Synthesis of potential prebiotics using *Pseudomonas syringae* DC3000 levansucrase Lsc3

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Plant-derived inulin-type oligofructans are considered effective prebiotics that function as specific growth substrates for beneficial gut bacteria. The effects of levan-type FOS (fructooligosaccharides) containing β-2,6 linkages are poorly studied as they are not commercially available. Few studies prove their enhanced prebiotic effect [1]. Polymeric levan has potential applications as anti-cancer, anti-inflammatory and immune stimulating agent.

Levansucrases (EC 2.4.1.10) are bacterial enzymes belonging to GH68 family of glycoside hydrolases. *Pseudomonas syringae* DC3000 encodes three levansucrases (Lsc1, Lsc2, Lsc3). Recombinant Lsc3 splits sucrose and synthesizes β-2,6-linked FOS, polymeric levan and also heterooligosaccharans when transfructosylating alternative acceptors [2]. Lsc3 has very high catalytic activity (k$_{\text{cat}}$ ~500 1/s), stability and therefore a high biotechnological potential.

In this study, we optimized levan and FOS production by Lsc3 protein. High-performance liquid chromatography (HPLC) system coupled with ELS detector was used to detect and quantify mono-, di- and oligosaccharides, levan was quantified spectrophotometrically.

We showed that pure Lsc3 produced up to 15.4 g of FOS per mg of protein under optimized conditions. Product yield and spectrum depended on substrate and enzyme concentrations, temperature and reaction time. Also, permeabilized bacterial cells expressing levansucrase were shown to serve as effective catalyst for FOS production.

We developed a method for enzymatic production of levan-type FOS from sucrose and a simple yeast-based method for the removal of monosaccharides that form as by-products of the reaction.

This work was supported by ERF grant 3.2.0701.12-0041 (SLOMR12215T) managed by Archimedes Foundation and an ETF grant GLOMR9072.
