = 1.59E-04), glycosyl transferase family 2 (log2FC = −5.22, FDR = 4.15E-04), amino acid and carbohydrate ABC transporters (log2FC = −5.14, FDR = 2.99E-13), and others. The enzyme lactate dehydrogenase was upregulated in SRS (none in RC), expressed by *A. oris* C505 (log2FC = −2.02), *A. gerencseriae* (log2FC = −1.82), *A. johnsonii* (log2FC = −3.53, FDR = 7.30E-06), *Actinomyces* OT178 (log2FC = −3.08, FDR = 3.06E-10), and *A. naeslundii* (log2FC = −2.65, FDR = 1.73E-06).

Figure 2 shows PCA plots that were based on the transcriptomic data mapped to each *Actinomyces* strain. It indicates larger differences among RC samples than among SRS samples, suggesting that metabolic functions in the caries lesions are less conserved than in the SRS samples.

**Metabolic pathways**

Figure 3 shows the number of overexpressed genes per group (SRS and RC). A higher number of genes involved in amino acid metabolism were overexpressed in RC samples. Nucleotide metabolism had a higher number of overexpressed genes in SRS. Both groups of samples (SRS and RC) indicated overexpression of genes associated with carbohydrate metabolism.

Figure 4 shows the starch and sucrose metabolism pathways, highlighting the overexpressed functions in SRS and RC. As can be observed, all overexpressed genes are different among groups. While in RC, *Actinomyces* spp. especially seem to be metabolizing simple sugars (as sucrose and galactose), in SRS they are metabolizing...
Fig. 4. Continued
Discussion
Many studies have investigated the microbiota associated with human tooth root surfaces in health and disease by culture-based approaches, with the aim of detecting the root caries pathogen (7, 9, 11–13, 17–22). It was believed that a unique microbiota from root surfaces with and without caries lesions would be comprised mainly of Actinomyces spp. The present analysis focused on transcriptional dynamics among eight abundant Actinomyces spp. in oral microbial populations. Overall, exposed root surface samples were collected from larger surface areas compared to the lesions, which could affect differential analyses; however, normalizations steps were applied, and results showed consistently high levels of gene expression in all Actinomyces strains in both sample groups (RC and SRS). Additionally, the very low number of differentially expressed genes (5.9%) indicates that the Actinomyces present have very similar metabolisms in both caries and caries-free sites. This means that Actinomyces spp. seem able to easily exploit this niche, and that they are able to colonize and proliferate in and on root surfaces in a healthy and cariogenic environment.

The analysed species of Actinomyces seem to have a small number of genes with significantly higher expression for putative cariogenic functions, except for Actinomyces OT178, which had some overrepresented functions in health-related biofilm. The present study found a high expression level of some genes related to cell wall metabolism (Fig. 1) in health biofilms and lesions. Other studies that used RNA-seq to identify the transcriptome/metatranscriptome of oral biofilms also found overrepresentation of elongation factors Tu and G, underlining their involvement in oral biofilm formation (14, 23, 24). Likewise, putative transposase and multidrug resistance protein were upregulated in RC. These genes take part in mechanisms that can transfer genes or plasmids from a pathogen to oral commensal bacteria. In bacteria, transposons can contain genes for antibiotic resistance or can modify the phenotype of other species by mutation, which can be related to the higher potential for genetic mobility when growing as a biofilm. In another study that evaluated the metatranscriptome of biofilms related to periodontal disease, transposases were overexpressed by Lactobacillus casei and Streptococcus mitis in pathogenic biofilms (25). Furthermore, because many hypothe-

Fig. 4. Starch and sucrose metabolism pathway obtained from Kyoto Encyclopedia of Genes and Genomes (KEGG) and displaying overexpressed genes (pink boxes) for root caries and sound root surfaces biofilms, based on the differential expression analysis of selected Actinomyces spp. 

Overexpressed in RC: Maltodextrin glucosidase (EC 3.2.1.20) expressed by A. johnsonii (Log2FC = 3.47; FDR = 4.54E-05) and by Actinomyces OT170 (Log2FC = 3.71; FDR = 1.53E-05). Sucrose phosphorylase (EC 2.4.1.7) expressed by Actinomyces OT170 (Log2FC = 2.32; FDR = 1.56E-05) and by A. naeslundii (Log2FC = 2.51; FDR = 1.85E-04). Alpha-amylose (EC 3.2.1.1) expressed by Actinomyces OT178 (Log2FC = 3.08; FDR = 5.95E-07).

