

Structural and Enzymatic Comparison of *Faecalibacterium prausnitzii* GH31 α -glycosidases

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The gut microbiome is home to thousands of species of bacteria, that are essential for human digestion, immunity, and physiology. *Faecalibacterium prausnitzii* makes up about 5% of a healthy human gut microbiome and a lower abundance of this bacterium has been found in patients with IBD and Crohn's disease. Among an extensive repertoire of carbohydrate active enzymes, *F. prausnitzii* has 2 GH31 α -glycosidases, which are from the same family as Sucrase-Isomaltase and Maltase-Glucoamylase, human digestive enzymes with overlapping and distinguishing substrate specificities. This project aims to characterize the substrate specificity and preference of *F. prausnitzii* GH31 α -glycosidases to better understand the structural features of GH31 enzymes and the biological capabilities of these bacteria. AlphaFoldV2.1.0 was used to create computational models of *F. prausnitzii* α -glycosidases, and the substrate specificity and kinetics parameters are reported. Structurally, these α -glycosidases have the same identified conserved N-terminal and $(\beta/\alpha)_8$ barrel domains, but FpAG1 has an additional conserved domain of unknown function at the C-terminus which is not found in the FpAG2 structure. Both FpAG1 and FpAG2 have α -glucosidase and oligo-1,6-glucosidase activity. The comparative kinetic studies show that FpAG1 has a greater preference for α -1,6 glycosidic linkages, and FpAG2 has a greater preference for α -1,4 glycosidic linkages. Gaining insight on the GH31 α -glycosidases as a component of *F. prausnitzii* metabolism can further our understanding of this community in the human gut microbiome.