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Increased Dextransucrase Production by Response Surface Methodology from *Leuconostoc* Species; Isolated from Fermented *Idli* Batter

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Abstract: A lactobacillus strain was isolated from i*dli* batter, an Indian fermented food. Strain was identified as *Leuconostoc mesenteroides* after biochemical and 16S rRNA sequencing studies. The isolated strain was found to produce dextransucrase. Hence a culture medium was developed using Placket-Burman and response surface methodology (RSM) for enhanced dextransucrase production. Plackett-Burman was applied to find the significant factors affecting enzyme production. RSM was applied further to optimize these significant medium constituents viz., sucrose, yeast extract, sodium acetate and beef extract for enhanced yield. The optimum values of the tested variables by response surface methodology were; sucrose, 13.75%; yeast extract, 0.53%; beef extract, 0.53% and sodium acetate, 1.51% found to be optimum for dextransucrase production. Response surface model had suggested a second-order equation which was validated experimentally. The model was very satisfactory as the coefficient of determination was 0.98. The dextransucrase production 489.19 DSU/ml showed 2.02 fold increase over the central point and 5.42 fold increase over the basal medium. The isolated strain can be used as a new source for maximum dextransucrase production.

Key words: Dextransucrase · Dextran · *Leuconostoc* · Response surface methodology

INTRODUCTION

Lactic acid bacteria (LAB) are one of the important classes of microorganisms that are known to produce several industrially important biomolecules. They produce lactic acid, acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes [1]. LABs are reported to show diverse behavior and hence have attracted attention as a potential source of new applications as well as for enzyme production [2]. Current challenges in the production of enzyme from LAB include not only strain improvement, but also enhanced production. Due to this reason biotechnologists are constantly in search of newer strain having higher enzyme productivity. Hence, fermented idli batter, was selected in present work as a source to isolate lactobacilli strains and test these for enzyme production. One of the strains was found to give significant dextransucrase activity and was used in the present study.

Dextransucrase (E. C. 2.4.1.5) from *Leuconostoc* species are used in industry for the production of oligosaccharides and dextran. These oligosaccharides are useful as food additives because of their desirable physiochemical properties in food and their prebiotics effect on intestinal bacteria. Oligosaccharides produced by dextransucrase find several applications in food, feed, pharmaceutical, or cosmetic industries [3]. Dextransucrase has gained importance as it catalyses formation of dextran by the transfer of D-glucosyl units to a growing polymer chain from sucrose; which acts as an inducer for the enzyme [4]. Different kinds of dextransucrase are produced by various LABs such as *Leuconostoc* and *Streptococcus* species. This enzyme has gained importance because it produces dextran having 95% linear α-(1→6) glucouspyronosyl linkages units and 5% α-(1→2), α-(1→3) and α-(1→4) branched linkage [5]. Dextran has significant commercial values in blood plasma substitute formulations. It is also used in many pharmaceutical, chemical and food industries, as an additive, emulsifier, drug carrier and stabilizer. Cross-linked dextran i.e. Sephadex is widely used for separation and purification of various products like proteins and enzymes [3,4]. Considering its wide clinical and commercial applications, efforts are ongoing to improve and optimize

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dextransucrase production. The effect of nutrients and other culture conditions such as effect of temperature, pH, minerals, carbon and nitrogen source on *Leuconostoc* species for enzyme and polysaccharide production has been reported [6-13].

In the last decade, statistical experimental methods such as Plackett-Burman and Response surface methodology (RSM) have been applied to optimize media for industrial purposes. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of various factors and searching for the optimum conditions. RSM has been successfully used in the optimization of bioprocesses [12,14-16].

The present study was aimed to optimize media component to increase dextransucrase production for *Leuconostoc* species, isolated from fermented *idli* batter. An optimal media composition has been optimized in two steps: (i) Plackett-Burman used for selection of the most influential media components, (ii) RSM was used for further optimization to enhance the yield using the influential process variables. To best of our knowledge there are limited or no reports on the production of dextransucrase from *Leuconostoc* species; isolated from fermented *Idli* batter.

