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Enhanced production of a novel dextran from *Leuconostoc mesenteroides* NRRL B-640 by Response Surface Methodology

Avishek MAJUMDER§, Sourabh BHANDARI, Ravi Kiran PURAMA§, Seema PATEL, Arun GOYAL*

Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India

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Abstract - In our earlier study dextran produced by *Leuconostoc mesenteroides* NRRL B-640 was reported to possess novel food gelling and thickening properties (Purama *et al.,* 2009). In the present study response surface methodology based experimental designs were applied to enhance the production of this novel dextran by *Leuconostoc mesenteroides* NRRL B-640. Eleven medium components were examined for their significance on dextran production using Plackett-Burman factorial design. Sucrose, peptone and beef extract were found to have significant effect on the dextran production. The combined effect of these nutrients on dextran production were studied using a 2^3 full-factorial central composite design, a second-order polynomial was established to identify the relationship between the output i.e. dextran produced and the three medium components. The optimal concentration of variables for maximum dextran production were 5%, w/v sucrose, 2.5%, w/v peptone, and 2.5%, w/v beef extract. The maximum concentration of dextran obtained by predicted model was 12.0 mg/ml that was in perfect agreement with the experimental determined value (12.2 ± 0.2 mg/ml). This value of dextran concentration was over 70 percent higher as compared to un-optimized medium that gave 7.0 ± 0.2 mg/ml of dextran.

Key words: dextran; *Leuconostoc mesenteroides*; response surface methodology; central composite design.

INTRODUCTION

Polysaccharides are polymers of many monosaccharides joined together by glycosidic bonds. They are very large, often branched macromolecules. They have a general formula of $C_n(H_2O)_{n-1}$ where n is usually a large number. Plant and seaweed polysaccharides are often used as emulsifiers, stabilizers, thickening and gelling agents. Microorganisms offer a more attractive alternative as they can be grown under controlled conditions and they produce a range of polysaccharides with unique properties. Dextran $(C_6H_{10}O_5)_n$ is a polysaccharide consisting of glucose monomers linked 95% mainly by α-(1→6) bonds which forms the linear part of the molecule and the branches results from α-(1→3), α-(1→2) or α-(1→4) bonds. Dextrans of various size and structure are synthesized depending on dextransucrase produced by a particular strain (Seymour and Knapp, 1980; Barker *et al.*, 1993; Leathers, 2002; Majumder *et al.*, 2009, Purama *et al.*, 2009). Dextrans have many industrial applications due to its non-ionic character and good stability under normal operating conditions. Dextrans are used in food and pharmaceutical industry or as stabilizers in cosmetics, as anti-carcinogenic agents, and immunostimulating agents and also as chromatographic media (Rodrigues *et al.*, 2003; Purama and Goyal, 2005; Naessens *et al.*, 2005). Dextran is synthesized by dextransucrase, an exocellular enzyme produced when *Leuconostoc mesenteroides* is grown on a sucrose rich medium. Dextransucrase catalyzes the sucrose hydrolysis to produce the polymer dextran and releasing the fructose free (Purama and Goyal, 2005).

The production of dextran is strongly influenced by the medium composition such as carbon sources, nitrogen sources and inorganic salts. It is difficult to search for the major factors and to optimize them for biotechnological processes including multi-variables. The traditional 'one factor at a time' technique used for optimizing a multivariable system is not only time consuming, but can also result in misleading conclusions. Response surface methodology (RSM) is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching optimum conditions of factors for desirable responses. RSM has been applied in many areas of biotechnology such as optimization of cultural medium, enzyme synthesis, aqueous two phase separation of proteins and for glucan production (Haider and Pakshirajan, 2007; Li *et*

