Screening Of Diverse Organic, Inorganic And Natural Nitrogen Sources For Dextran Production By *Weissella Sps* Using Plackett-Burman Design

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Abstract: Exopolysaccharides like dextran produced by different microorganisms have a wide range of applications in the food, pharmaceutical and other industries. Dextran and its derivatives like iron dextran, clinical dextran, food grade dextran are rapidly emerging as new and industrially significant products. Dextran a polymer of glucose is produced using sucrose rich media and also requires efficient nitrogen sources for production. In the present study diverse organic nitrogen sources like yeast extract, soya bean meal, meat extract, beef extract, casein hydrolysate, bacterial peptone, corn steep liquor, inorganic nitrogen sources like sodium nitrate, sodium nitrite, potassium nitrate, ammonium chloride, ammonium nitrate, ammonium sulphate, ammonium phosphate and natural nitrogen sources like Bengal gram, red gram, black gram, green gram, horse gram, soybeans, cow pea were screened using statistical design like Plackett-Burman. An eight experimental design of Plackett-Burman was used and seven sources were screened. Broth analysis indicated presence of more fructose and less glucose. Dextran was recovered from broth by alcohol precipitation. The results indicated that there was higher dextran production in organic nitrogen sources like casein hydrolysate, natural nitrogen sources like black gram. Though inorganic nitrogen sources did not show good yield, but comparatively ammonium sulphate gave a positive response. These studies indicate that organic and natural nitrogen sources can be used for optimization of production media for commercial production of dextran.

Keywords: Dextran, Dextransucrase, Fructose, Glucose, Plackett-Burman, Sucrose, *Weissella sp*

1 INTRODUCTION:

Dextran is structurally an exopolysaccharide [16], biochemically a branched glucan made up of glucose molecules joined into chains of varying length [11]. It is produced as low molecular weight and high low molecular weight dextrans. (From 10 to 150 kilo Daltons) [15]. It is produced by certain lactic acid bacteria like *Leuconostoc mesenteroides* [7],[12], *Lactobacillus brevis, Streptococcus mutants* and *Weissella sps* [8]. Dextran is of particular interest because of its use as blood-plasma volume expander [2]. It finds various other industrial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer [6]. Crossed linked Dextran known as sephadex [1] are widely used for separation and purification of various products like proteins in research and industry. In food industry it is being used as thickener for jam and ice cream [4]. It prevents crystallization of sugar, improves moisture retention, and maintains flavor and appearance of various food stuffs. Due to its numerous industrial applications it is being produced commercially using the strain of *Weissella sps*. Dextran production depends upon the composition of fermentation media. The study of factors affecting dextran production is an important strategy. The cell growth and the accumulation of product (Dextran) are strongly influenced by media composition such as carbon sources, nitrogen sources and inorganic salts [12]. In the present study diverse organic, inorganic and natural nitrogen sources were screened using statistical design like Plackett-Burman [13]. An eight experimental design of Plackett-Burman was used and seven nitrogen sources were screened.

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2 MATERIALS AND METHODS:

2.1 Isolation of Dextran producer Weissella sps:

Bacterial culture was isolated from Idli batter/black gram soaked water, using enrichment culture technique. Sample was inoculated into a cortezi medium [4] containing sucrose -40g, yeast extract-20g, K_2HPO_4-20g, $MgSO_4$. $7H_2O$ - 0.20g, $MnSO_4.H_2O$- 0.01g, Nacl- 0. 01g, $FeSO_4.7H_2O$- 0.01g the pH was adjusted at 6.5 and autoclaved at 121°C for 15 minutes and screened by using Mc.Clesky medium containing 0.05% sodium [9]. From diverse dextran producers obtained by primary screening *Weissella sp* was selected and used for this study due to its highest dextran producing characteristic. *Weissella sps* was identified by microscopic, biochemical tests like resistance to vancomycin and confirmed by 16s rRNA gene sequencing analysis.

2.2 Fermentation:

Broth studies for dextran production was done in 250ml Erlenmeyer flasks containing 50 ml cortezi medium to which different nitrogen sources were added according to Plackett-Burman design. The inoculum size was 5% and it contained 10^6 cells /ml. The flasks were incubated at 30°C for 24 hours and later at 4° C for another 24 hours. Duplicate flasks were set up according to the experimental design. The broth sample was tested for dextran production by anthrone method [10] and fructose by resorcinol method [14]. Fructose in broth was tested only to prove that dextran is a polymer of glucose and fructose is left in broth when sucrose is taken in the medium.

