Heterologous Expression and Characterization of Thermostable Levansucrase (*BsSacB*) from *Bacillus subtilis* BB03

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Abstract: Thermostable levansucrase encoding gene (sacB) with its promoter was isolated from Bacillus subtilis BB03. BsSacB is composed of 1419 bp, encoding 473 amino acid residues and with promoter regions TTGCAA (-35) and TAGAAT (-10). The amino acid sequence analysis of BsSacB showed conserved motifs of microbial fructosyltransferase belonging to Glycoside Hydrolase family 68 (GH68). Comparative analysis of the protein structure was carried out with 3D model of BsSacB constructed using PyMol software. BsSacB gene was expressed in Escherichia coli and the purified enzyme demonstrated thermo stability up to 50°C with a high hydrolytic activity at pH 6.0 and presence of Ca^{2+} promoted the activity by 15%. An attempt was made to further characterize the thermostable recombinant levansucrase from *B.subtilis* BB03 using crude sucrose rich substrates. Optimal levan production was seen with molasses $(1.94\pm0.04 \text{ g/L})$ at 15 h as compared to cane juice (1.0±0.01 g/L) at 12 h. An increase in levan formation with gradual decrease in hydrolytic activity was distinctly evident in both the molasses and juice media.

Keywords: *B.subtilis*, Cane Juice, Cane Molasses, *E.coli*, Levan and Levansucrase

Introduction

Levans are prebiotic agents with potential health benefits, selectively support intestinal health and act as low calorie sweeteners (Byun *et al.*, 2014; Kang *et al.*, 2009). They are used as blending component in cosmetics to provide cell-proliferating and skin moisturizing effects (Abdel-Fattah *et al.*, 2012). Due to their physical and biological functions as antitumor, anti-inflammatory agent, they have potential application in food and pharmaceutical industry (Yoon *et al.*, 2004; Kim *et al.*, 2005). Levans are water soluble fructo-oligosaccharides which form viscous solutions, hence used as emulsifier or encapsulating agent in cosmetics, biodegradable plastics, textile coatings and detergents (Kang *et al.*, 2009).

Levans are synthesized by bacterial levansucrases which belong to GH68 family. They synthesize fructan oligosaccharides and levan by transferring fructosyl group of non-activated sucrose into fructan polymers with β $(2\rightarrow 6)$ linkage (Seibel *et al.*, 2006). Levansucrases catalyze two different reactions-hydrolysis of sucrose and transfructosylation to form fructose polymers by releasing glucose (Ozimek *et al.*, 2006; Goldman *et al.*, 2008). *Bacillus* species like *B. methylotrophicus*, *B.subtilis*, *B. amyloliquefaciens and B. megaterium*, are reported to be good producers of levansucrases (Zhang *et al.*, 2014; Seibel *et al.*, 2006; Homann *et al.*, 2007; Rairakhwada *et al.*, 2010; Vaidya and Prasad, 2012). However, poor availability and low stability of characterized microbial enzymes generates the need for investigation on levansucrases with improved physico-chemical properties (Maiorano *et al.*, 2008).

For industrial exploitation of the potential of these enzymes, it is essential to gain knowledge about their



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