* Corresponding Author. Phone: 91-361-2582208;
Fax: 91-361-2582249; Email: arungoyl@iitg.ernet.in
§ Present address: Dr. Avishek Majumder, Department of System Biology, Enzyme and Protein Chemistry Group, Technical University of Denmark, Building 224, DK-2800 Kgs, Lyngby, Denmark; E-mail: avishek.majumder@gmail.com
Dr. Ravi Kiran Purama, Department of Biotechnology, Lund University, Lund, Sweden; E-mail: ravipurama@gmail.com

al., 2007; Liu and Wang, 2007; Majumder and Goyal, 2008). Extensive work has been done on optimization and modification of the fermentation processes for improved production of dextran (Jeanes, 1965; Lawford *et al.*, 1979; Alsop, 1983; Barker *et al*., 1993; Lazic *et al.*, 1993; Goyal and Katiyar, 1997; Behravan *et al.*, 2003.; Kralj *et al.*, 2004). The effect of nutrients and culture conditions for dextran production by various *Leuconostoc* strains under flask cultures and batch fermentation have been studied. The production of dextran from other strains of *Leuconostoc mesenteroides* using response surface methodology has been reported (Karthikeyan *et al.*, 1996; Majumder *et al.*, 2009). The dextran produced by *Leuconostoc mesenteroides* NRRL B-640 was reported to possess novel food gelling and food thickening properties (Purama *et al*., 2009). This strain produces more soluble dextran than the other strains as it contains more than 95% of linear α-(1→6) linkages (Purama *et al.*, 2009). From our earlier study we reported higher production of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640 using statistical methods (Purama and Goyal, 2008). The conditions favorable for dextransucrase production did not support the maximum dextran production. In the present study the production of dextran from *Leuconostoc mesenteroides* NRRL B-640 was carried out by optimization of medium components using statistical approach. The optimization of dextran production 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 dextran production. A central composite design (CCD) was used to identify the optimum levels of the significant variables to generate enhanced dextran production.

MATERIALS AND METHODS

Microorganism. *Leuconostoc 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 with glucose replaced by 2% sucrose as stab at 4 °C and sub-cultured every 2 weeks (Goyal and Katiyar, 1996). A loop 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.

Production and purification of dextran. The production of dextran was carried out in 250 ml Erlenmeyer flasks containing 100 ml medium as per the design inoculated with 1% culture inoculum. The inoculated flasks were incubated under orbital shaking at 200 rpm and 25 °C for 48 h. All the runs were replicated and the dextran content was estimated. The culture supernatant was obtained by centrifugation of the broth at 10000 *x g* at 4 °C for 10 min. The crude dextran was precipitated by the addition of 3 volumes of 95% (v/v) pre-chilled ethanol at 4 °C and centrifuged at 13000 *x g*. The process of precipitation was repeated to remove any trace impurities or free reducing sugars.

Estimation of dextran.
The polysaccharide content was determined by phenol-sulfuric acid method (Dubois *et al.*, 1956) in a micro titer plate (Fox and Robyt, 1991). To 25 µl of sample containing dextran in a microtitre plate, 25 µl of 5% (w/v) phenol was added. The plate was mixed at slow speed on a vortex mixer for 30 s. The plate was then placed onto ice bath and 125 µl of concentrated sulphuric acid was added to each well containing sample and phenol. The plate was again mixed for 30 s incubated in water bath at 80 °C for 30 min and cooled and the absorbance was determined at 490 nm on a microtitre plate reader. Standard curve was prepared using dextran (10 kDa) in the concentration range 0.1-1 mg/ml.

Plackett-Burman design. For screening purpose, various medium components and culture parameters were evaluated. Based on Plackett-Burman factorial design, each factor was examined in two levels: -1 for low level and +1 for high level (Plackett and Burman, 1946). Table 1 shows the factors under investigation as well as the levels of each factor used in the experimental design, whereas Table 2 represents the design matrix. Plackett-Burman experimental design is based on the first order polynomial model:

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

where Y is the response (dextran), β_0 is the model intercept, 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 response. In the present work, eleven assigned variables were screened in twelve experimental designs. All experiments were carried out in duplicate and the averages of the dextran concentration were taken as the response (Table 2). From the regression analysis the variables, which were significant at 90% level ($P < 0.1$) were considered to have greater impact on dextran production and were further optimized by a central composite design. The experimental design and statistical analysis of the data were done by using Minitab statistical software package (Release 15).

Central composite design. The central composite design (CCD) 2^3 full-factorial central composite experimental plan with three medium constituents i.e. sucrose, beef extract and peptone at five levels, was generated by Minitab statistical software (Release 15). All other nutrients in CCD were added at their median levels of the Placket-Burman design. In this study, the experimental plan consisted of 20 trials and the value of the dependent response was the mean of two replications. The relationships and interrelationships of the variables were determined by fitting the second order polynomial equation to data obtained from 20 experiments.

