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Invertase of *Saccharomyces cerevisiae* SAA-612: Production, characterization and application in synthesis of fructo-oligosaccharides

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ABSTRACT

Production and characterization of invertase from *Saccharomyces cerevisiae* SAA-612 were carried out and transfructosylation activity was also studied for the synthesis of fructo-oligosaccharides (FOS). Optimization of invertase production following Response Surface Methodology resulted in 24 fold increase in the production of invertase activity of *S. cerevisiae* SAA-612. Optimum temperature and pH for invertase activity were observed to be 40 °C and 6.0, respectively. The enzyme was found to be stable at 30 °C and 40 °C. K_m and V_{max} of invertase of *S. cerevisiae* SAA-612 were calculated to be 11 mM and 434.7 U/mg protein, respectively. The maximum FOS synthesis was observed with 250 mg sucrose and 2.5 U invertase in one ml reaction, 5.5 pH, 40 °C and 4–8 h of incubation. This investigation can be further extended to explore the prebiotic potential of synthesized FOS.

1. Introduction

Invertases or β-fructofuranosidases (EC.3.2.1.26) are members of the GH32 family of glycoside hydrolases that catalyse the hydrolysis of sucrose to give an equimolar mixture of monosaccharide D-glucose and D-fructose called invert sugar. Invertases are widely distributed in the biosphere and mainly isolated from plants (Alberto, Bignon, Sulzenbacher, Henrissat, & Czjzeks, 2004; Hussain, Rashid, Perveen, & Ashraf, 2009) like Japanese pear fruit (*Pyrus pyrifolia*), pea (*Pisum sativum*), oat (*Avena sativa*), sugarcane (*Saccharum officinarum*) and microorganisms e.g. *Saccharomyces cerevisiae* (Kulshrestha, Tyagi, Sindhi, & Yadavilli, 2013;; Ansari, Yadav, & Lal, 2013), *Aspergillus* sp. (Nyugen, Rezessy Szabo, Bhat, & Hoschke, 2005; Guimaraes, Terenzi, Polizeli, & Jorge, 2009), *Candida utilis* (Belcarz, Ginalska, Lobarzewski, & Penel, 2002), *Neurospora crassa, Fusarium oxysporium, Phytophthora meganosperma, Schizosaccharomyces pombe* and *Schwanniomyces occidentalis* (Silveira, Oliveira, Carvajal, & Bon, 2000).

Invertase is extensively used in confectioneries, food industries and in the production of high fructose sugar syrup from sucrose (Kumar, Kayalvizhi, & Gunasekaran, 2001; Uma, Gomathi, Muthulakshmi, & Gopalakrishnan, 2010). It is used to produce artificial honey and able to catalyse transfructosylation to produce fructo-oligosaccharides (FOS) such as kestose (GF2), nystose (GF3) and fructofuranosyl nystose (GF4). However, inulinase has also been used to synthesize fructo-oligosaccharides (FOS) from sucrose (Santos & Maugeri, 2007) or by the hydrolysis of inulin (Silva et al., 2013). Fructo-oligosaccharides are well known as neo-sugars and have numerous beneficial properties (Gill, Manhas, & Singh, 2006; Yun, 1996). It has a wide range of applications in food, bread, and beverage products (Kurakake et al., 2010; Park et al., 2003). FOS can be obtained synthetically from agave fructans by acid-catalyzed hydrolysis, from sucrose *via* microbial action of β-fructosyltransferases or by β-fructofuranosidases (Avila Fernandez, Galicia-Lagunas, Rodriguez-Alegria, Olvera, & Lopez-Munguia, 2011; Balken, Dooren, Tweel, Kamphuis, & Meijer, 1991; Ghazi et al., 2005, Chiang, Lee, Sheu, & Duan, 1997).

The formation of FOS via enzymatic methods is preferred due to high substrate specificity and selectivity of the enzymes. FOS from sucrose are considered as new alternative sweeteners with functional properties, also called soluble fibres, having a number of desirable characteristics such as low calories, no cariogenicity and safety for diabetics. Fructo-oligosaccharides are also known as prebiotics, since they stimulate the growth of probiotic organisms. The ingestion of fructo-oligosaccharides (FOS) has been shown to stimulate bifidobacteria in the lower gut. The gut microflora

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performs three major tasks: colonization resistance to pathogens, modulation of gastrointestinal and systemic immune responses, and nutritional support (Crittenden & Playne, 2006; Farthing, 2004). These prebiotics along with probiotics have many health benefits such as promotion of normal colon transit time, production of short-chain fatty acids (Swennen, Courtin, & Delcour, 2006), enhancement of mineral absorption (Beynen, Baas, & Hoekemeijer, 2002), favourable modulation of lipid levels (Fiordaliso, Kok, & Desager, 1995), improved gut mucosal barrier and immune function (Gibson, McCartney, & Rastall, 2005; Murasaki et al., 1999), influences on glucose and insulin levels (Swennen et al., 2006) and reduction in colon cancer risk (Pool-Zobel, 2005). *Saccharomyces cerevisiae* is the organism of choice for invertase production because of its capability to ferment sucrose. Present study is focused on characterization of invertase from *Saccharomyces cerevisiae* SAA-612 isolated from the traditional alcoholic beverage (*chhang*) of Himachal Pradesh and its use in the synthesis of fructo-oligosaccharides (FOS).