MATERIALS AND METHODS

Medium: The existing MRS medium was modified for the isolation of the LAB strain [17]. The modified sMRS media (glucose replaced by sucrose) was used with following composition: (g/l): proteose peptone, 10; yeast extract, 5; beef extract, 10; sucrose, 20; triammonium citrate, 2; sodium acetate, 5; tween 80, 1.0; MgSO4, 0.1; $MnSO_4$, 0.05. Medium pH was adjusted to 7.0 before steam sterilization (121°C, 15 psi and 20 min).

Isolation and Identification of the Isolated Strain: One gram of idli batter was transferred to 100 ml of sterile saline. One ml of the resulted suspension was then transferred to 9 ml of sterile saline and serial dilutions were prepared. One loopful culture from the final dilution was spread on to sMRS agar plates containing 0.05% of sodium azide for the inhibition of growth of yeast cells. The plates were incubated at 30°C for 48 h. The colonies were picked up and then suspended in saline and again one loopful culture was streaked on to sMRS agar plates. The process was repeated until there were no mixed cultures on each plate.

Physiological and biochemical tests of the isolated LAB strain were done as per Bergey's Manual of Systematic Bacteriology [18]. Cultures were further identified according to 16S rRNA studies. The 16S rRNA sequencing was done at National Center for Cell Science (NCCS), Pune, India. The obtained sequence was searched against the GenBank database and homology studies were performed to identify the isolate. Initially sequence was analyzed at National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov). Phylogenetic tree was constructed using ClustalW2 software from European Bioinformatics Institute website (http://www.ebi.ac.uk). The sequence was deposited at GenBank with accession no GC141831.

Maintenance of Culture and Inoculum Preparation: The isolated culture designated as UICT/LI18 was streaked on MRS slants and kept for incubation at 28±2°C for 24 h. These slants were kept at 4°C for further experiments and subcultured monthly. Inoculum was prepared in 250 ml Erlenmeyer flasks containing 100 mL sterile sMRS medium (180 rpm for 12 h at 25°C). Unless and otherwise mentioned all experiments were carried at 25°C and the optical density of the inoculum was adjusted to 1.0 before inoculation and 2% inoculum was used. Temperature and inoculum size were previously optimized by one-factor at a time method (data not shown).

Selection of the Significant Media Components for Process Modeling: The purpose of Plackett-Burman factorial design was to identify significant medium components affecting the dextransucrase production. This factorial design is important when large numbers of factors are to be considered for optimization. Twelve experiments were obtained for 11 factors namely sucrose, bacteriological peptone, yeast extract, beef extract, triammonium citrate, K_2HPO_4, $MgSO_4$, $MnSO_4$, sodium acetate, pH and one dummy variable. Each variable was represented at two levels, upper ("high (+)") and lower ("low (-)") levels of the range covered by each variable and the response [19]. Table 1 shows a 12-run Plackett-Burman experimental design. Experimental responses were analyzed by first order model from the following equation

$$Y = \beta_o + \Sigma \beta_i x_i$$

Where Y is the response for dextransucrase production, β_o is the model intercept and β_i is the linear coefficient and x_i is the level of the independent variable.

Table 1: Plackett-Burman design experiments for media components [a]

