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SECONDARY SCREENING OF VARIOUS CHEAPER NUTRIENTS 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 mesenteroides*, *Lactobacillus spp*, *Streptococcus mutans*, *Weissella confusa* 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. Selection of the best nutrients is one of the most critical stage in media optimization for dextran production. Plackett-Burman designs of 12 and 8 faciors were used to screen various cheaper carbon sources, nitrogen sources, micronutrients and macronutrients respectively for dextran production by *Weissella confusa*. The most influential or significant nutrients short listed by the first step screening based on regression coefficients and t-values obtained after subjecting the experimental data to statistical analysis using Indostat software were selected for second level screening. In the present study eleven significant nutrients like four cheaper carbon sources namely molasses, sugarcane juice, banana pulp and sapota, two nitrogen sources namely black gram and soyabeans, four micronutrients like $MgCl_2$, $MnCl_2$, $MgSO_4$ and $MnSO_4$ and one macronutrient like K_2HPO_4 were selected for second level screening using 12 experimental design of Plackett-Burman. Sugarcane juice as cheaper carbon (sucrose) source, black gram as nitrogen source, $MgCl_2$ and $MgSO_4$ as micronutrients and K_2HPO_4 as macronutrient showed maximum dextran production and this data was analysed by Indostat software and so were selected for further quantitative optimization.

Keywords: Carbon sources, Dextran, Nitrogen sources, Plackett-Burman, *Weisella confusa.*

1.0 Introduction

Dextran is a bacterial exopolysaccharide[1] which is biochemically a branched glucan made up of glucose molecules joined into chains of varying length [2]. It is produced as low molecular weight and high molecular weight dextrans (From 10 to 150 Kilo Daltons) [3]. It is produced by certain lactic acid bacteria like *Leuconostoc mesenteroides* [4,5] *Lactobacillus brevis*, *Streptococcus mutants* and *Weissella sps* [6]. Dextran is of particular interest because of its use as blood-plasma volume expander [7]. It finds various other industrial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer

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[8]. Crossed linked dextran known as sephadex [9] 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[10] 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 by commercially using the strain of *Leuconostoc mesenteroides*. The amount of dextran produced however is practically insufficient to meet the dextran requirements of the various industries, hence the need for the isolation of more dextran-producing organisms with potentials for industrial application. Dextran production depends upon composition of fermentation media. The study of factors affecting dextran production is an important strategy. The cell growth and the accumulation of product are strongly influenced by media composition such as carbon sources, nitrogen sources, micronutrients and macronutrients [5]. In the present study most efficient cheaper carbon, nitrogen sources, micronutrients and macronutrient were screened by using a multifactorial Placket-Burman [11] statistical design in an attempt to optimize suitable production media. The selected nutrients for second level screening were obtained after first level of screening for different nutrients using 12 and 8 experimental designs of Plackett-Burman. An twelve experimental design of Plackett-Burman was used to study effect of eleven different nutrients on dextran production by the isolate *Weissella confusa*.

2.0 Materials and Methods

2.1 Isolation of Dextran Producer *Weissella confusa*

Bacterial culture under study was isolated from idli batter/black gram soaked water, using enrichment culture technique. Sample was inoculated into a Cortezi medium[10]. From diverse dextran producers obtained by primary screening *Weissella confusa* was selected and used for this study due to its highest dextran producing ability. *Weissella confusa* 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 250 ml Erlenmeyer flasks containing 50ml Cortezi medium with sucrose as main carbon source. The fermentation parameters were studied for their influence on dextran production over a range to identify the most optimum factor. The flasks were incubated for 48 hours broth samples collected from different flasks and tested for dextran production.

2.3 Dextran Assay

Dextran produced was tested by anthrone method [12] and fructose by resorcinol method [13]. 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.

2.4 Recovery

Dextran was recovered from broth by alcohol precipitation. The precipitation step was repeated twicw till a which thick semi dry material was obtained. This was dried under vacuum over $CaCl_2$ at 30^0C and weighed[14]. Dextran yield was determined in grams/100ml of fermented broth. Molecular weight of dextran was analysed by HPLC using Agilent Zorbax GF-250, and it indicates presence of low molecular weight dextran [15].

