Screening of Diverse Micronutrients and Macronutrients For Dextran Production by *Weissella sp* Using Plackett-Burman Design

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Abstract- Exo-polysaccharides like dextran produced by *Weissella sp.* have a wide range of applications in the food, pharmaceutical and other industries. This biopolymer and its derivatives like iron dextran, clinical dextran are rapidly emerging as a new and industrially important products. Dextran a polymer of glucose is produced using sucrose rich media with nitrogen source. It also requires efficient micronutrients and macronutrients for production. In the present study diverse micronutrients (chlorides) like ferric chloride, cupric chloride, magnesium chloride, manganese chloride, calcium chloride, cobalt chloride, mercuric chloride (sulphates) like ferrous sulphate, copper sulphate, magnesium sulphate, manganese sulphate, zinc sulphate, calcium sulphate, potassium hydrogen sulphate and macronutrients (phosphate) like dipotassium hydrogen phosphate, potassium dihydrogen phosphate, ammonium dihydrogen phosphate, calcium phosphate, aluminium phosphate, disodium hydrogen phosphate and zinc phosphate 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 very less glucose as it was used for exopolysccharide production. Dextran was recovered from broth by alcohol precipitation. The results indicated that there was higher dextran production in chloride micronutrients like magnesium chloride, manganese chloride, sulphate micronutrients like magnesium sulphate, manganese sulphate and macronutrient like dipotassium hydrogen phosphate. These studies indicate that micronutrients and macronutrients significantly influence dextran production. These should be included in optimized production media for commercial production of dextran.

Index Terms- Dextran, Dextransucrase, Fructose, Glucose, Plackett-Burman, Sucrose, *Weissella sp.*

I. INTRODUCTION

Dextran is a bacterial exopolysaccharide [20], biochemically a branched glucan made up of glucose molecules joined into chains of varying length [10]. It is produced as low molecular weight and high low molecular weight dextrans (From 10 to 150 kilo Daltons) [17]. It is produced by certain lactic acid bacteria like Leuconostoc mesenteroides [6],[11] Lactobacillus brevis, Streptococcus mutants and Weissella sps [7]. 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 [5]. 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 [3] as it prevents crystallization of sugar, improves moisture retention, and maintains flavor and appearance of the food stuffs. As it has numerous industrial applications, it is being produced commercially using the strain of Leuconostoc mesenteroides. Dextran production depends upon the composition of fermentation media. The cell growth and the accumulation of product (Dextran) are strongly influenced by media composition such as carbon sources, nitrogen sources [19] and inorganic salts [11]. Therefore an eight experimental design of Plackett-Burman[12] was used to study interactive effect of seven different micro and macro nutrients on dextran production by the isolate Weissella sps .

II. 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 [3] containing sucrose as main cabon source and screened by using Mc.Clesky medium containing 0.05% sodium [8]. 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 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 [9] and fructose by resorcinol method [15]. 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 [4]. Product was assayed and found to contain glucose polymer (Dextran) by using anthrone method. Dextran

yield was determined in grams/100ml of fermented broth and results subjected to statistical analysis.

2.4 Experimental design (Plackett-Burman design): For screening purpose, different macro, micronutrients in either chloride or sulfate form and diverse phosphate sources were evaluated using Plackett-Burman statistical design. This design is a two level factorial design based on the first order model 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.

III. RESULTS

3.1 Screening of micro and macro nutrients by Plackett-Burman: In present study an eight Plackett-Burman statistical design was employed for screening the seven different micronutrients(chlorides) like ferric chloride, cupric chloride, magnesium chloride, manganese chloride, calcium chloride, cobalt chloride, mercuric chloride, (sulphates) like ferrous sulphate, magnesium sulphate , manganese sulphate, zinc sulphate, calcium sulphate, potassium hydrogen sulphate and macronutrients (phosphates) like dipotassium hydrogen phosphate, potassium dihydrogen phosphate, ammonium dihydrogen phosphate, calcium phosphate, aluminium phosphate, disodium hydrogen phosphate, zinc phosphate were screened 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 micro and macro nutrients 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 micronutrients (chloride) like magnesium chloride, manganese chloride (Table-1), (sulphate) like magnesium sulphate, manganese sulphate (Table-2), and macronutrients (phosphate) like dipotassium hydrogen phosphate (Table-3) influenced dextran production significantly(Table-4).