2.3 Recovery:

Dextran was recovered from broth by alcohol precipitation, dried under vacuum over $CaCl_2$ at 30^0C and weighed [5]. Product was assayed and found to contain glucose polymer (Dextran) by using anthrone method. Dextran yield was determined in grams/100ml of fermented broth, results subjected to statistical analysis.

2.4 Experimental design (Plackett-Burman design):

For screening purpose, various organic, inorganic and natural nitrogen sources have been evaluated using Plackett-Burman statistical design, which is a two level factorial design and allows the investigation of n-1 variables in at least n experiments. This design requires that the frequency of each level of a variable should be equal and that in each test the number of high and low variable should be equal. Then the effects of changing the other variables cancel out while determining the effect of a particular variable. The main effect was calculated as the difference between the average of measurements made at the high level setting (+1) and the average of measurements observed at low setting (-1) of each factor. This design is practical especially when the investigator is faced with large number of factors and is unsure of which settings are likely to produce optimal or near optimal responses. Plackett-Burman experimental design was based on the first order model.

3 RESULTS:

3.1 Screening of nitrogen nutrients by Plackett-Burman:

In present study an eight Plackett-Burman statistical design was employed for screening the seven different organic nitrogen sources like yeast extract, soya bean meal, meat extract, beef extract, casein hydrolysate, bacterial peptone, corn steep liquor, inorganic nitrogen sources like sodium nitrate, sodium nitrite, potassium nitrate, ammonium chloride, ammonium nitrate, ammonium sulphate, ammonium phosphate and natural nitrogen sources like bengal gram, red gram, black gram, green gram, horse gram, soya beans, cow pea for maximum production of dextran. The yield of dextran obtained in grams/ 100ml broth was tabulated and results were analyzed using Indostat software. The efficient organic, inorganic and natural nitrogen sources were selected based on highest positive regression coefficient and t–values. The most important nutrients under different categories were selected after statistical analysis, based on regression coefficients and highest t-values. Those with p-values less than 0.005 were considered to be significant and shortlisted for further optimization studies. The probability of the experiment was 0.00001 and highly significant. Nutrients with highest positive regression coefficients and their corresponding t-values were ranked first, second and so on. The cultured broth containing organic nitrogen sources like casein hydrolysate, bacterial peptone (Table -1), natural nitrogen sources like black gram, soya beans (Table-2) influenced dextran production significantly, and among inorganic nitrogen sources ammonium sulphate shows positive response (Table-3).

Table - 1: Plackett – Burman 8 Experimental design for 7 organic nitrogen sources for dextran production by *Weissella sps.*

Run	a	b	c	d	e	f	g	Dextran-yield Gram/ (100ml)-Set I	Dextran yield Gram / (100ml) -Set II	Average Dextran yield Gram / (100ml)	Reg. Coeff	t-Value
1	+	-	-	+	-	+	+	2.7	2.6	2.65	0.9125	55.7547
2	+	+	-	-	+	-	+	2.8	2.75	2.77	0.6250	38.1881
3	+	+	+	-	-	+	-	2.4	2.45	2.42	0.2625	16.0390
4	-	+	+	+	-	-	+	2.5	2.5	2.5	0.6500	39.7157
5	+	-	+	+	+	-	-	2.6	2.6	2.6	0.2750	16.8028
6	-	+	-	+	+	+	-	2.2	2.15	2.17	0.1250	7.6376
7	-	-	+	-	+	+	+	1.65	1.6	1.62	0.4625	28.2592
8	-	-	-	-	-	-	-	0.5	0.5	0.5	-0.025	-1.5275

(a)-Casein hydrolysate, (b)-Corn steep liquor (c) - Soya bean meal, (d)-Bacterial peptone, (e)-Yeast extract (f) - Beef extract. (g)-Meat extract Upper Limit (+) = 1.5% Lower Limit (-) = 0.5%

Table-2: Plackett – Burman 8 Experimental design for 7 natural nitrogen sources for dextran production by *Weissella sps.*