2.5 Experimental Design (Plackett-Burman)

For screening purpose various carbon, nitrogen sources, micronutrients and macronutrients have been evaluated using Plackett-Burman statistical design in the first step of screening. Plackett-Burman experimental design is based on the first order model and it was selected as it is a two level factorial design that allows the investigation of n-1 variables in just n experiments. In second step screening four different cheaper carbon sources like molasses, sugarcane juice, banana pulp, sapota, two nitrogen sources like black gram, soyabeans, four micronutrients like $MgCl_2$, $MnCl_2$, $MgSO_4$, $MnSO_4$ and one macronutrient that is K_2HPO_4 were added to the medium flasks according to the pattern of 12 experimental design. According to the design – indicated low level of nutrient and + indicated high level of nutrient and different nutrients had different levels as indicated in the table 1 (Table 1).

3.0 Results and Discussion

In the present study when a twelve Plackett-Burman statistical design was employed for secondary screening the four different cheaper carbon sources like molasses, sugarcane juice, banana pulp, sapota, two nitrogen sources like black gram, soyabeans, four micronutrients like $MgCl_2$, $MnCl_2$, $MgSO_4$, $MnSO_4$ and one macronutrient that is K_2HPO_4 were screened for production of dextran the yield varied for various flasks. Peak production was by 24 hrs and it reduced by 48hrs. Production of dextran by 24 hrs was Tconsidered and the yield of dextran obtained in grams/100ml broth was tabulated and results were analyzed using Indostat software. The efficient carbon, nitrogen sources, micronutrients and macronutrient were selected based on highest positive regression coefficient and t-values. Those with p-values less than 0.005 considered to be significant and short listed for further optimization studies. The probability of the experiment was 0.00001 and is highly significant. Nutrients

with highest positive regression coefficients and their corresponding t-values were ranked first, second and so on. Based on this aspect the significant nutrients that influenced dextran production were sugarcane juice as carbon source, black gram as nitrogen source, $MgCl_2$, $MgSO_4$ as micronutrients, and K_2HPO_4 as macronutrient (Table-2).

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. Carbon is an important constituent of the cellular components and it plays a central role in energy generation in living cells [5]. Sucrose being one of the most suitable carbon source for dextran production by lactic acid bacteria [16], including *Weissella confusa* [17].In this study sugarcane juice (sucrose) ranked 1st in significanct indicating its influencing effect on dextran production. This is of particular importance when considering the cost of dextran production which is mostly based on sucrose containing medium. Dextran though an exopolysaccharide needs enzyme dextran sucrase for production[18]. Diverse nitrogen sources may contain amino acids in different concentrations which may influence protein (enzyme dextran sucrase) production for dextran yield. The natural nitrogen source like black gram ranked 1st in significance indicating its influence on dextran production. Microorganisms require micronutrients and macronutrients to support the biosynthesis of proteins like enzymes (dextran sucrase), and structural proteins [3]. Micronutrients like $MgCl_2$ and $MgSO_4$ ranked 1st and 2nd in significance in affecting dextran production. Macronutrient like K_2HPO_4 was also found significant in dextran production.

Table-1: Plackett-Burman 12 experimental design for secondary screening of 11 different nutrients for dextran production by *Weissella confusa*

RUN	a	b	c	d	e	f	g	h	i	j	k	Dextran yield in gm/100ml I	Dextran yield in gm/100m II	Average dextran yield in gm/100ml
1	+ 1%	+ 1%	+ 1%	+ 1%	+ 1%	+ 1%	+ 0.05%	+ 0.05%	+ 0.05%	+ 0.05%	+ 0.05%	2.95	2.85	2.9
2	- 0.5%	+ 1%	- 0.5%	+ 1%	+ 1%	+ 1%	- 0.01%	- 0.01%	- 0.01%	+ 0.05%	- 0.01%	2.6	2.6	2.6
3	- 0.5%	- 0.5%	+ 1%	- 0.5%	+ 1%	+ 1%	+ 0.05%	- 0.01%	- 0.01%	- 0.01%	+ 0.05%	2.7	2.6	2.65
4	+ 1%	- 0.5%	- 0.5%	+ 1%	- 0.5%	+ 1%	+ 0.05%	+ 0.05%	- 0.01%	- 0.01%	- 0.01%	2.5	2.4	2.45
5	- 0.5%	+ 1%	- 0.5%	- 0.5%	+ 1%	- 0.5%	+ 0.05%	+ 0.05%	+ 0.05%	- 0.01%	- 0.01%	2.3	2.35	2.32