Run	Sucrose (A)	Bacteriological Peptone (B)	Yeast Extract (C)	Beef Extract (D)	Triammonium citrate (E)	K_2HPO_4 (F)	$MgSO_4$ (G)	$MnSO_4$ (H)	Sodium acetate (J)	pH (K)	Dummy (L)	Dextransucrase[b] (DSU/ml)
1	10(-1)	0.1(-1)	0.1 (-1)	2(+1)	1(+1)	1(+1)	0.005 (-1)	0.01(+1)	1(+1)	6 (-1)	1	90.16
2	30(+1)	0.1(-1)	2(+1)	0.1 (-1)	0.1 (-1)	0.1 (-1)	0.05(+1)	0.01(+1)	1(+1)	6 (-1)	1	146.80
3	10(-1)	0.1(-1)	2(+1)	2(+1)	1(+1)	0.1 (-1)	0.05(+1)	0.01(+1)	0.1 (-1)	8(+1)	-1	36.70
4	10(-1)	2(+1)	2(+1)	0.1 (-1)	1(+1)	0.1 (-1)	0.005 (-1)	0.001 (-1)	1(+1)	8(+1)	1	15.50
5	30(+1)	0.1(-1)	0.1 (-1)	0.1 (-1)	1(+1)	1(+1)	0.05(+1)	0.001 (-1)	1(+1)	8(+1)	-1	23.72
6	30(+1)	2(+1)	0.1 (-1)	2(+1)	1(+1)	0.1 (-1)	0.05(+1)	0.001 (-1)	0.1 (-1)	6 (-1)	1	164.83
7	30(+1)	2(+1)	0.1 (-1)	2(+1)	0.1 (-1)	0.1 (-1)	0.005 (-1)	0.01(+1)	1(+1)	8(+1)	-1	45.81
8	10(-1)	2(+1)	0.1 (-1)	0.1 (-1)	0.1 (-1)	1(+1)	0.05(+1)	0.01(+1)	0.1 (-1)	8(+1)	1	288.79
9	10(-1)	2(+1)	2(+1)	2(+1)	0.1 (-1)	1(+1)	0.05(+1)	0.001 (-1)	1(+1)	6 (-1)	-1	162.24
10	30(+1)	0.1(-1)	2(+1)	2(+1)	0.1 (-1)	1(+1)	0.005 (-1)	0.001 (-1)	0.1 (-1)	8(+1)	1	28.41
11	30(+1)	2(+1)	2(+1)	0.1 (-1)	1(+1)	1(+1)	0.005 (-1)	0.01(+1)	0.1 (-1)	6 (-1)	-1	168.25
12	10(-1)	0.1(-1)	0.1 (-1)	0.1 (-1)	0.1 (-1)	0.1 (-1)	0.005 (-1)	0.001 (-1)	0.1 (-1)	6 (-1)	-1	362.19

[a] Values in parentheses are coded variables, actual values in (%w/v)

[b] Readings are average of two determinations

Table 2: Central composite design (CCD) matrix for RSM showing observed and predicted yield of dextransucrase