Overexpressed in SRS: PTS system, beta-glucoside-specific IIB component (EC 2.7.1.69) expressed by Actinomyces OT170 (Log2FC = -2.57; FDR = 4.17E-08). Sucrose-6-phosphate hydrolase (EC 3.2.1.26) expressed by Actinomyces OT170 (Log2FC = -3.22; FDR = 1.94E-04). Fructokinase (EC 2.7.1.4) expressed by Actinomyces OT178 (Log2FC = -3.07; FDR = 1.10E-05) and by A. gerencseriae (Log2FC = -2.17; FDR = 1.96E-04). UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) expressed by Actinomyces OT178 (Log2FC = 1.83E-04). Glycogen phosphorylase (EC 3.2.1.9) expressed by A. oris C505 (Log2FC = -1.97; FDR = 6.53E-05). Glucosamine phosphorylase (EC 2.4.1.1) expressed by A. gerencseriae (Log2FC = -2.06; 3.62E-04), Actinomyces OT178 (Log2FC = -1.98; FDR = 3.62E-04), A. oris C505 (Log2FC = -1.92; FDR = 4.07E-04), A. naeslundii (Log2FC = -1.79; FDR = 5.79E-04), and Actinomyces OT170 (Log2FC = -1.72; FDR = 6.42E-05). 1,4-alpha-glucan-branching (EC 2.4.1.18) protein expressed by A. oris C505 (Log2FC = 2.67; FDR = 2.82E-06). Alpha-1,6-glucosidase expressed by Actinomyces OT178 (Log2FC = -3.28; FDR = 2.26E-06) (EC 3.2.1.33), 4-alpha-glucanotransferase (amylosomal) (EC 2.4.1.25) expressed by A. johnsonii (Log2FC = 2.91; FDR = 3.30E-04). Alpha-1,4-glucan-maltose-1-phosphate maltosyltransferase (EC 2.4.99.16) expressed by Actinomyces OT178 (Log2FC = -2.20; FDR = 8.72E-07).
tical proteins were upregulated in both conditions, their functions should be studied to clarify the relationship of *Actinomyces* to the development of root caries, even though *Actinomyces* previously have been described as the most prevalent bacteria in root surfaces (2, 7, 12, 13, 19, 26).

It was suggested that microorganisms involved in root caries are less dependent on refined carbohydrates than in coronal caries (9). In the present study, many genes related to glycolysis/gluconeogenesis pathways were identified, concluding that *Actinomyces* generate energy mainly from glycolysis in both healthy and carious root sites but using different enzymes (Figs. 3 and 4). This suggests the importance of sugar availability for the genus’ prevalence in the plaque, despite reports of their low saccharolytic activities (27). Likewise, this also suggests that the metabolism of carbohydrates is the most altered function of *Actinomyces* in a biofilm in homeostasis and dysbiosis. *Actinomyces* cells metabolize carbohydrates to organic acids and can also accumulate intracellular polysaccharides, which represent a cariogenic trait in these bacteria.

The main route to degrade glucose is the Embden-Meyerhof-Parnas (EMP) pathway, which is widely distributed in saccharolytic bacteria. However, it was already shown that *A. naeslundii* cells operate in this pathway in a manner different from that of the oral streptococci, which comprise the other dominant part of the saccharolytic bacteria in dental plaque and whose glycolysis has been studied intensively (28). *Actinomyces* use the compounds of polyphosphate and phosphoenolpyruvate carboxykinase (GTP) (also called pekA in *Actinomyces* OT448) as phosphate donors for hexokinase synthesis and phosphofructokinase phosphatase instead of ATP. GTP converts oxaloacetate into phosphoenolpyruvate and carbon dioxide. Further metabolism will generate the end products lactate, formate, acetate, and succinate through the regular EMP pathway (27, 28) like most other saccharolytic bacteria. These specific initial steps of phosphorylation could be implicated in the pathogenesis because this mechanism gives some advantage to *Actinomyces* cells for energy saving. In the results of this study, phosphoenolpyruvate carboxykinase was one of the higher expressed genes in RC and SRS, with no differential expression (very similar gene expression in both conditions).

