Phylogenetic Analysis, Molecular Modeling and Activity Profiling of an Alpha -L-Fucosidase from a Bovine Gut Metagenome

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ABSTRACT

Glycoconjugates are molecules which have a carbohydrate moiety bonded to lipids or proteins. Fucosylated glycoconjugates in particular fucosylated oligosaccharides play a significant role in a wide variety of biological processes. Alpha-L-fucosidases are glycoside hydrolases found in organisms ranging from bacteria, to fungi, mollusks, ascidians and mammals. It has recently been shown that alpha-L-fucosidases have also transglycosylation properties. There is now widespread scientific interest in glycoside hydrolases for the purpose of synthesis of fucosylated glycoconjugates useful for biomedical research. The aim of this study was to subject an alpha-L-fucosidase gene *(Mt-fuc)* derived from a bovine gut metagenomic library to phylogenetic analysis, molecular modeling and to profile its transfucosylation activity. Mt-fuc gene was cloned and expressed in pQE30 expression vector and Transfucosylation probed using substrate *p*NPαFuc. Based on sequence similarity search on Pfam-A, Mt-fuc was found to be more similar to alpha-L-fucosidases belonging to O-Glycosyl hydrolases family 29 (GH29). Phylogenetic analysis showed that Mt-fuc is closely related to bacterial alpha-L-fucosidases with the following identities: *Bacteroides caccae* 48%, *Flavobacterium* sp. 47%, *Geobacillus* sp. 47%, *Rhizobium* sp. 15%, and *Roseiflexus* sp. 10% respectively. Comparative sequence analysis of Mt-fuc amino acid sequence with representative vertebrate and invertebrate alpha-L-fucosidases of GH29 showed that Mt-fuc had an alpha-L-fucos domain similar to that of GH29. It however lacked the GH29 signature pattern *PxxLxxxKWExC* and the sequence *WxDx* that contains the catalytic nucleophile aspartate (D). This indicates that Mt-fuc is a novel alpha-L-fucosidase with a high similarity to GH29 members. Structural analysis of the Mt-fuc 3D model using the crystal structure of *Thermotoga maritima* alpha-fucosidase in Complex with L-fucose resulted in 117 residues of Mt-fuc aligning to the template. The model revealed that Mt-fuc has the fold

responsible for L-fucose (substrate) binding. Transfucosylation activity was observed following Thin Layer Chromatography (TLC) analysis of the reaction mixture between Mt-fuc enzyme extract and substrate *p*NPαFuc incubated at 55° C for 16 hours. It was shown that Mt-fuc is a novel alpha-L-fucosidase with a 48% identity to the closest related alpha-L-fucosidase in public protein data bases. Mt-fuc transfucosylation activity was confirmed although mild, highlighting the importance of continuing further work on this enzyme.