	Media components (%w/v)[a]				Dextransucrase (DSU/ ml)[b]	
Run	Sucrose (A)	Beef extract (B)	Yeast extract (C)	Sodium acetate (D)	Experimental	Predicted
1	22.50 (0)	1.025 (0)	1.025 (0)	1.025 (0)	252.02	250.88
2	31.25 (+1)	1.512 (+1)	1.512 (+1)	1.512 (+1)	98.15	95.48
3	13.75 (-1)	0.537 (-1)	1.512 (+1)	0.537 (-1)	252.09	242.79
4	22.50 (0)	1.025 (0)	2.000 (+2)	1.025 (0)	215.29	207.46
5	22.50 (0)	1.025 (0)	1.025 (0)	1.025 (0)	241.25	250.88
6	31.25 (+1)	1.512 (+1)	0.537 (-1)	1.512 (+1)	190.49	201.83
7	22.50 (0)	1.025 (0)	1.025 (0)	1.025 (0)	258.32	250.88
8	5.00 (-2)	1.025 (0)	1.025 (0)	1.025 (0)	232.72	244.69
9	22.50 (0)	1.025 (0)	1.025 (0)	0.050 (-2)	255.69	256.20
10	22.50 (0)	1.025 (0)	1.025 (0)	1.025 (0)	244.58	250.88
11	31.25 (+1)	0.537 (-1)	0.537 (-1)	1.512 (+1)	259.46	267.36
12	13.75 (-1)	1.512 (+1)	1.512 (+1)	1.512 (+1)	220.15	219.96
13	22.50 (0)	1.025 (0)	0.050 (-2)	1.025 (0)	450.55	447.60
14	22.50 (0)	1.025 (0)	1.025 (0)	1.025 (0)	246.58	250.88
15	31.25 (+1)	0.537 (-1)	1.512 (+1)	0.537 (-1)	175.55	184.90
16	13.75 (-1)	1.512 (+1)	0.537 (-1)	1.512 (+1)	372.84	372.24
17	13.75 (-1)	0.537	1.512 (+1)	1.512 (+1)	276.82	281.33
18	22.50 (0)	2.000 (+2)	1.025 (0)	1.025 (0)	242.44	245.86
19	40.00 (+2)	1.025 (0)	1.025 (0)	1.025 (0)	39.15	16.39
20	13.75 (-1)	0.537 (-1)	0.537 (-1)	0.537 (-1)	365.15	376.57
21	22.50 (0)	0.050 (-2)	1.025 (0)	1.025 (0)	358.51	344.31
22	22.50 (0)	1.025 (0)	1.025 (0)	2.000 (+2)	272.19	260.90
23	13.75 (-1)	1.512 (+1)	0.537 (-1)	0.537 (-1)	301.21	285.77
24	31.25 (+1)	1.512 (+1)	0.537 (-1)	0.537 (-1)	231.44	235.67
25	13.75 (-1)	1.512 (+1)	1.512 (+1)	0.537 (-1)	209.01	209.86
26	31.25 (+1)	0.537 (-1)	1.512 (+1)	1.512 (+1)	85.65	103.13
27	13.75 (-1)	0.537 (-1)	0.537 (-1)	1.512 (+1)	495.89	491.49
28	31.25 (+1)	1.512 (+1)	1.512 (+1)	0.537 (-1)	199.25	205.69
29	22.50(0)	1.025 (0)	1.025 (0)	1.025 (0)	262.55	250.88
30	31.25 (+1)	0.537 (-1)	0.537 (-1)	0.537 (-1)	270.52	272.75

[a] Values in parentheses are coded variables

[b] Readings are average of two determinations

This model does not describe the interaction among factors and it is used to screen and evaluate important factors that influence the response. From the regression analysis of the variables, the factors having significant effect on dextransucrase production were further optimized by RSM.

Optimization Using Response Surface Methodology: RSM was adopted for improving dextransucrase production using Design-Expert Version 6.0.10, Stat-Ease Inc. Minneapolis, USA software; to find the interactive effects of the four variables found to be significant from the Plackett-Burman experiments. The central composite design (CCD) of response surface method was used to obtain data that fits a full second order polynomial model. The coded terms and actual values are presented in Table 2. Regression analysis was performed on the data obtained. A second-order polynomial equation was used to fit the data by multiple regression procedure. The three dimensional graphical representation of model equation represents the individual and interactive effect of the test variables on the response. For a four factor system the model equation is

$$Y = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{23} BC + \beta_{24} BD + \beta_{34} CD$$

Where Y (dextransucrase) is the predicted response; β_o is the intercept; β_1, β_2, β_3 and β_4 are the linear coefficients; β_{11}, β_{22}, β_{33} and β_{44} are the squared coefficients; β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} are the interaction coeff icients and A, B, C, D, A^2, B^2, C^2, D^2, AB, AC, AD, BC, BD and CD are independent variables. The proportion of variance explained by the polynomial models obtained was given by multiple coefficient of determination, R^2. The fitted polynomial equation was expressed as three dimensional response surface plots to find the concentration of each factor for maximum dextransucrase production. These diagram shows relationship between the responses and the experimental levels of each factor used in the design. To optimize level of each factor for maximum response 'Numerical optimization' process was employed. The combination of different optimized parameters, which gave maximum dextransucrase yield, was tested experimentally to validate the model.

