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SCREENING OF DIVERSE SUCROSE RICH CHEAPER SOURCES FOR DEXTRAN PRODUCTION BY *WEISSELLA CONFUSA* USING PLACKETT-BURMAN DESIGN

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Abstract – Dextran a bacterial exopolysaccharide and a polymer of glucose is produced by different microorganisms like *Leuconostoc mesentiroides, Lactobacillus spp, Weissella spp* etc. It has 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. In the present study different carbon sources like sucrose, glucose, maltose and lactose were studied for their effect on dextran production and dextransucrase activity. Results indicated that 4% sucrose concentration had an inducing effect on both dextran production and dextransucrase activity. Further study was done to screen for cheap sucrose source using statistical design like Plackett-Burman. A twelve experimental design of Plackett-Burman was used and eleven sources like guava, pineapple, sugarcane, apple, orange, sapota, white grapes, banana, black grapes, sweet lime and pomegranate were screened. Broth analysis indicated presence of more fructose and less glucose as glucose was used for dextran production. Dextran was recovered from broth by alcohol precipitation. The results indicated that there was higher dextran production in sugarcane and sapota. These studies indicate that such sucrose rich cheaper sources can be used for commercial production of dextran.

Keywords: Dextran, dextransucrase, Fructose, Glucose, Plackett-Burman, Sucrose, *Weissella spp*

I. INTRODUCTION

Dextran is a bacterial exopolysaccharide [14], biochemically a branched glucan made up of glucose molecules joined into chains of varying length [12]. It is produced as low molecular weight and high low molecular weight dextrans (From 10 to 150 Kilo Daltons) [16]. It is produced by certain lactic acid bacteria like *Leuconostoc mesenteroides* [11] *Lactobacillus brevis*, *Streptococcus mutants* and *Weissella sps* [10]. 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 [8]. Crossed linked dextran known as sephadex [1] is 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 flavour 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 [11], nitrogen sources [18]. However, research on dextran produced by *Weissella sp* using low cost carbon sources has not previously been reported. Cheap and abundant carbon sources would be interest for economical production of dextran. Therefore various carbon sources were screened for both dextran production and dextransucrase activity. A statistical method like Plackett-Burman [6] is an efficient design for screening of nutrients at different concentrations along with their interactive effects in consideration. In the present study twelve experimental design of Plackett-Burman was used to study effect of eleven different cheap sucrose sources on dextran production by the isolate *Weissella confusa*.

II. MATERIALS AND METHODS

A. *Isolation of Dextran producer Weissella confusa*

Bacterial culture was isolated from idly batter/black gram soaked water, using enrichment culture technique. Sample was inoculated into a cortezi medium [3]. From diverse dextran producers obtained by primary screening *Weissella confusa* was selected and used for this study due to its highest dextran producing characteristic. *Weissella confusa* was identified by microscopic, biochemical tests like resistance to vancomycin and confirmed by 16s rRNA gene sequencing analysis.

B. *Fermentation*

Broth studies for dextran production was done in 250ml Erlenmeyer flasks containing 4% different carbon sources to which yeast extract was added as nitrogen source. The inoculum size was 5% and it contained 10^6cells/ml. The flasks were incubated at 30^0C for 24 hours and later at 4^0 C for another 24 hours. Broth studies were done for studying inducer effect of different carbon sources at 4% concentration for highest dextran production and dextransucrase activity. Then the effective inducer's effect was studied at different concentrations. Further broth studies were done for screening different cheaper sucrose sources for dextran production using Plackett-Burman 12 experimental design. The broth sample was tested for dextran production by anthrone method [13] 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.

C. *Enzyme assay*

Dextransucrase activity was determined by measuring the reducing sugar released from sucrose [7]. Units of dextransucrase activity are represented in DSU/ml/hr. One unit of enzyme activity was defined as the enzyme quantity that converts 1.0 milligram of sucrose into fructose and dextran in 1hour under standard conditions [9].

D. Recovery

Dextran was recovered from broth by alcohol precipitation, dried under vacuum over $CaCl_2$ at 30^0 C 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 were subjected to statistical analysis.

E. Experimental design (Plackett-Burman)

For screening purpose various cheap sucrose sources have been evaluated using Plackett-Burman statistical design is based on the first order model and is a two level factorial design that allows the investigation of n-1 variables in at least n experiments. This design is practical especially when large number of factors are to be studied along with their interactive effects to screen for the most significant factors.

III. RESULTS

A. Screening of carbon sources

In the present study different carbon sources like sucrose, glucose, maltose and lactose were studied for their inducer effect for maximum production of dextran. Sucrose influenced dextran production significantly (Tab-1).

B. Effect of sucrose at different concentration on dextran production and dextransucrase activity

Sucrose at different concentrations when studied for its inducer effect on dextran production and dextransucrase activity, it was found that 4% sucrose concentration was effective (Tab-2).

C. Screening of cheaper sucrose sources by Plackett-Burman

In present study a twelve Plackett-Burman statistical design was employed for screening the eleven different cheaper sucrose sources like guava, pineapple, sugarcane juice, apple, orange, sapota, white grapes, banana, black grapes, sweet lime and pomegranate were screened for maximum production. The yield of dextran obtained in grams/100ml broth was tabulated and results were analysed using Indostat software. The efficient cheaper sucrose 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 highest positive 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 so is highly significant. Nutrients with highest positive regression coefficients and their corresponding t-values were ranked first, second and so on. The sucrose source most effective is sugarcane juice followed by sapota and apple (Tab-3 and Tab-4)

Table-1: Effect of different carbon sources (4%) on dextran yield by *Weissella confusa*.