TABLE 1 - Assigned concentration of variables at different levels in Plackett-Burman design for dextran production

Sample No.	Variables with designation	Lower level (% w/v)	Higher level (% w/v)
1	Sucrose (X_1)	1.0	6.0
2	Yeast extract (X_2)	0.5	3.0
3	K_2HPO_4 (X_3)	1.0	4.0
4	Peptone (X_4)	0.5	3.0
5	Beef extract (X_5)	0.5	3.0
6	Tween 80 (X_6)	0.1	1.0
7	$MgSO_4$ (X_7)	0.01	0.1
8	$MnSO_4$ (X_8)	0.001	0.01
9	$FeSO_4$ (X_9)	0.001	0.01
10	$CaCl_2$ (X_{10})	0.001	0.01
11	NaCl (X_{11})	0.001	0.01

TABLE 2 - Plackett-Burman design for 11 variables with coded values along with the observed results for dextran production

Run order	Sucrose	Yeast extract	K_2HPO_4	Peptone	Beef extract	Tween 80	$MgSO_4$	$MnSO_4$	NaCl	$CaCl_2$	$FeSO_4$	Dextran (mg/ml)
1	1	-1	1	-1	-1	-1	1	1	1	-1	1	10.085
2	1	1	-1	1	-1	-1	-1	1	1	1	-1	13.986
3	-1	1	1	-1	1	-1	-1	-1	1	1	1	5.000
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	13.665
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	16.385
6	1	1	1	-1	1	1	-1	1	-1	-1	-1	13.323
7	-1	1	1	1	-1	1	1	-1	1	-1	-1	3.869
8	-1	-1	1	1	1	-1	1	1	-1	1	-1	10.675
9	-1	-1	-1	1	1	1	-1	1	1	-1	1	11.900
10	1	-1	-1	-1	1	1	1	-1	1	1	-1	15.400
11	-1	-1	-1	-1	-1	1	1	1	-1	1	1	4.900
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	4.975

$$Y = \beta_o + \sum_{i}^{k} \beta_i x_i + \sum_{i}^{k} \sum_{j} \beta_{ij} x_i x_j + \sum_{i}^{k} \beta_{ii} x_i^2 \quad (2)$$

where Y is the predicted response, k is the number of factor variables, β_o is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient. X_i is the factor variable in its coded form. The following equation was used for coding the actual experimental values of the factors in the range of (-1 to +1):

$$X = \frac{\left[actual - \left(low - level + high - level\right)/2\right]}{\left(high - level - low - level\right)/2} \quad (3)$$

Statistical analysis of the data was performed by software package Minitab 15 to evaluate the analysis of variance (ANOVA) to determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case. Response surfaces were drawn for the experimental results obtained from the effect of different variables on dextran production in order to determine the individual and cumulative effects of these variables and the mutual interactions between them.

Experimental validation of the optimized dexran production. To validate the optimization of the medium components for the culture medium, five tests were carried out using the optimized medium components in 100 ml medium contained in 250 ml flasks to confirm the results from the analysis of the response surface method.

RESULTS

Evaluation of factors affecting dextran production

The dextran produced was estimated in the cell supernatant of *Leuconostoc mesenteroides* NRRL B-640 culture broth at 48 h as there was no sucrose left and no dextran production was observed after 48 h. Under the standard conditions using medium of Tsuchiya *et al.* (1952) the strain produced 7.0 ± 0.2 mg/ml dextran in the broth. The aim was to enhance and maximize the production of this novel dextran by statistical techniques. In the present study all assays were carried out in duplicate and the average values were reported. The data in Table 2 showed a wide variation in dextran production from 3.869 mg/ml to 16.385 mg/ml in the twelve trials. This variation reflected the importance of medium optimization to attain higher yields. On analysis of regression coefficients and *t*-value of 11 ingredients (Table 3), the factors which showed positive effect on dextran production were sucrose, peptone, beef extract, Tween 80, $MnSO_4$ and NaCl while yeast extract, K_2HPO_4, $MgSO_4$, $FeSO_4$ and $CaCl_2$ had a negative effect on the dextran production. Variables with confidence level greater than 90% were considered as significant. Sucrose and peptone were significant at 99.99% level and showed high production of dextran. Beef extract was also significant at a confidence level of 98%. K_2HPO_4 and yeast extract were also found to be significant although with a negative coefficient. The polynomial model describing the correlation between 11 factors and the dextran yield could be presented as:

$$Y_{mg/ml} = 10.3880 + 3.3073X_1 - 0.7536X_2 - 0.9075X_3 + 1.6186X_4 + 1.7980X_5 + 0.1750X_6 - 0.0119X_7 + 0.4427X_8 - 0.3472X_9 - 0.3600X_{10} - 0.1190X_{11}$$

