

GENE EXPRESSION OF BACTERIAL COLLAGENOLYTIC PROTEASES IN ROOT CARIES

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BACKGROUND AND AIM

It is unknown whether bacteria play a role in the collagen matrix degradation that occurs during caries progression. Our aim was to characterize the expression level of genes involved in bacterial collagenolytic proteases in root biofilms with and without caries.

METHODS

CLINICAL SAMPLES COLLECTION



n=10

SOUND ROOT SURFACE

Biofilm samples



n=9

ROOT CARIES

Biofilm + Soft active carious dentin samples

RNA EXTRACTION AND SEQUENCING

RNA extraction: total RNA was extracted, followed by DNase on-column digestion.

rRNA removal: Ribo-Zero™ Meta-Bacteria Kit (solution-based hybridization capture), to isolate the mRNA.

Libraries preparations: Illumina TruSeq library prep protocols (Illumina, San Diego, CA) - RNA fragmentation, cDNA synthesis, DNA fragments repair, amplifications by PCR and purification, with adapters ligation of a different index for each sample.

Sequencing: Illumina HiSeq2500 (Illumina Inc.) sequencer (2 x 100bp sequence reads).

BIOINFORMATIC AND DATA ANALYSIS

Genes encoding putative bacterial collagenolytic proteases were identified in genomes of 162 bacteria.

Normalization and differential expression analysis was performed on all metatranscriptomes (FDR<10⁻³) was also carried out using the R package DESeq2.

RESULTS

A total of 201 genes coding for bacterial collagenolytic proteases were identified in 113 bacterial species. The majority of the proteases expressed genes belonged to peptidase U32 family (protease PrtC).

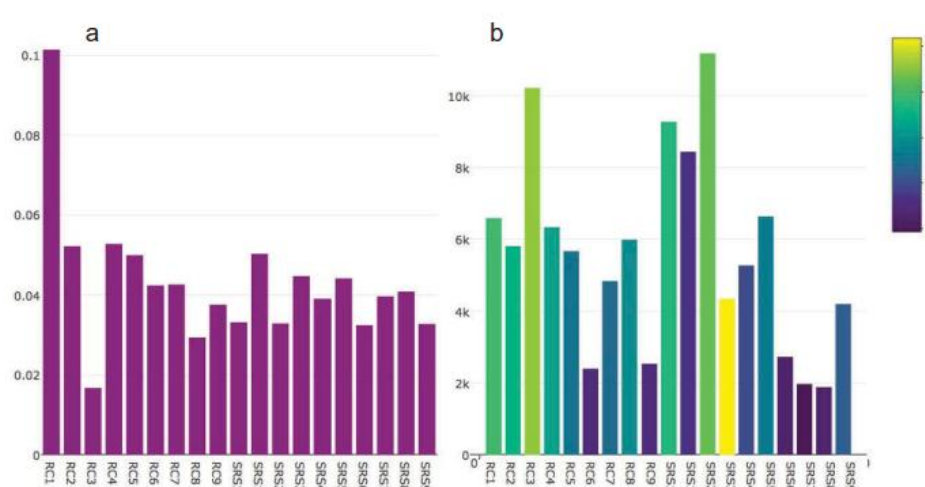


Figure 1. Bacterial collagenolytic proteases present in samples from sound root surfaces (SRS) and root caries (RC). (a) Proportion (%) of bacterial collagenolytic proteases based on the total read count per sample; (b) Number of reads mapped to bacterial collagenolytic protease genes (yellow = sample with more total reads per sample; blue = sample with less total reads per sample).