Analysis of Dextransucrase: The fermented broth was centrifuged (11400 *g*, 4°C and 30 min) to obtain clear supernatant. The supernatant was used for dextransucrase estimation. Dextransucrase activity was determined by measuring the total amount of reducing sugars produced (calculated as fructose) using 3, 5-di-nitrosalicylic acid (DNSA) method [20]. Sucrose (10%) in acetate buffer (0.05 M, pH 5.2) was used as a substrate. For estimation of the enzyme activity, 0.9 ml of substrate solution was mixed with 0.1 ml of enzyme broth and incubated at room temperature for 15 min. The reaction was stopped by addition of 1 ml of DNSA reagent. The reaction mixture was heated in a boiling water bath for 15 min. After cooling, 10 ml of distilled water was added to each tube and mixed on a vortex mixer. The reducing sugars produced were determined spectrophotometrically at 540 nm using Hitachi UV-VIS spectrophotometer.

One unit (U) of dextransucrase activity is defined as the amount of enzyme that liberates one μmol of reducing sugar from sucrose in one min at pH 5.2 and 30°C. The relation between these two units was found to be 1U/ml = 20.52 DSU/ml [21].

RESULTS AND DISCUSSION

Isolation and Identification of the Isolated Strain: In the present study, cultures were isolated from fermented idli batter. These isolates were maintained as glycerol stocks and revived in MRS media. The culture UICT/LI18 in unoptimized sMRS basal media produced 90.15 ± 0.84 DSU/ml amount of enzyme. Hence this strain was selected for further studies. The culture was Gram-positive and had non-motile cocci. It does not utilize arginine, no indole production, no nitrate reduction. Gas production was observed. Slime production was observed when grown on gelatin-sucrose agar plates. The biochemical tests showed that culture was catalase negative, can utilize glucose, fructose, sucrose, galactose, sorbitol, raffinose, lactose, mannose, inulin and adenine but not xylose and L-arabinose. The 16S rRNA study showed high homology with *Leuconostoc mesenteroides* species (EU074838). On the basis of 16S rRNA gene sequence study the isolate was identified as *Leuconostoc mesenteroides*.

Selection of Significant Factors by Plackett-burman Design: Plackett-Burman design was adopted to select most significant medium components. Table 1 shows the design along with response of different experimental trials. The standard analysis of variance (ANOVA) results calculated from experimental runs shown in Table 3. Contrast coefficients allow determination of the effect of each constituent. A large coefficient either positive or negative indicates that a factor has a large impact on

Table 3:ANOVA results for the CCD quadratic model for the response

Source	Sum of squares	DF	Mean square	F value	Prob>F
Model	253441.1	14	18102.93	105.7545	< 0.0001
A	78180.19	1	78180.19	456.7162	< 0.0001
B	14540.08	1	14540.08	84.94084	< 0.0001
C	86502.03	1	86502.03	505.3311	< 0.0001
D	33.20554	1	33.20554	0.193981	0.6659
A^2	24825.97	1	24825.97	145.0294	< 0.0001
B^2	3349.034	1	3349.034	19.56452	0.0005
C^2	10070.39	1	10070.39	58.82959	< 0.0001
D^2	100.7072	1	100.7072	0.588315	0.4550
AB	2886.107	1	2886.107	16.86018	0.0009
AC	2109.335	1	2109.335	12.3224	0.0032
AD	14475.1	1	14475.1	84.56122	< 0.0001
BC	3349.226	1	3349.226	19.56565	0.0005
BD	809.2603	1	809.2603	4.72757	0.0461
CD	5832.759	1	5832.759	34.07405	< 0.0001
Residual	2567.684	15	171.1789		
Lack of fit	2223.925	10	222.3925	3.234716	0.1035
Pure error	343.7589	5	68.75179		