TABLE 3 - Statistical analysis of Plackett-Burman design showing coefficient values, *t* and *P*-value for each variable

Variable	Dextran production		
	Coefficient	*t* Stat	*P*-value
Intercept	10.3880	99.70	0.000
Sucrose	3.3073	31.74	0.000
Yeast extract	-0.7536	-7.23	0.000
K_2HPO_4	-0.9075	-8.71	0.000
Peptone	1.6186	15.54	0.000
Beef extract	1.7980	17.26	0.000
Tween 80	0.1750	1.68	0.121
$MgSO_4$	-0.0119	-0.11	0.911
$MnSO_4$	0.4427	4.25	0.001
$FeSO_4$	-0.3472	-3.33	0.007
$CaCl_2$	-0.3600	3.45	0.005
NaCl	0.1190	1.14	0.277

From regression analysis of above, the variables which were significant at more than 90% level were considered to have high impact on dextran production and were considered for analysis.

Optimization of medium composition by RSM

At the end of above screening experiments three nutritional factors were believed to play a significant role in dextran production. A central composite design (CCD) with twenty experiments was carried out. The respective low and high levels of each variable are given in Table 4 and the CCD design with dextran produced in each case is given in Table 5. The results of the second order response surface model fitting in the form of ANOVA are given in Table 6 (for dextran production). To test the fit of the model equation, the regression based determination coefficient R^2 was evaluated (Haider and Pakshirajan, 2007; Liu and Wang, 2007). The nearer the values of R^2 to 1, the model would explain better for variability of experimental values to the predicted values (Li *et al.*, 2007). The model presented a high determination coefficient (R^2 = 0.9066) explaining 90.66% of the variability in the response i.e. dextran production (Table 4). The coefficients of regression were calculated and the following regression equation was obtained:

$$Y = 7.53618 + 2.54224\,X_1 + 1.22370\,X_2 + 1.20463\,X_3 + 0.73225\,X_1X_2 + 0.38275\,X_1X_3 - 1.41225\,X_2X_3 + 0.00639\,X_1^2 - 0.50980\,X_2^2 + 0.08417\,X_3^2$$

The statistical significance of above equation was checked by *F*-test, the results of ANOVA for dextran production are shown in Table 6. The results demonstrated that the model is highly significant, and is evident from Fischer's, *F* test with a very low probability value ($P_{model} > F$ = 0.0001) (Table 4). A low value of coefficient of variation was observed (CV = 17.06%) which indicated precision and reliability of the experiments. Model coefficients estimated by regression analysis for each variable representing their effect on dextran concentration is shown in Table 5.

The ANOVA of quadratic regression model demonstrated that the model is highly significant, and is evident from the Fisher's *F*-test with a very low probability value ($P_{model} > F$ = 0.0001) (Table 6). At the same time, relatively lower value of coefficient of variation (CV = 17.06%) indicates good precision and reliability of the experiments carried out. The significance of each coefficient was determined by *t*-values and *P*-values which are listed in Table 7. The larger the magnitude of *t*-test and value and smaller the *P*-value indicates the high significance of the corresponding coefficient (Karthikeyan *et al.*, 1996). The result showed that among the independent variables, X_1 sucrose has significant effect with a positive coefficient [Table 7, Eq (2)]. The increase in its concentration can increase the product yield. Among the interactions, X_1X_2 (Sucrose x Peptone) and X_1X_3 (Sucrose x Beef extract) has positive coefficient; while X_2X_3 (Peptone x Beef extract) has a negative coefficient.