Sl.No	Carbon sources	Dextran yield gm/100ml-set I	Dextran yield gm/100ml-set II	Average Dextran yield gm/100ml-III
1	Sucrose	1.3	1.3	1.3
2	Glucose	0.5	0.4	0.45
3	Maltose	0.6	0.6	0.6
4	Lactose	0.5	0.5	0.5

Table -2: Effect of sucrose at different concentration on dextran yield and dextransucrase activity by *Weissella confusa.*

Sl.No	Concentration of sucrose	Dextransucrase activity DSU/ml	Dextran yield gm/100ml	Fructose released gm/100ml
1	2%	1.30	0.5	0.2
2	4%	2.92	1.3	0.6
3	6%	2.50	1.1	0.4
4	8%	2.10	0.9	0.35
5	10%	1.95	0,75	0.3

Table-3: Plackett-Burman 12 Experimental design for screening of cheap carbon (sucrose) sources for dextran production by using *Weissella confusa.*

RUN	a	b	c	d	e	f	g	h	I	j	k	Dextran yield in gm/100ml – set I	Dextran yield in gm/100ml – set II	Average dextran yield in gm/100ml
1	+	+	+	+	+	+	+	+	+	+	+	2.8	2.8	2.8
2	-	+	-	+	+	+	-	-	-	+	-	2.6	2.55	2.57
3	-	-	+	-	+	+	+	-	-	-	+	3.0	2.95	2.97
4	+	-	-	+	-	+	+	+	-	-	-	2.5	2.45	2.47
5	-	+	-	-	+	-	+	+	+	-	-	2.2	2.1	2.15
6	-	-	+	-	-	+	-	+	+	+	-	2.8	2.75	2.77
7	-	-	-	+	-	-	+	-	+	+	+	2.1	2.15	2.12
8	+	-	-	-	+	-	-	+	-	+	+	1.95	2.0	1.97
9	+	+	-	-	-	+	-	-	+	-	+	2.45	2.5	2.47
10	+	+	+	-	-	-	+	-	-	+	-	2.5	2.5	2.5
11	-	+	+	+	-	-	-	+	-	-	+	2.7	2.65	2.67
12	+	-	+	+	+	-	-	-	+	-	-	2.65	2.6	2.62

a-Guava, b-Pine apple, c- Sugarcane, d-Apple, e-Orange, f-Sapota, g- White grapes, h- Banana, i- Black grapes, j-Sweet lime, k-Pomegranate UPPER LIMIT (+) = 0.5%, LOWER LIMIT (-) = 0.25

Table – 4: Regression coefficient and t-values of different cheap carbon (sucrose) sources.

Sources	Reg.coefficient	t-value
Guava	-0.0354	-4.9262
Pine apple	0.0187	2.6080
Sugarcane	0.2146	29.8468*
Apple	0.0354	4.9262
Orange	0.0062	0.8693
Sapota	0.1687	23.4718*
White Grapes	-0.0063	-0.8693
Banana	-0.0354	-4.9262
Black Grapes	-0.0188	-2.6080
Sweet lime	-0.0521	-7.2444
Pomegranate	-0.0062	-0.8693

Note: * Indicate the significant cheap carbon (sucrose) sources influencing dextran production.

IV. DISCUSSION

An optimized culture medium significantly influences commercial production as it ensures that the required nutrients are present in appropriate forms and at non-inhibitory concentrations. Carbon is an important constituent of the cellular components and it plays a central role in energy generation in living cells [11]. Sucrose being one of the most suitable carbon source for dextran production by lactic acid bacteria [17], including *Weissella confusa* [5]. In this study sucrose at 4% concentration induced highest dextran production and dextransucrase activity. This is of particular importance when considering the cost of dextran production which is mostly based on sucrose containing medium. Taking the fact that various cheaper sucrose sources are cost effective they were screened using statistical methods like Plackett-Burman [6] 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. Diverse sucrose sources may contain carbon in different concentrations which may influence production for dextran. The cheaper sucrose rich sources like sugarcane juice and sapota ranked 1st and 2nd in significance indicating their influence on dextran production, as sucrose is broken down by dextransucrase and dextran polymer is produced from glucose leaving free fructose in broth by producer organism. The present study was useful in screening efficient and cheaper carbon (sucrose) source for dextran production in less number of experiments. This saves both time and chemicals a very important aspect in design of optimum medium for industrial production.

V. CONCLUSION

An efficient isolate that produced more amount of exo-polysaccharide by 48 hours in sucrose rich medium and was identified morphologically, biochemically and by 16s-rRNA sequencing as *Weissella confusa.* Carbon source like sucrose at 4% concentration had inducer effect on dextransucrase activity and dextran production as indicated by the results. Cheaper sucrose sources like sugarcane juice and sapota influenced dextran production by *Weissella confusa* as indicated by high dextran production and reconfirmed by high fructose levels in broth, indicating use of glucose for dextran production. The isolate can be commercially exploited for dextran production.

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