Adhesion of bacteria to tooth surfaces is an essential step in the initiation of bacterial pathogenesis. It is already known that *Actinomyces* spp. can colonize the cervical surfaces and the gingival margin, contributing also to periodontal disease (3, 4). Numerous adhesion factors have been studied and implicated in the virulence of these bacteria, especially for periodontal diseases. Bacteria express a diverse array of fimbriae that are involved in bacterial adherence (29) and interbacterial adhesive interactions (30). Fimbriae type 1 and type 2 may confer some advantage in the establishment of *Actinomyces* (29). Different fimbriae-binding properties may explain the presence of *Actinomyces* in root sites. High expression of genes that code Type-2 fimbriae were found in both conditions, which relates to epithelial adhesion. Perhaps this confers advantages for *Actinomyces* adhesion in root surfaces (relationship with the gingival tissue) and also for the community biofilm co-adhesion. Also, collagen-binding protein was highly expressed and could be involved in the *Actinomyces* adhesion to root sites. It was previously demonstrated that the strains of *Actinomyces* that possess fimbriae exhibited strong binding to collagen. Therefore, the avidity of *Actinomyces* spp. for collagen would seem to be at least partially responsible for the high proportions of these organisms found on root surfaces (31).

The role of this genus in root caries development or health maintenance may be potentially complex because its species could be capable of surviving in both sites by being able to adapt the metabolism and utilize the available substrate. Several stress-related genes were highly expressed in RC, which means that these species developed mechanisms to survive in that inhospitable environment. One of these mechanisms was the use of lactate as a carbon source. The acidic environment within carious lesions is unlikely to be homogenous despite lactic acid being the major organic acid present (23). Lactate metabolism is an important pH regulating mechanism that facilitates the pH neutralization-producing propionate, acetate, CO₂, and H₂. Bacteria that utilize organic acids as their energy source may contribute to maintaining health by serving as an acid sink (32). Lactate could be converted to pyruvate by NAD-independent lactate dehydrogenase and further to acetyl CoA by pyruvate dehydrogenase. It was demonstrated that *A. naeslundii* strains degrade lactate, aerobically, to acetate and CO₂ through the conversion of lactate into pyruvate by a NAD-independent lactate dehydrogenase (28). The results of this study showed that the lactate dehydrogenase was upregulated in SRS in *Actinomyces* OT178, *A. naeslundii*, *A. johnsonii*, *A. gerencseriae*, and *A. oris* C505. This mechanism may have implications for *Actinomyces*’ ability to preserve the pH homeostasis in root surfaces’ biofilm.

In conclusion, the results of the present study showed that *Actinomyces* metabolism is very high and very similar in both health and caries root surface samples. *Actinomyces* OT178 seems to have some specific functions in health-related biofilms. As determined by past evidence, which found a high prevalence of *Actinomyces* in both sound and root caries sites, these results indicate their presence as commensals but do not preclude them from contributing to cariogenicity through the expression of fimbriae components for adhesion, genetic mobility, and energy saving for sugar metabolism. However, it is
important to point out that these functions in biofilms may be compensated by other non-Actinomyces species, and thorough gene expression analysis of the whole microbial community could help explain these interactions.