TABLE 4 - Experimental range and levels of independent variables

Variable	Symbol	Range and Levels				
		-2	-1	0	1	2
Sucrose (%, w/v)	X_1	1.0	2.0	3.5	4.98	6.0
Peptone (%, w/v)	X_2	0.5	1.0	1.75	2.5	3.0
Beef extract (%, w/v)	X_3	0.5	1.0	1.75	2.5	3.0

TABLE 5 - Full factorial central composite design matrix of three variables in coded units and the experimentally observed response

Run No.	X_1	X_2	X_3	Dextran (mg/ml)
1	0	0	0	7.82
2	1	-1	1	11.83
3	-1	1	-1	6.19
4	0	0	0	8.23
5	0	-2	0	2.72
6	0	0	0	7.47
7	2	0	0	10.58
8	1	1	1	12.14
9	1	1	-1	12.69
10	0	0	-2	4.28
11	0	0	0	7.31
12	0	2	0	7.12
13	-1	-1	1	6.72
14	0	0	0	7.68
15	1	-1	-1	5.42
16	-1	1	1	5.42
17	0	0	2	8.92
18	0	0	0	7.11
19	-2	0	0	2.18
20	-1	-1	-1	3.16

TABLE 6 - ANOVA for quadratic model

Source	SS	DF	MS	*F*-value	Prob (*P*) > *F*
Model	153.989	9	17.1099	10.79	0.000
Residual (error)	15.858	10	1.5858		
Lack of fit	15.065	5	3.1030	19.00	0.003
Pure error	0.793	5	0.1585		
Total	168.847	19			

R^2 = 0.9066, CV = 17.06, Adj R^2 = 0.8226.
SS: sum of squares, DF: degrees of freedom, MS: mean square.

TABLE 7 - Model coefficient estimated by multiple linear regression

Model Term	Parameter estimate	Standard error	Computed *t*-value	*P*-value
Intercept	7.536	0.5136	14.673	0.000
X_1	2.54224	0.3408	7.461	0.000
X_2	1.22370	0.3408	3.591	0.005
X_3	1.20463	0.3408	3.535	0.005
X_1^2	0.00639	0.3317	0.019	0.985
X_2^2	-0.50980	0.3317	-1.537	0.155
X_3^2	0.08417	0.3317	0.254	0.805
X_1X_2	0.73225	0.4452	1.645	0.131
X_1X_3	0.38275	0.4452	0.860	0.410
X_2X_3	-1.41225	0.4452	-3.172	0.010

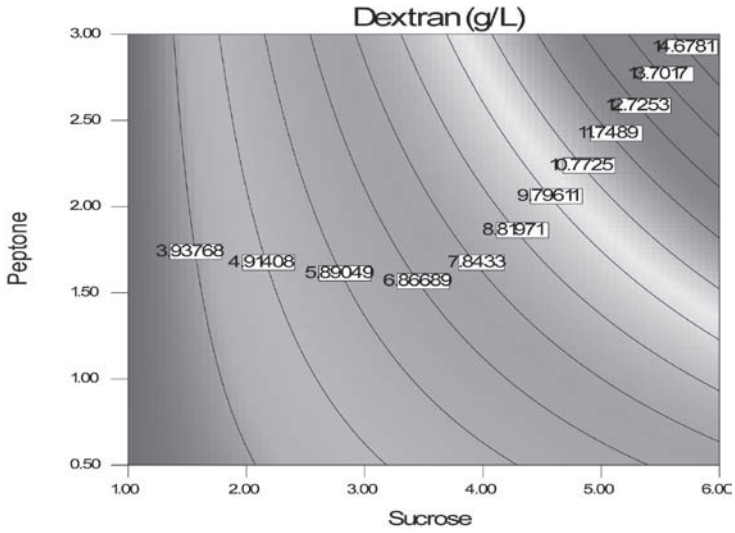

FIG. 1 - Contour plot of the combined effects of sucrose and peptone on dextran production by *Leuconostoc mesenteroides* NRRL B-640. Fixed level: beef extract = 1.75.

