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Screening and optimization of nutritional factors for higher dextransucrase production by *Leuconostoc mesenteroides* NRRL B-640 using statistical approach

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Abstract

To improve dextransucrase production from *Leuconostoc mesenteroides* NRRL B-640 culture medium was screened and optimized using the statistical design techniques of Plackett–Burman and response surface methodology (RSM). Plackett–Burman design with six variables viz. sucrose, yeast extract, K_2HPO_4, peptone, beef extract and Tween 80 was performed to screen the nutrients that were significantly affecting dextransucrase production. The variables sucrose, K_2HPO_4, yeast extract and beef extract showed above 90% confidence levels for dextransucrase production and were considered as significant factors for optimization using response surface methodology. 2^4-central composite design was used for RSM optimization. The experimental results were fitted to a second-order polynomial model which gave a coefficient of determination $R^2 = 0.95$. The optimized composition of 30 g/l sucrose, 18.9 g/l yeast extract, 19.4 g/l K_2HPO_4 and 15 g/l beef extract gave an experimental value of dextransucrase activity of 10.7 U/ml which corresponded well with the predicted value of 10.9 U/ml by the model.

Keywords: Dextransucrase; *Leuconostoc mesenteroides*; Response surface methodology; Central composite design

1. Introduction

Dextran is synthesized by dextransucrase from sucrose which belongs to a class of glucosyltransferases. Glucosyltransferases are produced mainly by two bacterial genera *Leuconostoc* and *Streptococcus* (Remaud-Simeon et al., 2000). The major glucosyltransferases are dextransucrase, alternansucrase, mutansucrase which produce dextran, alternan and mutan, respectively, which differ in their linkage pattern of the D-glucose residues (van Hijum et al., 2006). Dextrans are a class of homopolysaccharides composed of D-glucans with contiguous α-(1→6) glycosidic linkages in the main chains and α-(1→2), α-(1→3) or α-(1→4) branched glycosidic linkages (Robyt, 1995). Dextrans have several applications in pharmaceutical, food, photochemical and fine chemical industries (Lacaze et al., 2007; Purama and Goyal, 2005; Naessens et al., 2005). The low molecular weight dextrans (6%) are used as a blood plasma substitute. High molecular weight dextrans are used to increase the puffiness of bakery products (Lacaze et al., 2007).

In addition to catalyzing the synthesis of dextran from sucrose, dextransucrase also catalyzes the transfer of a D-glucopyranosyl group from sucrose to other acceptor molecules resulting in the generation of oligosaccharides (Naessens et al., 2005; Goulas et al., 2004). Maltose, isomaltose and galactose are the known acceptor molecules for dextransucrase which in presence of sucrose, synthesize oligosaccharides such as maltooligosaccharides, isomaltooligosaccharides and galactooligosaccharides, respectively (Seo et al., 2007). Oligosaccharides are used in food, feed, pharmaceutical or cosmetics as stabilizers, as anti-carcinogenic agents, antioxidants, immunostimulating

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agents and prebiotic compounds (Seo et al., 2007; Naessens et al., 2005; Chung and Day, 2002; Goulas et al., 2004). As there are enormous applications of dextran, dextransucrase is required in higher amounts at the expense of optimum levels of nutrients.

Several authors have described the modification of nutrients and culture conditions for dextransucrase production by various *Leuconostoc* strains under flask cultures and batch fermentation (Behravan et al., 2003; Santos et al., 2000; Goyal and Katiyar, 1997, 1996; Barker and Ajongwen, 1991; Tsuchiya et al., 1952). All the parameters affecting the dextransucrase production reported were, using the univariate experiments (Behravan et al., 2003; Santos et al., 2000; Goyal and Katiyar, 1997; Barker and Ajongwen, 1991). Univariate at a time approach is time consuming and it does not account for the interactions among the medium components. The statistical approach for medium optimization is believed to be a better alternative to univariate at a time approach and have been extensively used recently (Tanyildizi et al., 2005; Tari et al., 2006; Majumder and Goyal, 2008).

In the present report, the production of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640 was studied by statistical approach for screening and optimization of medium components. The optimization of dextransucrase production from *L. mesenteroides* NRRL B-640 was studied by a sequential study of factorial Plackett–Burman design followed by central composite design (CCD). The factorial design of Plackett–Burman was used to screen the most significant factors affecting enzyme production. A central composite design (CCD) was used to identify the optimum levels of the significant variables to generate optimal response.