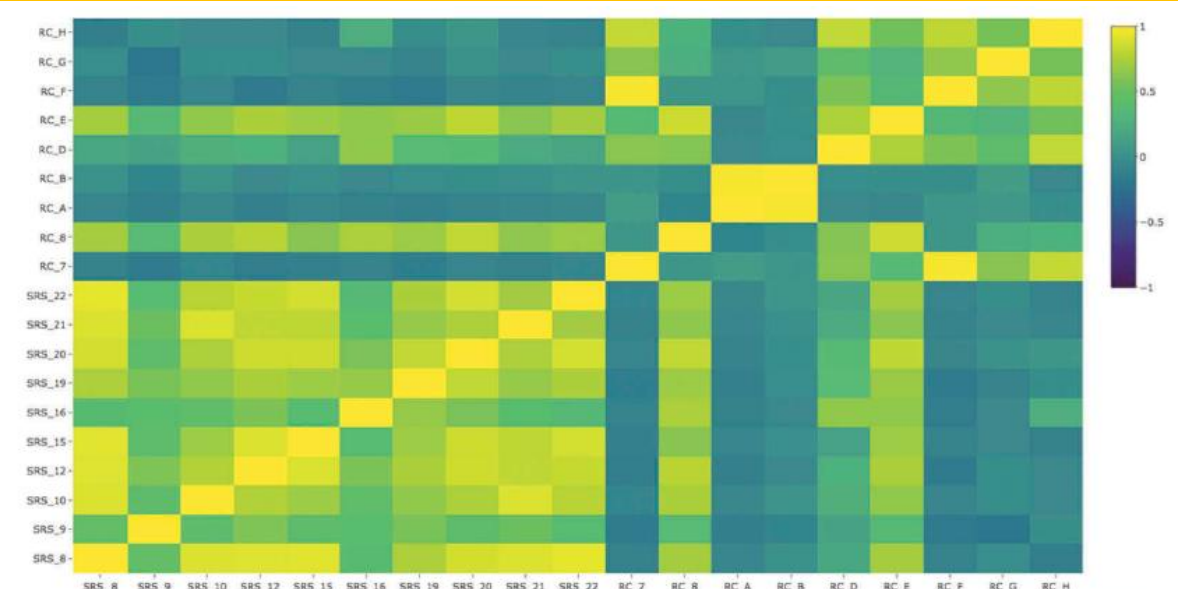


Figure 2. Heatmap showing the distances between the samples as calculated from the normalized count data of the gene expression of bacterial collagenolytic proteases. RC = root caries samples; SRS = sound root surfaces samples.

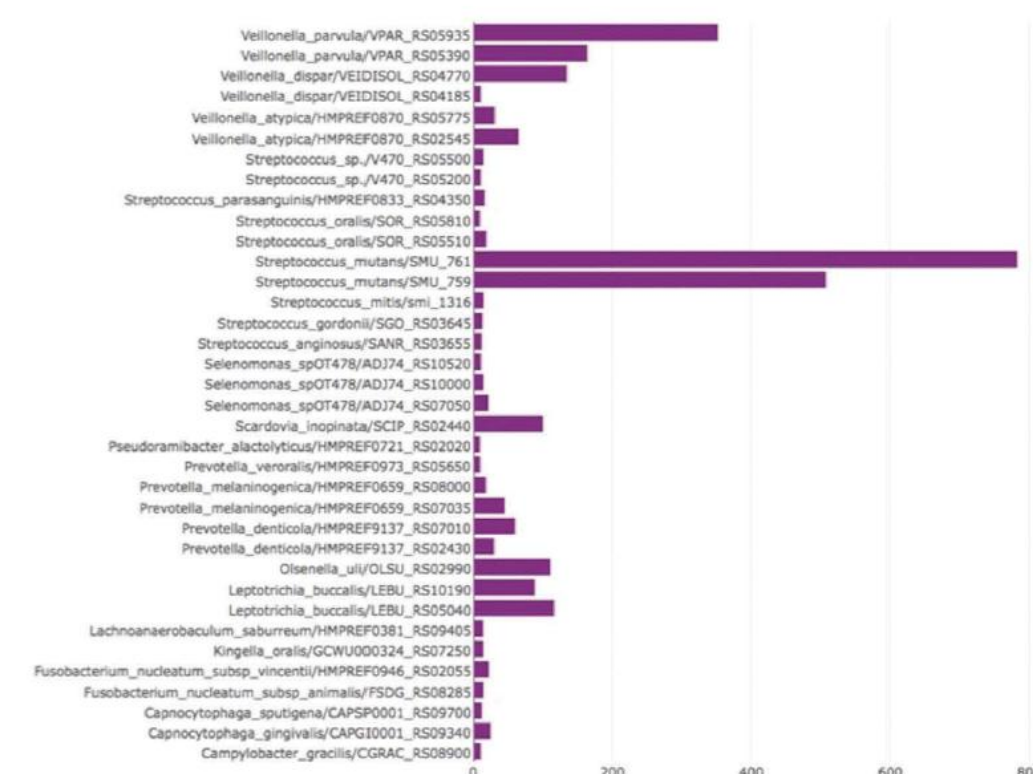


Figure 3. Gene expression level (median of expression value) of putative bacterial collagenolytic proteases (presented as 'bacterial species name/gene locus tag') in root caries. Only genes that had gene expression level >10 are displayed.