R^2: 0.9998; Adj R^2: 0.9806; Pred R^2: 0.9458; CV: 5.18

response. A coefficient close to zero means that a factor has little or no effect. The P-value is the probability that the magnitude of a contrast coefficient is due to random process variability and it serves as a tool for checking the significance of each of the coefficients. A low P-value indicates a 'real' or significant effect. ANOVA for dextransucrase production (Y, DSU/ml) indicated the 'F-value' to be 421.46, which implied the model to be significant. ANOVA indicated the R^2 value of 0.99 for response Y. This again ensured a satisfactory adjustment of the model to the experimental data and indicated that the model could explain 99% variability in the response. The adequate precision which measures the signal to noise ratio was 63.94 for response, which indicates an adequate signal. A ratio of > 4 is desirable. This model can be used to navigate the design space for response Y. The 'Pred R-Squared' of 0.96 is in reasonable agreement with 'Adjusted R-Squared' of 0.99 for Y. The model equation can be shows as

Dextransucrase, Y (DSU/ml) = + 742.66 - 3.14 × sucrose + 13.80 × bacteriological peptone - 36.63 × yeast extract - 41.85 × beef extract - 99.08 × triammonium citrate - 1.89 × K_2HPO_4 + 417.62 × $MgSO_4$ -104.61 × sodium acetate - 54.62 pH - 5.36 D1

Sucrose was selected as an essential carbon source as it acts as an inducer for dextransucrase synthesis. Yeast extract and beef extract were selected as the nitrogen source as they found to be significant for dextransucrase production from *Leuconostoc* species. Sodium acetate acts as a buffering agent and helps to maintaining the pH in the fermentation process [4,7]. Sucrose, beef extract, yeast extract and sodium acetate were selected as significant components for further optimization by RSM.

Response Surface Methodology: Optimum levels of the above mentioned factors and the effect of their interactions on enzyme production were determined by CCD. Table 2 shows the details of the actual and coded values employed in the RSM as well as the predicted and observed responses for enzyme production (Y). Second order regression equation provided the levels of dextransucrase production as a function of initial values of sucrose, beef extract, yeast extract and sodium acetate which can be predicted by the following equation.

Dextransucrase (Y; DSU/ml) = + 436.43 + 12.40 × A - 248.37 × B - 329.04 × C + 257.55 × D - 0.39 A^2 + 46.49 × B^2 + 80.62 × C^2 + 8.06 × D^2 + 3.14 × A × B + 2.69 × A × C - 7.05 × A × D + 60.87 × B × C -29.92 × B × D- 80.33 × C × D

Where

A = sucrose, B = beef extract,
C = yeast extract, D = sodium acetate.

Table 4:ANOVA results for the Plackett Burman model for the response

Source	Sum of squares	DF	Mean square	F value	Prob>F
Model	135142.5	10	13514.25	421.46	0.0379
A	11892.15	1	11892.15	370.87	0.0330
B	2065.43	1	2065.439	64.41	0.0789
C	14534.48	1	14534.48	453.27	0.0299
D	18969.44	1	18969.44	591.58	0.0262
E	23858.42	1	23858.42	744.05	0.0233
F	8.75	1	8.756726	0.27	0.6934
G	1059.56	1	1059.564	33.04	0.1097
J	26596.23	1	26596.23	829.44	0.0221
K	35812.22	1	35812.22	1116.85	0.0190
L	345.78	1	345.78	10.78	0.1882
Residual	32.06	1	32.06		

