Poster Presentation

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Optimizing enzyme behaviour through protein engineering

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Galactooligosaccharides (GOS) constitute an important class of prebiotic compounds used by the food industry as active ingredients with potential health benefits. GOS are enzymatically produced from lactose using β-galactosidases through a reaction known as transgalactosylation. Many studies have been conducted in an attempt to increase GOS yields by controlling the reaction conditions using β-galactosidases from a range of microorganisms. In this study, we have used high-throughput protein engineering for two GOS producing β-galactosidases, BbgIII and BbgIV from Bifidobacterium bifidum in an effort to enhance transgalactosylation activity (over hydrolysis) thus favouring GOS synthesis. A total of 36 and 11 C- and N-terminus deletion mutants were designed for BbgIII and BbgIV, respectively. The mutant constructs ranged from highly active to completely inactive enzymes. Selected constructs were tested for their transgalactosylation activity. An increase ranging between 5 and 10% (of total carbohydrates) was obtained with the mutant enzymes. Additionally, up to 2-fold increase in the higher degree of polymerization of GOS products was observed for selected mutants compared to the native enzyme. Structure determination of two highly active constructs at 2.0 Å resolution indicated that truncations affected the oligomeric state of the enzymes, which may have implications for activity.

Keywords: beta-galactosidase, galactooligosaccharides, transgalactosylation