2. Materials and methods

2.1. Chemicals

The chemicals used in the present study were purchased from Sigma Aldrich (USA) and HiMedia (Mumbai, India). All the chemicals were of analytical grade.

2.2. Microorganism

The nine yeast strains screened for invertase enzyme were procured from Research Laboratory-II of Department of Biotechnology, Himachal Pradesh University, Shimla, India.

2.3. Production of invertase enzyme

Several production media (M1-M7) were tested and the most productive medium (M3) composed of sucrose (20.0 g/l), yeast extract (10.0 g/l), ammonium sulphate (1.0 g/l), magnesium sulphate (0.75 g/l) and potassium hydrogen phosphate (3.5 g/l) was used for the production of invertase. Pre-cultivation of *S. cerevisiae* SAA-612 was done in a medium containing yeast extract (1%), peptone (2%) and dextrose (2%) at 30 °C for 16–24 h with continuous shaking (150 rpm). Preculture (2% v/v) was seeded into 50 ml of M3 medium and incubated for 24 h at 30 °C in incubator shaker. After 24 h of incubation, production medium was centrifuged at 10,000 g for 10 min at 4 °C. The supernatant was collected and used for assay of the invertase activity. Cells of *S. cerevisiae* SAA-612 were also tested for invertase activity.

2.4. Invertase assay

The invertase assay was based on the reduction of bright yellow coloured solution of 3, 5-dinitrosalicylate (DNS) to dark orange-coloured solution of 3-amino-5-nitrosalicylate resulting from enzymatic hydrolysis of sucrose. The absorbance was recorded at 540 nm and compared with the standard curve of glucose. One unit of invertase activity (U) was defined as the amount of enzyme required for the conversion of one micromole of substrate to product per min under the standard assay conditions.

2.5. Response Surface Methodology to optimize culture conditions of invertase from S. cerevisiae SAA-612

2.5.1. Plackett-Burman design

Design Expert (Version 9.0.3.1) was used for Plackett-Burman design and regression analysis. Optimized media components (maltose, yeast extract, ammonium sulphate, magnesium sulphate, potassium hydrogen phosphate) and other physiochemical parameters (pH, temperature, incubation time and inoculum size) were screened for their effect on invertase activity using Placket-Burman design. The concentration of different independent variables which showed positive effect (maltose, yeast extract, magnesium sulphate and incubation time) on the production of invertase enzyme was optimized using central composite design (CCD). The statistical model was validated for the production of invertase production by performing experiment at shake flask under predicted set of conditions.

2.6. Optimization of reaction conditions for invertase enzyme

2.6.1. Optimization of buffer system

In order to select an appropriate buffer for assay of invertase activity different buffers (0.1 M) viz. Citrate buffer (pH 3–6), borate buffer (pH 5–7), potassium phosphate buffer (pH 6–8), tris-HCl buffer (pH 6–9), sodium carbonate buffer (pH 9–10), sodium phosphate buffer (pH 6.5–7.5) and borax buffer (pH 9–10) were used.

2.6.2. Effect of molarity of buffer

The optimum molarity of buffer was evaluated by assaying the enzyme activity at molarity values ranging from 0.01 M to 1.0 M at pH 6.

2.6.3. Time course of reaction for invertase activity

The enzyme reaction was carried out for different time periods ranging from 5 to 60 min to work out optimum incubation time for the assay of invertase enzyme.

2.6.4. Reaction temperature

The enzyme reaction was carried out at different temperatures (25 °C-70 °C) to find out the optimum reaction temperature for invertase activity.

2.6.5. Substrate and enzyme concentrations

Varied concentrations of sucrose were used in the range from 0.005 M to 0.5 M in the reaction to study the effect of substrate concentration on the activity of invertase. Different concentrations of the enzyme were used in the range 0.1 μg/ml to 2 μg/ml and the enzyme activity was assayed.

2.6.6. Thermal stability

Thermostability of enzyme was evaluated by preincubating it at 30 °C, 40 °C, 50 °C, 60 °C and 70 °C for 6 h. The residual activity was measured after an interval of 1 h.

2.7. Synthesis and detection of fructo-oligosaccharides

Synthesis of fructo-oligosaccharides (FOS) was carried out in batch mode at 40 °C using invertase of *S. cerevisiae* SAA-612 according to the method described by Khandekar et al. (2014). The experiment was carried out using various concentrations of sucrose (150–500 mg/ml), enzyme (0.5–5.0 U/ml) at pH 5.5 and 40 °C. The synthesized product formed was analyzed by thin layer chromatography according to the procedure of Jork, Funk, Fischer, and Wimmer (1990).

3. Results

Among nine yeast strains screened, *S. cerevisiae* SAA-612 showed highest invertase activity (15 U/mg protein) and was