R^2: 0.9998; Adj R^2: 0.9974; Pred R^2: 0.9658; CV: 4.43

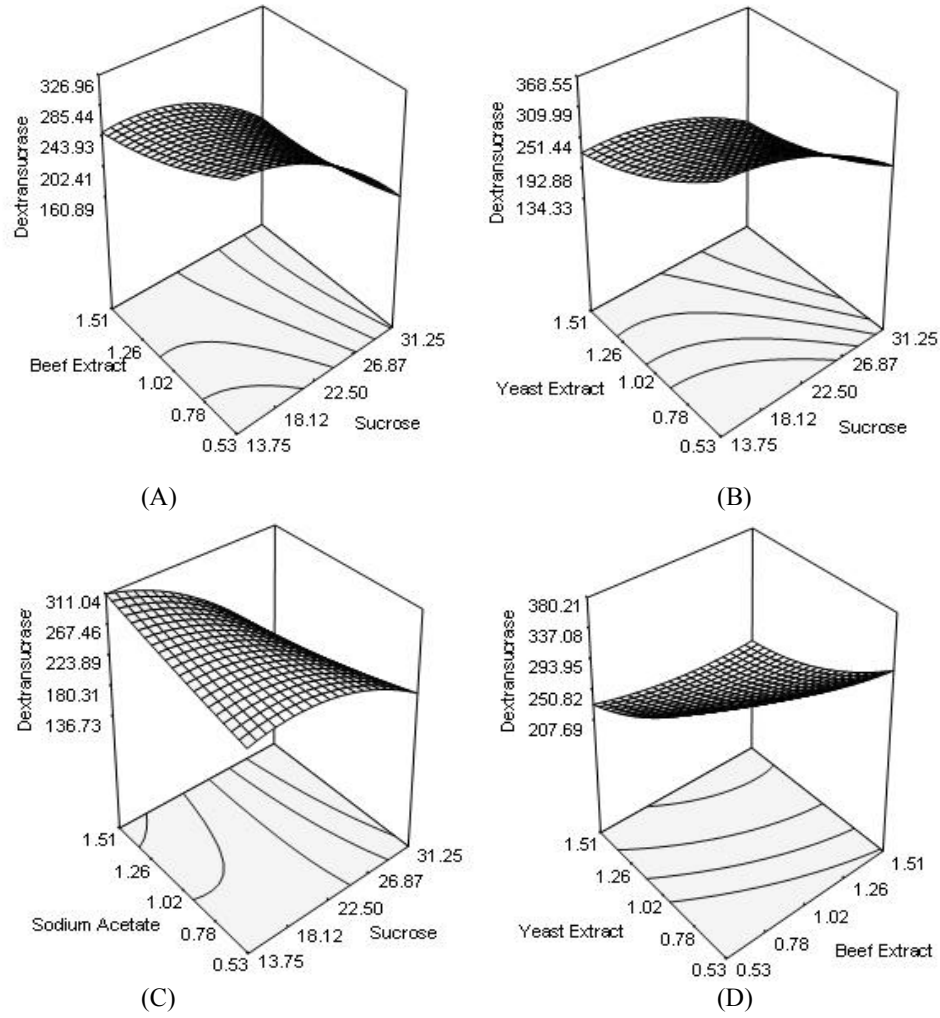

Fig. 1: Three dimentional response surface plots, interactive effect of (A) beef extract and sucrose, (B) yeast extract and sucrose, (C) sodium acetate and sucrose, (D) yeast extract and beef extract on dextransucrase (DSU/ml). Other two components are at their central values [central values (% w/v) are sucrose 22.50, beef extract 1.025, yeast extract 1.025 and sodium acetate 1.025].

Table 5: Comparison of dextransucrase production by UICT/L18 with reported study

Strain	Source	Dextransucrase (DSU/ml)	Reference
L. mesenteroides B-512F	Wine	86	Tsuchiya et al., 6
		450	Barker & Ajongwen., 1991, 7
		66	Rodrigues et al., 2003, 11
		35.57	Qader et al., 2005 13
L. Mesenteroides PCSIR-4	Vegetables	108.26	Qader et al., 2005 13
L. Mesenteroides FT 045 B	Alcohol and Sugar Mill	3.2	Contiero et al., 2005, 10
L. mesenteroides UICT/LI18 (Present study)	Idli batter	90.15 ± 0.84489.19 ± 3.28	Before optimizationAfter RSM