Run	a	b	c	d	e	f	g	Dextranyield Gram / (100ml) -Set I	Dextranyield Gram / (100ml) -Set II	Average Dextran yield Gram / (100ml)	Reg. Coeff	t-Value
1	+	-	-	+	-	+	+	2.2	2.3	2.25	0.3188	18.1944
2	+	+	-	-	+	-	+	1.9	1.9	1.9	0.3937	22.4755
3	+	+	+	-	-	+	-	2.75	2.7	2.72	0.8313	47.4482
4	-	+	+	+	-	-	+	2.5	2.45	2.47	0.4063	23.1890
5	+	-	+	+	+	-	-	2.4	2.4	2.4	0.3438	19.6214
6	-	+	-	+	+	+	-	2.3	2.35	2.32	0.6813	38.8861
7	-	-	+	-	+	+	+	2.7	2.7	2.7	0.3438	19.6214
8	-	-	-	-	-	-	-	0.5	0.5	0.5	0.0062	0.3568

(a)-Chenna Powder, (b)-Red Gram, (c)-Black Gram, (d)-Green Gram, (e)-Horse Gram, (f)-Soya beans (g)-Cow Pea.
Upper Limit = 1.5% Lower Limit = 0.

Table-3: Plackett – Burman 8 Experimental design for 7 inorganic nitrogen sources for dextran production by *Weissella sps.*

Run	a	b	c	d	e	f	g	Dextran yield Gram/(100ml) -Set I	Dextran yield Gram / (100ml) - Set II	Average Dextran yield Gram /(100ml)	Reg. Coeff	Reg. Coeff
1	+	-	-	+	-	+	+	2.3	2.3	2.3	0.2125	0.2125
2	+	+	-	-	+	-	+	2.4	2.35	2.37	0.2250	0.2250
3	+	+	+	-	-	+	-	1.8	1.8	1.8	0.2625	0.2625
4	-	+	+	+	-	-	+	2.0	2.1	2.05	0.4875	0.4875
5	+	-	+	+	+	-	-	2.4	2.4	2.4	0.8625	0.8625
6	-	+	-	+	+	+	-	2.65	2.7	2.67	0.5250	0.5250
7	-	-	+	-	+	+	+	2.75	2.7	2.72	0.5000	0.5000
8	-	-	-	-	-	-	-	0.6	0.55	0.57	0.0000	0.0000

(a)-Potassium Nitrate, (b)-Sodium Nitrate, (c)-Sodium Nitrite (d)-Ammonium Nitrite, (e)-Ammonium (f)-Ammonium Phosphate Sulphate, (g)-Ammonium Chloride
Upper Limit (+) = 1.5% Lower Limit (-) = 0.5%

Table 4: Plackett-Burman regression coefficient and t-values of different nitrogen sources

Organic nitrogen sources			Natural nitrogen sources			Inorganic nitrogen sources		
Sources	Reg. coeff	t-value	Sources	Reg. coeff	t-value	Sources	Reg. coeff	t-value
Casein hydrolysate	0.9125	55.7547*	Chenna Powder	0.3188	18.1944	Potassium Nitrate	0.2125	11.2444
Corn steep liquor	0.6250	38.1881	Red Gram	0.3937	22.4755	Sodium Nitrate	0.2250	11.9059
Soya bean meal	0.2625	16.0390	Black Gram	0.8313	47.4482*	Sodium Nitrite	0.2625	13.8902
Bacterial peptone	0.6500	39.7157*	Green Gram	0.4063	23.1890	Ammonium Nitrite	0.4875	25.7961
Yeast extract	0.2750	16.8028	Horse Gram	0.3438	19.6214	Ammonium Sulphate	0.8625	45.6392
Beef extract	0.1250	7.6376	Soya beans	0.6813	38.8861*	Ammonium Phosphate	0.5250	27.7804
Meat extract	0.4625	28.2592	Cow pea.	0.3438	19.6214	Ammonium Chloride	0.5000	26.4575

Note: * Indicate the significant nitrogen sources influencing dextran production.

