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A novel glycosidic hydrolase from the commensal *Streptococcus salivarius*: synergistic enzymatic approaches to oral biofilm degradation

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Caries and oral diseases present significant public health challenges. (1) Oral diseases cannot be explained alone by infection with any single causative agent. But should instead be viewed as a state of dysbiosis favouring the overgrowth of certain pathogenic species, (2) most notably *Streptococcus mutans* and related organisms. (2-3) The oral biofilm (OBF) these species produce is essential to the disease process, (3) and its presence greatly reduces the effectiveness of existing treatments. (3) In contrast to the aforementioned closely related *Mutans Streptococci* (MS), commensal microorganisms (MOs) such as *Streptococcus salivarius*, are associated with inhibiting the growth of cariogenic species, promoting oral eubiosis and contributing to mucosal health. (2) Despite significant similarities with MS, *S. salivarius* possesses distinct characteristics that contribute to its reduced virulence and enhance its ability to compete with pathogenic MOs (2)—lending to its different overall effect on oral health. One potential unexplored feature is the presence of genes encoding not only a dextranase enzyme—active against one polysaccharide component of the OBF matrix and typically found in these MOs—but also a novel glycosidic hydrolase enzyme from a family with a strong predilection for activity against mutan, the other, more structurally significant polysaccharide component. (3) Combinations of similar enzymes have already been successfully applied *in vitro* to control OBFs. (3) Therefore, a thorough study—in comparison to known enzymes—on the molecular characteristics, protein structure, and efficacy as a treatment against OBFs would allow for the testing of the hypothesis that the enzymes sourced from competing MOs will possess superior biofilm-degrading potential, under the relevant conditions, compared to those sourced from other environments. Such a study would also aid in the formulation of new treatments for oral diseases and contribute to a broader understanding of protein structure-function relationships and the evolutionary processes behind such enzymes. Protein structures for *S. salivarius* dextranase (GH66) and mutanase (GH87) were predicted using the alphahold algorithm; and distinctly disordered N- and C-terminal regions (based on PLDDt score) were noted. Primers containing Ligation-Independent-Cloning (LIC) sites were designed for enzyme coding genes with and without disordered regions; genomic DNA from the *S. salivarius* was successfully extracted using the (Wizard® Genomic DNA Purification Kit).

Palavras-chave: Oral-disease; Biofilm; Enzymes.

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