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References

1. Beck J. The epidemiology of root surface caries. J Dent Res 1990; 69: 1216–21.
2. Syed SA, Loesche WJ, Pape HL, Grenier E. Predominant cultivable flora isolated from human root surface caries plaque. Infect Immun 1975; 11: 727–31.
3. Sumney DL, Jordan HV. Characterization of bacteria isolated from human root surface carious lesions. J Dent Res 1974; 53: 343–51.
4. Socransky SS, Hubersak C, Propas D. Induction of periodontal destruction in gnotobiotic rats by a human oral strain of Actinomyces naeslundii. Arch Oral Biol 1970; 15: 993–5.
5. Jordan HV, Hammond BF. Filamentous bacteria isolated from human root surface caries. Arch Oral Biol 1972; 17: 1333–44.
6. Beighton D, Lynch E, Heath MR. A microbiological study of primary root-caries lesions with different treatment needs. J Dent Res 1993; 72: 623–9.
7. Emison CG, Klock B, Sanford CB. Microbial flora associated with presence of root surface caries in periodontally treated patients. Scand J Dent Res 1988; 96: 40–9.
8. Emison CG, Raval N, Birkhed D. Effects of a 12-month prophylactic programme on selected oral bacterial populations on root surfaces with active and inactive carious lesions. Caries Res 1993; 27: 195–200.
9. Ellen RP, Banting DW, Fillery ED. Longitudinal microbiological investigation of a hospitalized population of older adults with high root surface caries risk. J Dent Res 1985; 64: 1377–81.
10. Keltjens H, Schaeken T, van der Hoeven H, Hendriks J. Epidemiology of root surface caries in patients treated for periodontal diseases. Community Dent Oral Epidemiol 1988; 16: 171–4.
11. Van Houte J, Jordan HV, Laraway R, Kent R, Soparkar PM, DePaola PF. Association of the microbial flora of dental plaque and saliva with human-root surface caries. J Dent Res 1990; 69: 1463–8.
12. Nyvad B, Kilian M. Microflora associated with experimental root surface caries in humans. Infect Immun 1990; 58: 1628–33.
13. Preza D, Olsen I, Aas JA, Willemsen T, Grinde B, Paster BJ. Bacterial profiles of root caries in elderly patients. J Clin Microbiol 2008; 46: 2015–21.
14. Do T, Sheehy EC, Mulli T, Hughes F, Beighton D. Transcriptomic analysis of three Veillonella spp. present in carious dentine and in the saliva of caries-free individuals. Front Cell Infect Microbiol 2015; 5: 25.
15. Love M, Anders S, Huber W. Differential analysis of count data – the DESeq2 package. bioRxiv 2014; 15: 550.
16. Zeng L, Choi SC, Danko CG, Siepel A, Stanhope MJ, Burne RA. Gene regulation by CepA and catabolite repression explored by RNA-Seq in Streptococcus mutans. PLoS One 2013; 8: e60465.
17. Schüpbach P, Osterwalder V, Guggenheim B. Human root caries: microbiota in plaque covering sound, carious and arrested carious root surfaces. Caries Res 1995; 29: 382–95.
18. Preza D, Olsen I, Willemsen T, Boches SK, Cotton SL, Grinde B, et al. Microarray analysis of the microflora of root caries in elderly. Eur J Clin Microbiol Infect Dis 2009; 28: 509–17.
19. Brown LR, Billings RJ, Kaster AG. Quantitative comparisons of potentially cariogenic microorganisms cultured from non-carious and carious root and coronal tooth surfaces. Infect Immun 1986; 51: 765–70.
20. Brailsford SR, Lynch E, Beighton D. The isolation of Actinomyces naeslundii from sound root surfaces and root carious lesions. Caries Res 1998; 32: 100–6.
21. Beighton D, Lynch E. Relationships between yeasts and primary root-caries lesions. Gerodontology 1993; 10: 105–8.
22. Benítez-Páez A, Belda-Ferre P, Simón-Soro A, Mira A. Microbiota diversity and gene expression dynamics in human oral biofilms. BMC Genomics 2014; 15: 311.
23. Peterson SN, Meissner T, Su AI, SnERSDU E, Ong AC, Schork NJ, et al. Functional expression of dental plaque microbiota. Front Cell Infect Microbiol 2014; 4: 108.
24. Frias-Lopez J, Duran-Pinedo A. Effect of periodontal pathogens on the metatranscriptome of a healthy multispecies biofilm model. J Bacteriol 2012; 194: 2082–95.
25. Brailsford SR, Shah B, Simons D, Gilbert S, Clark D, Ines I, et al. The predominant aciduric microflora of root-caries lesions. J Dent Res 2001; 80: 1828–33.
26. Takahashi N, Kalfas S, Yamada T. Phosphorylating enzymes involved in glucose fermentation of Actinomyces naeslundii. J Bacteriol 1995; 177: 5806–11.
27. van Houte J, Lopman J, Kent R. The predominant cultivable flora of sound and carious human root surfaces. J Dent Res 1994; 73: 1727–34.
28. Takahashi N, Yamada T. Glucose and lactate metabolism by Actinomyces naeslundii. Crit Rev Oral Biol Med 1999; 10: 487–503.
29. Mishra A, Wu C, Yang J, Cisar JO, Das A, Ton-That H. The Actinomyces oris type 2 fimbrial shaft FimA mediates co-aggregation with oral streptococci, adherence to red blood cells and biofilm development. Mol Microbiol 2010; 77: 841–54.
30. Ruhl S, Eidt A, Melzl H, Reischl U, Cisar JO. Probing of microbial biofilm communities for coadhesion partners. Appl Environ Microbiol 2014; 80: 6583–90.
31. Liu T, Gibbons RJ, Hay DJ, Skobe Z. Binding of Actinomyces viscosus to collagen: association with the type 1 fimbrial adhesin. Oral Microbiol Immunol 1991; 6: 1–5.
32. Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, et al. Bacterial 16S sequence analysis of severe caries in young permanent teeth. J Clin Microbiol 2010; 48: 4121–8.