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## **Gene expression of bacterial collagenolytic proteases in root caries.**

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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. We collected samples from active cavitated root caries lesions (RC, n = 30) and from sound root surfaces (SRS, n = 10). Total microbial RNA was isolated and cDNA sequenced on the Illumina Hi-Seq2500. Reads were mapped to 162 oral bacterial reference genomes. Genes encoding putative bacterial collagenolytic proteases were identified. Normalization and differential expression analysis was performed on all metatranscriptomes (FDR<10<sup>-3</sup>). Genes encoding collagenases were identified in 113 bacterial species the majority were peptidase U32. In RC, *Streptococcus mutans* and *Veillonella parvula* expressed the most collagenases. Organisms that overexpressed collagenolytic protease genes in RC (Log2FoldChange>8) but none in SRS were *Pseudoramibacter alactolyticus* [HMPREF0721\_RS02020; HMPREF0721\_RS04640], *Scardovia inopinata* [SCIP\_RS02440] and *Olsenella uli* DSM7084 [OLSU\_RS02990]. 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.