Table 4 shows ANOVA results for the RSM quadratic equation for response Y. According to the present model A, B, C, A^2, B^2, C^2, AB, AC, AD, BC, BD and CD are significant model terms. ANOVA for dextransucrase production (Y, DSU/ml) indicated the 'F-value' to be 105.75, which implied the model to be significant. Model terms having values of 'Prob>F' less than 0.05 are considered significant, whereas those greater than 0.10 are insignificant. The 'Lack of Fit P-value' of 0.1035 implies Lack of fit is not significant relative to pure error and that the model fits. ANOVA indicated the R^2 value of 0.98 for response Y. This again ensured a satisfactory adjustment of the quadratic model to the experimental data and indicated that this model could explain 98% response variability. The adequate precision which measures the signal to noise ratio was 51.35. A ratio of > 4 is desirable. This model can be used to navigate the design space for the response Y. The 'Pred R-Squared' of 0.94 is in reasonable agreement with the 'Adjusted R-Squared' of 0.98 for Y. A good correlation between observed and predicted results reflected the accuracy and applicability of central composite design for process optimization.

Dextransucrase yield for different levels of significant variables was predicted from the respective surface response plots (Fig. 1A-D). Each plot represents an infinite number of combinations of two test variables with the other two maintained at their respective central values. When all variables were kept at their central values dextransucrase yield was 241.25 DSU/ml. Fig. 1A shows the response for the interactive factors, sucrose and beef extract, where yeast extract and sodium acetate were kept at central level. Dextransucrase yield for this interaction was 326.96 DSU/ml; corresponding to the high amount of sucrose and beef extract. Fig. 1B shows interaction between yeast extract and sucrose where beef extract and sodium acetate were kept at central values. Increase in concentration of yeast extract and sodium acetate results in dextransucrase yield of 368.55 DSU/ml. Fig. 1C shows the interaction between sucrose and sodium acetate, where beef extract and yeast extract were kept at central values. Increase in the sucrose and sodium acetate concentration results in 311.04 DSU/ml. Fig. 1D shows the interaction between beef extract and yeast extract which results in high production of dextransucrase i.e. 380.21 DSU/ml, when sucrose and sodium acetate were kept at central values. It can be seen from three dimensional plots that at lower and higher concentration of sodium acetate, yeast extract, sucrose and beef extract results in lower dextransucrase yield. Higher sucrose concentration shows inhibitory action for dextransucrase yield due to increased viscosity during fermentation which results in mass transfer limitation of nutrients. Thus it is essential to balance these all nutrients to achieve maximum dextransucrase yield [7]. The RSM model showed that a medium containing sucrose, 13.75%; beef extract, 0.53%; yeast extract, 0.53% and sodium acetate, 1.51% was optimum for the dextransucrase production. Validation was carried out in shake flasks under conditions predicted by the model. The predicted yield was 494.54 DSU/ml. On experimentation, 489.19 DSU/ml dextransucrase was obtained. The experimental values were found to be very close to the predicted values and hence, the model was successfully validated.

In order to assess potential of the new isolate UICT/L18 as dextransucrase producer, dextransucrase activity obtained in this work were compared with those reported in the literature (Table 5). It can be seen that depending upon the strain used the activity varies from 3.2 DSU/ml to 450 DSU/ml. In this work, the isolated strain UICT/L18 gave 90.15 ± 0.84 DSU/ml in simple basal medium that was enhanced to 489.19 ± 2.33 DSU/ml by use of statistical optimization tool. Thus, it can be said that the isolate UICT/L18 is an excellent dextransucrase producer.

CONCLUSION

In the present study a lactic acid bacterial strain was isolated from Indian traditional fermented *Idli* batter. The strain was identified as *Leuconostoc mesenteroides* by biochemical and 16S rRNA study. Sequential statistical strategies, Plackett-Burman design followed by RSM were used successfully to find the optimum values of the significant factors to achieve maximum dextransucrase production. The predicted yield was 494.54 DSU/ml. On experimentation, 489.19 DSU/ml dextransucrase yield was

obtained. The experimental values were found to be very close to the predicted values and hence, the model was successfully validated. The dextransucrase production showed about 2.04 fold increases over the central point and 5.50 fold increases over the basal medium. The isolated strain can be used for the production of dextransucrase enzyme. Further it is important to discover newer lactic acid bacterial strain that produces enzymes that could be of industrial value.

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