Table - 1: Plackett – Burman 8 Experimental design for 7 micronutrients (chloride) 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)
1	+	-	-	+	-	+	+	1.8	1.8	1.8
2	+	+	-	-	+	-	+	1.7	1.8	1.75
3	+	+	+	-	-	+	-	2.0	1.9	1.95
4	-	+	+	+	-	-	+	2.6	2.6	2.6
5	+	-	+	+	+	-	-	2.9	2.9	2.9
6	-	+	-	+	+	+	-	2.7	2.7	2.7
7	-	-	+	-	+	+	+	2.6	2.6	2.6
8	-	-	-	-	-	-	-	0.6	0.7	0.65

(a)-Ferric chloride, (b)-Cupric chloride, (c)-Magnesium chloride, (d)-Manganese chloride, (e)-Calcium chloride, (f)-Cobalt chloride, (g)-Mercuric chloride
Upper Limit (+) = 0.05% Lower Limit (-) = 0.01%

Table-2: Plackett – Burman 8 Experimental design for 7 micronutrients (sulphate) 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)
1	+	-	-	+	-	+	+	2.3	2.3	2.30
2	+	+	-	-	+	-	+	2.4	2.3	2.35
3	+	+	+	-	-	+	-	2.8	2.7	2.75
4	-	+	+	+	-	-	+	2.2	2.2	2.20
5	+	-	+	+	+	-	-	2.8	2.9	2.85
6	-	+	-	+	+	+	-	2.5	2.5	2.5
7	-	-	+	-	+	+	+	1.7	1.8	1.75
8	-	-	-	-	-	-	-	0.5	0.6	0.55

(a)-Ferrous sulphate (b)- Cupper suphate (c)- Magnesium sulphate (d)- Manganese sulphate (e)- Zinc sulphate (f) Calcium sulphate (g)- Potassium hydrogen sulphate

Upper Limit (+) = 0.05% Lower Limit (-) = 0.01%

Table-3: Plackett – Burman 8 Experimental design for 7 macronutrients (phosphate) 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)
1	+	-	-	+	-	+	+	2.6	2.7	2.65
2	+	+	-	-	+	-	+	2.5	2.6	2.55
3	+	+	+	-	-	+	-	2.6	2.7	2.65
4	-	+	+	+	-	-	+	1.9	1.9	1.90
5	+	-	+	+	+	-	-	2.8	2.6	2.6
6	-	+	-	+	+	+	-	2.2	2.0	2.1
7	-	-	+	-	+	+	+	2.0	2.0	2.0
8	-	-	-	-	-	-	-	0.6	0.6	0.6

(a)-Dipotassium Hydrogen Phosphate (b)- Potassium di hydrogen Phosphate (c) Ammonium di hydrogen Phosphate (d)- Calcium Phosphate (e)- Aluminium Phosphate (f) – Di-Sodium hydrogen Phosphate (g)- Zinc Phosphate

Upper Limit (+) = 0.5% Lower Limit (-) = 0.05%

Table-4: Regression coefficient and t-values of different micro and macro nutrients

Phosphate			Chloride			Sulfate		
Sources	**Reg. coeff**	**t-value**	**Sources**	**Reg. coeff**	**t-value**	**Sources**	**Reg .coeff**	**t-value**
Dipotassium Hydrogen Phosphate	2.1944	21.3324*	Ferric Chloride	-0.9375	-1.6550	Ferrous Sulphate	9.0625	12.2861
Potassium Di hydrogen Phosphate	0.6944	6.7508	Copper Chloride	6.5625	11.5852	Copper Sulphate	7.8125	10.5915
Ammonium Di hydrogen phosphate	0.7500	7.2908	Magnesium Chloride	19.6875	34.7557*	Magnesium Sulphate	22.1875	30.0798*
Calcium Phosphate	0.8611	8.3710	Manganese Chloride	19.0625	33.6523*	Manganese Sulphate	19.0625	25.8432*
Aluminium	0.8611	8.3710	Calcium	18.4375	32.5490	Zinc	7.1875	9.7442

Phosphate			Chloride			Sulphate		
Di Sodium Hydrogen Phosphate	0.9167	8.9110	Cobalt Chloride	7.1875	12.6886	Calcium Sulphate	9.6875	13.1334
Zinc Phosphate	0.5833	5.6706	Mercuric Chloride	3.4375	6.0684	Potassium Hydrogen Sulphate	5.9375	8.0495

Note: * Indicate the significant micro and macro nutrients influencing dextran production.

IV. 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[13]. Taking the fact that phosphate sources play a significant role various micro and macro nutrients were screened using statistical methods like Plackett-Burman [14] 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 micro and macro nutrients to support the biosynthesis of proteins like enzymes, and structural proteins. Dextran though an exopolysaccharide needs the enzyme dextransucrase for production [11]. Diverse micro and macro nutrients in different concentrations influence protein (enzyme dextran sucrase) production for dextran yield. Calcium in different forms either chloride or sulfate influences both enzyme production and its stability as indicated by earlier research [16] The statistical method of screening facilitated identification of most significant micro and macro nutrients for dextran production[18] 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.

V. CONCLUSION

An efficient primary isolate was isolated that produced more amount of exo-polysaccharide by 48 hours in sucrose rich medium. The isolate was morphologically, biochemically and 16s-rRNA sequencing identified as *Weissella sp*. Diverse micro, macro nutrients and phosphate sources had influence on dextran production as indicated by the results. Magnesium chloride, manganese chloride, magnesium sulphate, manganese sulphate and dipotassium hydrogen phosphate as micronutrients and macronutrients had influenced dextran production by *Weissella sp* as indicated by high fructose levels in broth, as glucose is used for dextran production. The isolate can be commercially exploited for dextran production.

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