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Evaluation of efficient carbon, nitrogen sources, micro and macro nutrients for dextran production by *Weissella cibaria* CMGDEX3 by utilizing a modified multifactorial Placket-Burman statistical design

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Abstract: In the present study previously isolated *Weissella cibaria* CMG DEX3 capable of producing high molecular weight, water soluble dextran (Ahmed *et al.,* 2012) is characterized for most efficient less expensive carbon, nitrogen sources, micro and macro nutrients by utilizing a multifactorial Placket-Burman statistical design for optimization of dextran production. A twelve run Plackett-Burman experimental model with slight modification was utilized to evaluate the impact of ten diverse nutrients on the production of dextran by the bacterial isolate *Weissella cibaria* CMG DEX3.

Keywords: Dextran, *Weissella cibaria* CMG DEX3, Optimization, Plackett-Burman design.

INTRODUCTION

Dextrans can be referred as the glucose homo-polysaccharides with a substantial feature of consecutive α- linkage at number 1 and 6 in the chains, which accounts for almost 50% of the total chain linkages. These α- Dextrose glucans have also side-chains, branched from α linkage at position 1 and 3 or/and α -1 and 4 or α- 1 and 2 in branched chain (Bounaix *et al*., 2010). The structure of dextran and its type are dependent on the particular strain of the microbes or bacteria which produces it and also the definite dextransucrase is involved in the production. Dextran is polysaccharides which have high molecular mass and are prepared using sucrose (Kim and Robyt, 1995). Dextran can be synthesized using various species of bacteria such as *Acetobacter, Leuconostoc, Streptococcus, Weissella spp.*

The *Weissella* genus is a group of Gram-positive, heterofermentative, cocci or rod shaped lactic acid producing bacteria. *Weissella* strains are found in diverse environments such as soil, plants, dairy, fermented products (Wolter *et al*., 2014). The demand of the dextran is primarily accomplished by culturing the species of *L. mesenteroides* in the culture media which contain high amount of sucrose, the peptone is used as the organic source containing nitrogen, many growth factors, and specific minerals like phosphate in the trace amount (Vandamme *et al.,* 2002). There is a need to explore new indigenous bacterial species and evaluate their nutritional requirements for the production of dextran.

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MATERIALS AND METHODS

Isolation and purification of dextran producing W. cibaria CMGDEX 3

Weissella cibaria CMG DEX3 a potent dextran producer was previously isolated from cabbage, structure and physical characteristics of produced dextran were studied in detail by (Ahmed *et al*., 2012).

Media Composition, Dextran production, extraction and purification

Production of Dextran by CMGDEX 3 was carried out in medium containing g/l of sucrose 150, Bactopeptone 5, yeast extract 5, K_2HPO_4 15, $MnCl_2$ 0.01, NaCl 0.01, $CaCl_2$ 0.05 and pH was adjusted to 7. Dextran production, extraction and purification were done as previously described by (Ahmed *et al*., 2012).

Plackett-Burman Experimental Design

Primary screening of carbon, nitrogen sources, micro and macro nutrients have been done in the initial step. In order to analyze the experiment statistically experimental design Plackett-Burman is used. It is commonly known that Plackett-Burman is based on the first order linear model with two level factorial designs which allows to examining the 'n-1' factors among 'n' number of experiments. Whereas, the in next step screening ten different cheaper carbon sources like dates, sugarcane juice, banana pulp, sapota, two nitrogen sources like black gram, soybeans, three micronutrients like $MgCl_2$, $MnCl_2$, $MgSO_4$, $MnSO_4$, and one macronutrient K_2HPO_4 were added to the medium flasks according to the pattern of 12 experimental design (fig. 1). As per design negative sign indicates low level and positive sign indicates high level of nutrient, various supplements had various levels as shown in the (table 1).

STATISTICAL ANALYSIS

To perform analysis statistical software Minitab@14 is used and the Plackett-Burman design is established subjected to multiple linear regression model, output is reported in table 2.