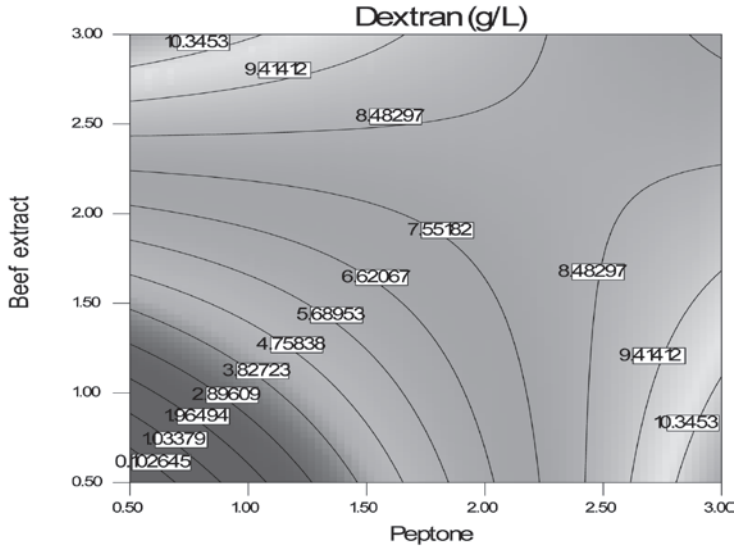

FIG. 2 - Contour plot of the combined effects of peptone and beef extract on dextran production by *Leuconostoc mesenteroides* NRRL B-640. Fixed level: sucrose = 3.5.

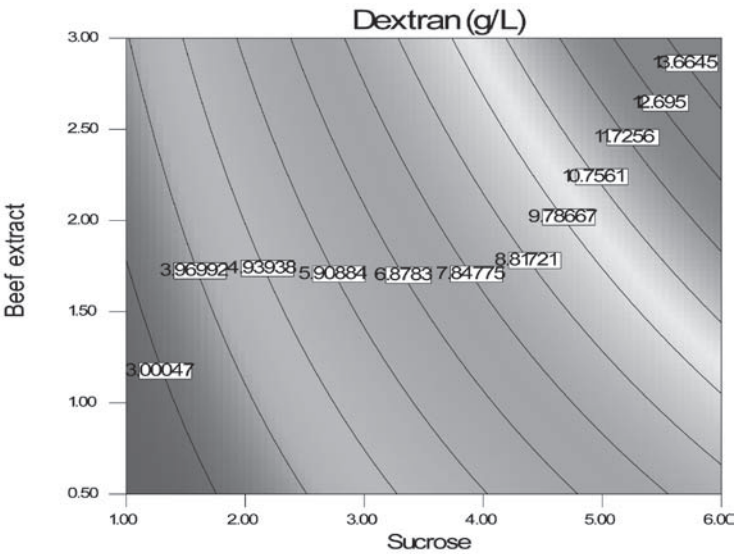

FIG. 3 - Contour plot of the combined effects of sucrose and beef extract on dextran production by *Leuconostoc mesenteroides* NRRL B-640. Fixed level: peptone = 1.75.

TABLE 8 - Comparison of dextran production by various media

Medium	Dextran (mg/ml)
Unoptimized medium (Tsuchiya *et al.,* 1952)	7.0 ± 0.2
Optimized medium for dextransucrase (Purama and Goyal, 2008)	5.5 ± 0.2
Optimized medium for dextran	12.2 ± 0.2

The 2-Dimensional contour plots are the graphical representations of the regression equation. The plots are presented in Figs. 1-3. From the contour plots it is convenient to understand the interactions between the two nutrients and to find out also their optimum levels. There was an increase in dextran production when high concentrations of sucrose (4-6% w/v) and peptone (2-3% v/v) were used (Fig. 1). The plot depicting the interaction of peptone and beef extract is given in Fig. 2. The plot indicated that there is no interaction among the independent variable corresponding to the contour and also a smaller *P*-value (0.011) showed that there is relatively no interaction between two parameters. Maximal activity was obtained with high levels of beef extract (2-3%) and high levels of sucrose (5-6%) (Fig. 3).

The concentration of ingredients for the medium selected from Minitab statistical software were as follows: 5%, w/v sucrose, 2.5%, w/v beef extract, 2.5%, w/v and peptone. The maximum dextran concentration predicted obtained using above selected variables was 12 mg/ml and the dextran concentration experimentally obtained was 12.2 ± 0.2 mg/ml (Table 8) which showed a perfect agreement with the above predicted model. The dextran production was also compared with a medium optimized for dextransucrase production from *Leuconostoc mesenteroides* NRRL B-640 (Purama and Goyal, 2008). 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 a value of 5.5 ± 0.2 mg/ml of dextran concentration (Table 8).