6	- 0.5%	- 0.5%	+ 1%	- 0.5%	- 0.5%	+ 1%	- 0.01%	+ 0.05%	+ 0.05%	+ 0.05%	- 0.01%	2.2	2.3	2.25
7	- 0.5%	- 0.5%	- 0.5%	+ 1%	- 0.5%	- 0.5%	+ 0.05%	- 0.01%	+ 0.05%	+ 0.05%	+ 0.05%	2.35	2.4	2.37
8	+ 1%	- 0.5%	- 0.5%	- 0.5%	+ 1%	- 0.5%	- 0.01%	+ 0.05%	- 0.01%	+ 0.05%	+ 0.05%	2.4	2.4	2.4
9	+ 1%	+ 1%	- 0.5%	- 0.5%	- 0.5%	+ 1%	- 0.01%	- 0.01%	+ 0.05%	- 0.01%	+ 0.05%	2.5	2.4	2.45
10	+ 1%	+ 1%	+ 1%	- 0.5%	- 0.5%	- 0.5%	+ 0.05%	- 0.01%	- 0.01%	+ 0.05%	- 0.01%	2.7	2.6	2.65
11	- 0.5%	+ 1%	+ 1%	+ 1%	- 0.5%	- 0.5%	- 0.01%	+ 0.05%	- 0.01%	- 0.01%	+ 0.05%	2.75	2.7	2.72
12	+ 1%	- 0.5%	+ 1%	+ 1%	+ 1%	- 0.5%	- 0.01%	- 0.01%	+ 0.05%	- 0.01%	- 0.01%	2.5	2.4	2.45

a- Molasses, b- Sugarcane, c- Banana pulp, d- Sapota,

e- Black gram, f- Soyabeans

g- Magnesium chloride, h- Manganese chloride, i- Magnesium sulphate
j- Manganese sulphate k- Dipotassium hydrogen phosphate
Note - Upper limit - (+), Lower limit - (-)

Table-2: Regression coefficient and t-values calculated from dextran production obtained using combination of different nutrients in second step screening using Plackett-Burman design

S.No	Ingredient	Reg.Coeff	t-value
1	Intercept	2.5188	235.0586
2	Molasses	0.0313	2.9164
3	Sugarcane	0.0898	8.3602*
4	Banana	0.0854	7.9714
5	Sapota	0.0646	6.0271
6	Black gram	0.0354	3.3052*
7	Soyabean	0.0312	2.9164

8	Magnesium chloride	0.0396	3.6941*
9	Manganese chloride	-0.0104	-0.9721
10	Magnesium sulphate	0.0104	0.9721*
11	Manganese sulphate	-0.0604	-5.6383
12	Dipotassium hydrogen phosphate	0.0646	6.0271*

Note- * Indicate significant nutrient

4.0 Conclusion

A potential dextran producer was isolated and identified by microscopic, cultural, biochemical and by 16s-rRNA sequencing as *Weissella confusa*. Carbon source like sugarcane juice, nitrogen source like black gram, micronutrients like $MgCl_2$ and $MgSO_4$ and macronutrient like K_2HPO_4 influenced dextran production as indicated by results. There was a 2-3 fold increase in dextran production and it is of low molecular weight. This is significant commercially as not only the production increased but also the optimized medium had cheaper nutrient sources. Thus the present optimized medium could be used for commercial production of low molecular weight dextran which can have application in food industry and also for clinical purpose.

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