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CHARACTERISATION OF FOUR ALPHA-L-FUCOSIDASES FROM ALPACA FECAL MICROBIOME METAGENOMIC LIBRARY

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α-L-Fucose (Fuc), a unique carbohydrate with an L-configuration and lacking a C-6 hydroxyl group, is a key component of oligosaccharides and the predominant terminal sugar in glycoconjugates[1]. Fucosylated glycoconjugates, which are vital to various organisms, play a crucial role in host-microorganism interactions, cell communication, neurological and immunological processes[1,2].

α-L-Fucosidases, exo-acting glycoside hydrolases, catalyze the removal of α-L-fucose from oligosaccharides and glycoconjugates. The enzymes are widespread in organisms and tissues. They help gut microorganisms to colonize and adapt to the gut environment[1]. Human α-L-fucosidase deficiency leads to fucosidosis and cancer. These enzymes have applications in medicine, research, and biotechnology, particularly in enzymatic oligosaccharide synthesis through transfucosylation[2].

Four new fucosidases Fuc25A, Fuc25C, Fuc25D and Fuc25E were obtained screening metagenomic library from alpaca fecal microbiome. Most of their homologues are proteins from Clostridia class bacteria. Phylogenetic analysis revealed close relations between Fuc25A, Fuc25D, and Fuc25E, while Fuc25C was more distant. Recombinant proteins were synthesized in *Escherichia coli* and purified. Kinetic parameters determined using *p*-nitrophenyl-fucopyranoside as a substrate showed Fuc25D with the highest catalytic efficiency (0,364 $\mu M^{-1}s^{-1}$) and Fuc25C the lowest (0,001 $\mu M^{-1}s^{-1}$). We determined that all fucosidases are mesophilic with optimal activity at neutral pH. They were most stable in a slightly alkaline environment (pH 8) and 0 °C. All four fucosidases also demonstrated transfucosylation activity.

[1] Wu, Haiyang et al., Structure and function of microbial alpha-l-fucosidases: a mini review. Essays in biochemistry vol. 67,3 (2023): 399-414.
[2] Ma, Bing et al., Fucosylation in prokaryotes and eukaryotes. Glycobiology vol. 16,12 (2006): 158R-184R.