2. Methods

2.1. Microorganism and cultivation conditions

L. mesenteroides NRRL B-640 was procured from Agricultural Research Service (ARS-Culture collection), USDA, Peoria, USA. Ingredients required for maintenance and enzyme production media were from Hi-Media Pvt. Ltd., India. The culture was maintained in modified MRS (DeMan et al., 1961) with sucrose replaced by glucose as stab at 4 °C and sub-cultured every two weeks. A loop full of culture from stab was transferred to 5 ml of medium as described by Tsuchiya et al. (1952). The cultures were grown at 25 °C with 200 rpm. One percent of the culture inoculum was used to inoculate 100 ml enzyme production medium. The culture broth was centrifuged at 9200*g* for 10 min at 4 °C to separate the cells. The cell free extract was analyzed for enzyme activity.

2.2. Dextransucrase activity assay

The assay of dextransucrase was carried out in 1 ml of a reaction mixture in 20 mM sodium acetate buffer, pH 5.4, containing 146 mM (5%) sucrose and using the cell free extract (10–20 μl) as the enzyme source. The reaction mixture was incubated at 30 °C for 15 min. Aliquots (0.2 ml), from the reaction mixture were analyzed for reducing sugar concentration. The enzyme activity was measured by estimating the liberated reducing sugar by the Nelson–Somogyi procedure (Nelson, 1944; Somogyi, 1945). One unit (U) of dextransucrase activity is defined as the amount of enzyme that liberates 1 μmol of reducing sugar per min at 30 °C in 20 mM sodium acetate buffer, pH 5.4.

2.3. Optimization procedure and experimental design

2.3.1. Screening of factors affecting dextransucrase production

Plackett–Burman factorial design was employed for screening the important nutrients for dextransucrase production. Six nutrients sucrose, yeast extract, K_2HPO_4, peptone, beef extract and Tween 80 were used to determine the key ingredients significantly affecting the dextransucrase production. Based on Plackett–Burman factorial design, each factor was examined at two levels: −1 for low level and +1 for high level, and a center point was run to evaluate the linear and curvature effects of the variables (Plackett and Burman, 1946). Table 1 shows the Plackett–Burman experimental design with six factors under investigation as well as levels of each factor used in the experimental design and the response. Plackett–Burman experimental design is based on the first-order polynomial model:

$$Y = \beta_0 + \sum \beta_i x_i \quad (1)$$

where Y is the response (productivity or enzyme activity), β_o is the model intercept and β_i is the linear coefficient, and x_i is the level of the independent variable. This model does not describe interaction among factors and it is used to screen and evaluate the important factors that influence the

Table 1
Plackett–Burman design showing six variables with coded values along with the observed results for dextransucrase production

Run order	Sucrose (g/l)	Yeast extract (g/l)	K_2HPO_4 (g/l)	Peptone (g/l)	Beef extract (g/l)	Tween 80 (g/l)	Activity (U/ml)
1	30 (+)	10 (−)	30 (+)	1 (−)	5 (−)	1 (−)	5.89
2	30 (+)	30 (+)	10 (−)	5 (+)	5 (−)	1 (−)	7.39
3	10 (−)	30 (+)	30 (+)	1 (−)	15 (+)	1 (−)	2.78
4	30 (+)	10 (−)	30 (+)	5 (+)	5 (−)	2 (+)	6.42
5	30 (+)	30 (+)	10 (−)	5 (+)	15 (+)	1 (−)	6.89
6	30 (+)	30 (+)	30 (+)	1 (−)	15 (+)	2 (+)	9.48
7	10 (−)	30 (+)	30 (+)	5 (+)	5 (−)	2 (+)	0.96
8	10 (−)	10 (−)	30 (+)	5 (+)	15 (+)	1 (−)	2.86
9	10 (−)	10 (−)	10 (−)	5 (+)	15 (+)	2 (+)	0.18
10	30 (+)	10 (−)	10 (−)	1 (−)	15 (+)	2 (+)	5.58
11	10 (−)	30 (+)	10 (−)	1 (−)	5 (−)	2 (+)	0.62
12	10 (−)	10 (−)	10 (−)	1 (−)	5 (−)	1 (−)	0.44
13	20 (0)	20 (0)	20 (0)	3 (0)	10 (0)	1.5 (0)	6.61
14	20 (0)	20 (0)	20 (0)	3 (0)	10 (0)	1.5 (0)	5.97
15	20 (0)	20 (0)	20 (0)	3 (0)	10 (0)	1.5 (0)	6.40