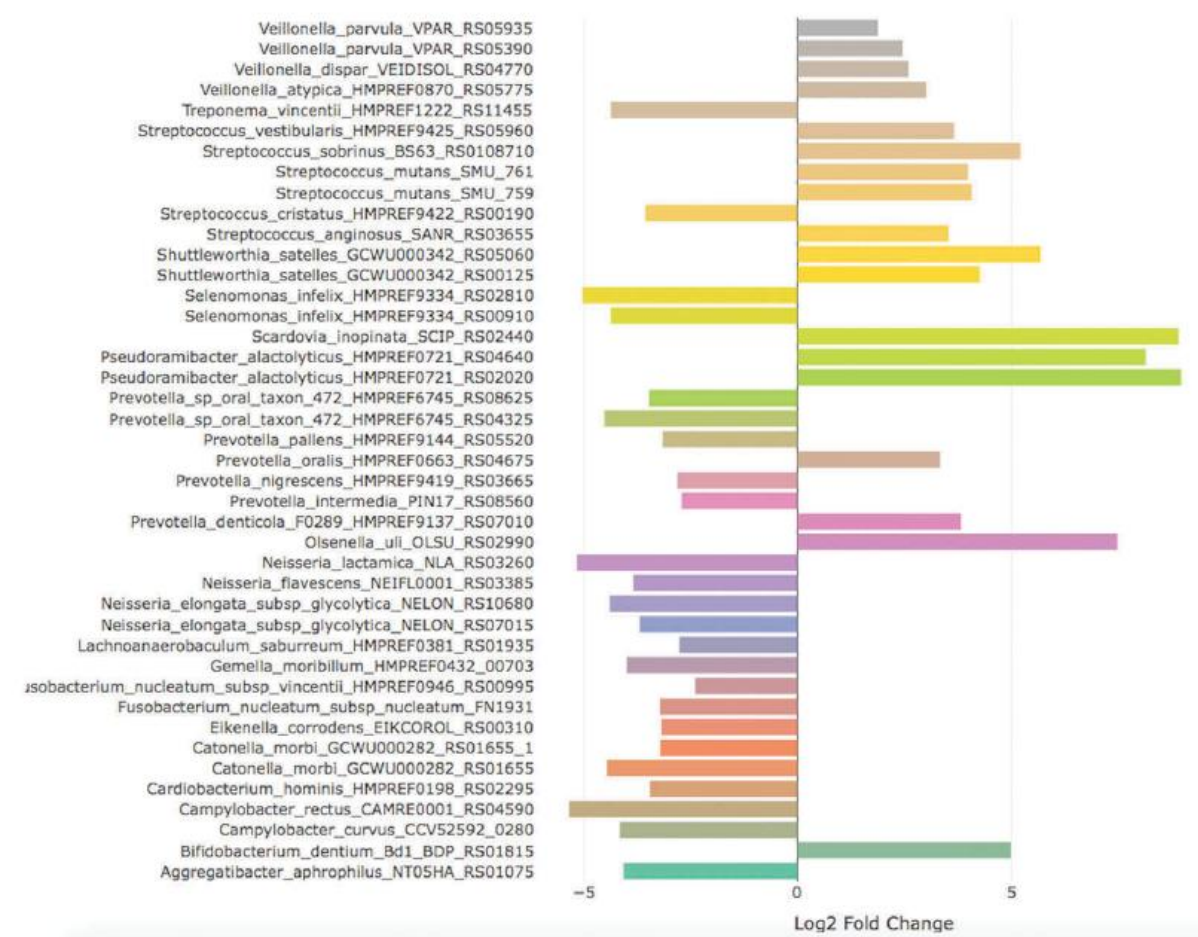


Figure 4. Genes with significant differential expression coding for bacterial collagenolytic proteases (presented as 'bacterial species name/gene locus tag') in the metatranscriptome analysis of root biofilms. Positive log2FoldChange means up-regulated genes in root caries, while negative log2FoldChange means up-regulated in sound root surfaces.

CONCLUSION

- ✓ Our findings suggest that the U32 proteases may be related to carious dentine.
- ✓ The contribution of a small number of species to dentine degradation should be further investigated.
- ✓ These proteases may have potential in future biotechnological and medical applications, serving as targets for the development of therapeutic agents.

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