Table 1: Output of the Plackett-Burman 12 experimental design for screening of 10 different nutrients for dextran production by *Weissella cibaria CMGDEX3*

RUN	A	B	C	D	E	F	G	H	J	K	Dextran yield in gm/100ml	Dextran yield in gm/100ml	Average dextran yield in gm/100ml
1	1	1	1	1	1	0.05	0.05	0.05	0.05	0.05	3.9	3.8	3.85
2	-0.5	1	1	1	1	-0.01	-0.01	-0.01	0.05	-0.01	3.6	3.5	3.55
3	-0.5	-0.5	-0.5	1	1	0.05	-0.01	-0.01	-0.01	0.05	3.6	3.7	3.65
4	1	-0.5	1	-0.5	1	0.05	0.05	-0.01	-0.01	-0.01	3.5	3.3	3.35
5	-0.5	1	-0.5	1	-0.5	0.05	0.05	0.05	-0.01	-0.01	3.3	3.3	3.35
6	-0.5	-0.5	-0.5	-0.5	1	-0.01	0.05	0.05	0.05	-0.01	3.3	3.2	3.25
7	-0.5	-0.5	1	-0.5	-0.5	0.05	-0.01	0.05	0.05	0.05	3.5	3.3	3.37
8	1	-0.5	-0.5	1	-0.5	-0.01	0.05	-0.01	0.05	0.05	3.3	3.3	3.3
9	1	1	-0.5	-0.5	1	-0.01	-0.01	0.05	-0.01	0.05	3.5	3.3	3.35
10	1	1	-0.5	-0.5	-0.5	0.05	-0.01	-0.01	0.05	-0.01	3.6	3.6	3.6
11	-0.5	1	1	-0.5	-0.5	-0.01	0.05	-0.01	-0.01	0.05	3.7	3.85	3.77
12	1	-0.5	1	1	-0.5	-0.01	-0.01	0.05	-0.01	-0.01	3.5	3.3	3.35

a- Dates, b- Sugarcane juice, c- Black gram, d- Sapota, e- Beans, f- $MgCl_2$, g- $MnCl_2$, h- $MgSO_4$, i- $MnSO_4$, j- K_2HPO_4, Note - Upper limit - (+), Lower limit - (-)

Table 2: Statistical analysis applied on Plackett-Burman design for dextran production by *Weissella cibaria* CMGDEX3 using combination of different cheap Carbon sources, Nitrogen sources, Macro and Micronutrients

Term	Effect	Coef	SE Coef	T-value	P-Value	VIF
Constant		3.47	0.18	19.28	0.033	
A	0.063	0.032	0.18	0.18	0.889	1
B	0.063	0.032	0.18	0.18	0.889	1
C	0.12	0.06	0.18	0.33	0.795	1
D	-0.213	-0.107	0.18	-0.59	0.659	1
E	0.08	0.04	0.18	0.22	0.861	1
F	-0.127	-0.063	0.18	-0.35	0.785	1
G	0.077	0.038	0.18	0.21	0.866	1
H	0.08	0.04	0.18	0.22	0.861	1
J	0.04	0.02	0.18	0.11	0.93	1
K	0.117	0.058	0.18	0.32	0.8	1

S = 0.623538 R-Sq = 47.63% , whereas T –value and p – value represent t-statistics and probability value respectively.

Table 3: Analysis of variance for the optimization of dextran production by Weissella cibaria CMGDEX 3 using Plackett-Burman 12 experimental design

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	10	0.3536	0.3536	0.09	0.992
Linear	10	0.3536	0.3536	0.09	0.992
A	1	0.012	0.012	0.03	0.889
B	1	0.012	0.012	0.03	0.889
C	1	0.0432	0.0432	0.11	0.795
D	1	0.1365	0.1365	0.35	0.659
E	1	0.0192	0.0192	0.05	0.861
F	1	0.0481	0.0481	0.12	0.785
G	1	0.0176	0.0176	0.05	0.866
H	1	0.0192	0.0192	0.05	0.861
J	1	0.0048	0.0048	0.01	0..930
K	1	0.0408	0.0408	0.11	0.8
Error	1	0.3888	0.3888		
Total	11	0.7424			

*Note: Author calculations

RESULTS

Ten variables with 12 runs Plackett-Burman design without interaction is used to evaluate the significant variables which can influence the yield of dextran, table 1.