4 DISCUSSION:

An optimized culture medium is necessary for commercial production as it ensures that the required nutrients are present in appropriate forms and at non-inhibitory optimum concentrations. Taking the fact nitrogen sources play a significant role various nutrients were screened using statistical methods like Plackett-Burman [13] as it is a rapid and reliable method of not only short listing nutrients but also understanding their interactions at varying concentrations. The method is significant and time saving as it screens up to n-1 variables in just n number of experiments. Microbes require nitrogen to support the biosynthesis of proteins like enzymes, and structural proteins. Dextran though an exopolysaccharide needs the enzyme dextran sucrase for production [11]. Diverse nitrogen sources may contain amino acids in different concentrations which may influence protein (enzyme dextran sucrase) production for dextran yield. The statistical method of screening facilitated identification of most significant nitrogen sources for dextran production. The organic nitrogen sources like casein hydrolysate and peptone, natural sources like black gram and soya beans ranked 1st and 2nd in significance indicating their influence on dextran production. These are good nitrogen sources that could serve as source [3] of amino acids significant for biosynthesis of dextran sucrase which is necessary for dextran production. The present study was useful in screening a cheaper nitrogen sources for dextran production in less number of experiments. The statistical design allowed to efficiently screen n-1 variables in just n number of experiments saving both time and chemicals a very important aspect in design of production medium.

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6 REFERENCES:

[1]. P.Andrews,"Estimation of the molecular weight of proteins by Sephadex Gel-filteration", *Biochem.J*, 91, 222-233, 1964.

[2]. J.Anthony, and M.B. Leonsins, "A Valuable plasma volume expander", *S.A. Medical Journal*, 546-549, 1952.

[3]. J.Behravan, B. S. F. Bazzaz, and Z. Salimi," Optimization of Dextran production *by Leuconostoc mesenteroides* NRRL B-512 using cheap and local sources of carbohydrate and nitrogen". *Biotechnology and Applied Biochemistry* 38: 267-269, 2003.

[4]. M.Cortezi, R. Monti and J.Contiero, "Temperatures effects on dextransucrase production by *Leuconostoc mesenteroides* FT045 B isolated from alcohol and sugar mill plant", *Afr J Biotechnol*, 4, 279-285, 2005.

[5]. Farwa Sarwat, Shah Ali UL Qader, Afsheen Aman and Nuzhat Ahmed, "Production and Characterization of a unique dextran from an indigenous *Leuconostoc mesenteroides* CMG713", *International Journal of Biological Sciences*, 4, 379-386, 2008.

[6]. A. Lakshmi Bhavani and J. Nisha, "Dextran-The polysaccharide with versatile uses", *International Journal of Pharma and Biosciences*, Vol-1, pp.569-573, 2010.

[7]. T. D. Leathers, G. T. Hayman, and G. L. Cote, "Rapid Screening of *Leuconostoc mesenteroides* Mutants for Elevated Proportions of Alternan to Dextran". *Current Microbiol*. 31: 19-22, 1995.

[8]. N.H.Maina,M. Tenkanen, H.Maaheimo, R.Juvonen, L.Virkki, "NMR spectroscopic analysis of exopolysaccharides produced by *Leuconostoc citreum* and *Weissella confuse*". *Carbohydr. Res*. 343:1446–1455, 2008

[9]. C.S. McClesky, L.W. Faville and Barnett, "Characteristics of *Leuconostoc mesenteroides* from cane sugar", *J. Bacteriol*. 54, 697-708.1947.

[10]. D.L. Morris," Quantitative determination of carbohydrates with Dreywoods Anthrone reagent", *Science,* 107, 254-255, 1948.

[11]. M. Naessens, A. Cerdobbel, W. Soetaert.and E. J. Vandamme," *Leuconostoc dextransucrase* and dextran;production properties and application". J.Chem. *Technol.Biotechnol*.80;845-860,2005.

[12]. A.A. Onilude, O. Olaoye, I.F. Fadahunsi, A. Owoseni, E.O. Garuba and T. Atoyabi, "Effects of cultural conditions on dextran production by *Leuconostoc spp*", *International Food Research Journal,* 20(4), 1645-1651, 2013.

[13]. R.L. Plackett and J.P.Burman, "The design of optimal multifractional experiments". *Biometrika*, Vol-33.

[14]. P. J. Roe, J.N. Eistein and N.P. Goldstein, "A Photometric method for the determination of insulin in plasma and urine", *J.Biol.Chem*, 178, 839, 1949.

[15]. Shah Ali UL Qader, Lubna Iqbal, Afsheen Aman, Erum Shireen and Abid Azhar, "Production of dextran by newly isolated strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9". *Turkish Journal of Biochemistry,* 31, 21-26, 2005.

[16]. A. H. Tallgren, U. Airaksinen, R. von Weissenberg, H. Ojamo, J. Kuusisto, and M. Leisola.. "Exopolysaccharide-Producing Bacteria from Sugar Beets*". Appl. Environ. Microbiol*. 65, no. 2: 862-64, 1999.