DISCUSSION

Dextran is required in higher amounts at the expense of optimum levels of nutrients as there are enormous applications of dextran. There are reports on the optimization studies for production of dextransucrase or dextran from other strains of *Leuconostoc mesenteroides*. The present study reports the production of a novel dextran from *Leuconostoc mesenteroides* NRRL B-640 using statistical designs of experiments. In our previous studies it was reported that the controlled pH (Purama *et al.*, 2007) and aeration (Purama *et al.*, 2008) do not affect this strain for dextranscurase production. Among all the studied variables, sucrose, peptone and beef extract significantly affected the dextran production from *Leuconostoc mesenteroides* NRRL B-640. The proposed model equation illustrated the quantitative effect of variables and also of the interactions among the variables on the dextran production. Under optimal medium composition (50 g/l sucrose, 25 g/l peptone and 25g/l beef extract), the experimentally obtained value of dextran concentration 12.2 ± 0.2 mg/ml (Table 8) perfectly matched with predicted value of 12.0 mg/ml. Sucrose is the known inducer of dextransucrase and is also the substrate for dextran production. Besides good enzyme activity and stability, dextran synthesis is related to the sucrose available to be polymerized. The amount of sucrose available will determine the maximal dextran formed. A lower value of dextran concentration (5.5±0.2 mg/ml) was obtained with the medium that was optimized for dextransucrase production (Purama and Goyal, 2008). In the present study, the optimized medium for dextran production from *Leuconostoc mesenteroides* NRRL B-640 required 5% w/v sucrose for maximum dextran production, whereas, only 3% sucrose was enough for maximum dextransucrase production (Purama and Goyal, 2008). The presence of higher sucrose concentration (50 g/l) in the newly optimized medium is thus justified for higher dextran production as its synthesis involves the breakdown of sucrose. The results showed the requirement of higher nitrogenous sources (peptone and beef extract each at 25 g/l,) for maximum dextran production. The beef extract contains some amount of sugar that might also have contributed to the higher production of dextran. K_2HPO_4 acts as buffering agent for the culture medium and thus promoted the microbial growth and dextransucrase release from *Leuconostoc mesenteroides* NRRL B-640 (Purama and Goyal, 2008), but it did not affect the dextran production in the present study. The statistically optimized medium applied to dextran production yielded over 70 percent higher dextran (12.2 ± 0.2 mg/ml) as compared to un-optimized medium that gave 7.0 ± 0.2 mg/ml of dextran. The present results showed that the statistical method is a powerful tool for maximizing dextran production from *Leuconostoc mesenteroides* NRRL B-640 that can also be applied for large scale production.

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REFERENCES

Alsop R.M. (1983). Industrial production of dextrans. In: Bushell M.E., Ed., Progress in Industrial Microbiology, Vol. 18, Elsevier, New York, pp. 1-42.

Barker P.E., Gantesos G., Ajongwen J. (1993). A novel approach to the production of clinical-grade dextran. J. Chem. Technol. Biotechnol., 57: 21-26.

Behravan J., Bazzaz B.S.F., Salimi Z. (2003). Optimization of dextran production by *Leuconostoc mesenteroides* NRRL B-512F using cheap and local sources of carbohydratre and nitrogen. Biotechnol. Appl. Biochem., 38: 267-269.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Fox J.D., Robyt J.F. (1991). Miniaturization of three carbohydrate analyses using a microsample plate reader. Anal. Biochem., 19: 593-596.

Goyal A., Katiyar S.S. (1996). Regulation of dextransucrase productivity *from Leuconostoc mesenteroide*s NRRL B-512F by the maintenance media. J. Gen. Appl. Microbiol., 42: 81-85.

Goyal A., Katiyar, S.S. (1997). Effect of certain nutrients on the production of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. J. Basic Microbiol., 37: 197-204.

Haider M.A., Pakshirajan K. (2007). Screening and optimization of media constituents for enhancing lipolytic activity by a soil microorganism using statistically designed experiments. Appl. Biochem. Biotechnol., 141: 377-390.

Jeanes A., (1965). Preparation of dextrans from growing *Leuconostoc* cultures. Methods Carbohydr. Chem., 5: 18-126.

Karthikeyan R.S., Rakshit S.K., Baradarajan A. (1996). Optimization of batch fermentation conditions for dextran production. Bioproc. Eng., 15: 247-251.