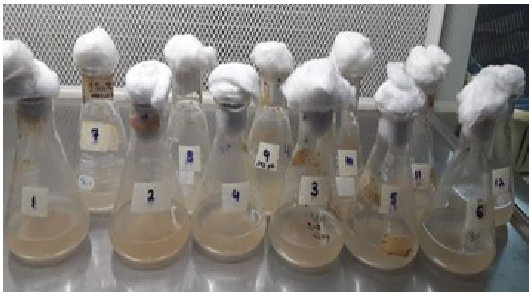

Fig. 1: Screening low-cost carbon sources, nitrogen sources, micronutrients, macronutrient according to the pattern of Plackett-Burman 12 experimental design

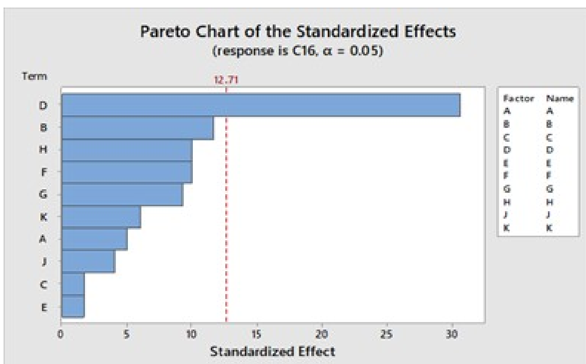

Fig. 2: Pareto chart for dextran production by *Weissella cibaria* CMGDEX3 using combination of different nutrients

The result displays that p-value of the constant is 0.03 indicates that statistical model is significant itself. Whereas, the p-value of all variables are greater than 0.05 (level of significance) represent that all factors analyzed are statistically insignificant, although they play varying role in dextran production. The multiple linear regression model is given by equation 1.

$$Y = 3.47 + 0.032A + 0.032B + 0.06\,C - 0.017D + 0.04E - 0.063F + 0.038G + 0.04\,H + 0.02\,I + 0.058\,J \quad ..(1)$$

Furthermore, multicollinearity does not exist among the factor as the VIF is less than 1, table 2.

Table 3 shows ANOVA for the optimization of dextran production by *Weissella cibaria* CMGDEX using linear model given in equation 1.

Next, to identify the effects of consider factors on dextran yield Pareto chart is plotted. Along x-axis factors standardized effects are taken. Whereas, ranking of the factors is taken along y-axis giving to their importance. It is observed from the Pareto chart that the factor sapota has more weightage followed by sugarcane, $MgSO_4$, $MgCl_2$ and $MnCl_2$. Whereas, the factors black grams and beans are less important in the production of dextran.

DISCUSSION

Dextran is a high molecular mass biopolymer of D-glucose which is being used in several industries including food, pharmacological and chemical as adjuvant, emulsifier, carrier and stabilizer. Keeping in view the versatile uses of dextran in different fields, the present study was conducted to statistically optimize the production of dextran using cheap carbon, nitrogen sources along with micro and macronutrients. Ten factors were investigated to determine the optimum medium components suitable for dextran production. The dextran yield from the twelve runs by using Plackett Burman design is shown in table 1. The regression analysis on dextran production is presented in table 2, all the tested factors have positive effect on the dextran except “C” and “E” which show negative impact. This shows that “C” and “E” which are nitrogen sources do not contribute in the optimization of the dextran production; this finding does not support to the findings of (Srinivas and Padma, 2015). Furthermore, p- values of t- statistics of each product are greater than 0.05 level of significance indicates that all factors analyzed are not statistically significant hence they play varying role in dextran production.

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