Kralj S., Van Geel-Schutten G.H., Dondroff M.G., Kirsanovs S., Van Der Maarel M.J.E.C., Dijkhuizen L. (2004). Glucan synthesis in the genus *Lactobacillus*: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains. Microbiology, 150: 3681-3690.

Lawford G.R., Kligerman A., Williams T., Lawford H.G. (1979). Deaxtran biosynthesis and dextransucrase production by continuous culture of *Leuconostoc mesenteroides*. Biotechnol. Bioeng., 21: 1121-1131.

Lazic M.L., Velzkovic V.B., Vucetic J.I., Vrvic M.M., (1993). Effect of pH and aeration on dextran production by *Leuconostoc mesenteroides*. Enzyme Microb. Technol., 15: 334-338.

Leathers T.D. (2002). Dextran: polysaccharides from prokaryotes. In: Vandamme E.J., De Baets S., Steinbüchel A., Eds, Biopolymers, Polysaccharides I, Wiley-VCH, Weinheim, pp. 299-321.

Li J., Ma C., Ma Y., Li Y. (2007). Medium optimization by combination of response surface methodology and desirability function: an application in glutamine production. Appl. Microbiol. Biotechnol., 74: 563-571.

Liu G.-C., Wang X.-L. (2007). Optimization of critical medium components using response surface methodology for biomass and extracellular polysaccharide production by *Agaricus blazei*. Appl. Microbiol. Biotechnol., 74: 78-83.

Majumder A., Goyal A. (2008). Enhanced production of exocellular glucansucrase from *Leuconostoc dextranicum* NRRL B-1146 using response surface method. Bioresour. Technol., 99: 3685-3691.

Majumder A., Goyal A. (2009) Rheological and gelling properties of a novel glucan from *Leuconostoc dextranicum* NRRL B-1146. Food Res. Int., 42: 525-528.

Majumder A., Singh A., Goyal A. (2009). Application of response surface methodology for glucan production from *Leuconostoc dextranicum* and its structural characterization. Carbohydr. Polym., 75: 150-156.

Naessens M., Cerdobbel A., Soetaert W., Vandamme E.J. (2005). *Leuconostoc* dextransucrase and dextran: production, properties and applications. J. Chem. Technol. Biotechnol., 80: 845-860.

Plackett R.L., Burman J.P. (1946). The design of optimum multifactorial experiments. Biometrika, 33: 305-325.

Purama R.K., Goyal A. (2005). Dextransucrase production by *Leuconostoc mesenteroides*. Ind. J. Microbiol., 2: 89-101.

Purama R.K., Goyal A. 2008. Screening and optimization of nutritional factors for higher dextransucrase production by *Leuconostoc mesenteroides* NRRL B-640 using statistical approach. Bioresour. Technol., 99: 7108-7114.

Purama R.K., Singh G., Majumder A., Dasu V.V., Goyal A. (2007). Dextransucrase production from *Leuconostoc mesenteroides* NRRL B-640 in batch fermentation. Int. J. Chem. Sci., 5: 1497-1504.

Purama R.K., Singh G., Majumder A., Dasu V.V., Goyal A. (2008). Dextransucrase production by *Leuconostoc mesenteroides* NRRL B-640 in bioreactor: effect of aeration and mathematical modelling. J. Appl. Biosci. Biotechnol., 4: 9-14.

Purama R.K., Goswami P., Khan A.T., Goyal A. (2009). Structural analysis and properties of dextran produced by *Leuconostoc mesenteroides* NRRL B-640. Carbohydr. Polym., 76, 30-35.

Rodrigues S., Lona L.M.F., Franco T.T. (2003). Effect of phosphate concentration on the production of dextransucrase by *Leuconostoc mesenteroides* B-512F. Bioproc. Biosyst. Eng., 26: 57-62.

Tsuchiya H.M., Koepsell H.J., Corman J., Bryant G., Bogard M.O., Feger V.H., Jackson R.W. (1952). The effect of certain cultural factors on production of dextransucrase by *Leuconostoc mesenteroides*. J. Bacteriol., 64: 521-527.

Seymour F.R., Knapp R.D. (1980). Structural analysis of dextrans from strains of *Leuconostoc* and related genera that contain 3-O-α-glucosylated-D-glucopyranosyl residues at the branched points, or in consecutive, linear position. Carbohydr. Res., 81: 105-129.