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Effect of Process Parameters on Dextran Production by *Weissella Confusa*

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Abstract: ***Dextran a bacterial polysaccharide and a polymer of glucose is produced by different microorganisms like Leuconostoc mesenteroides, Lactobacillus sps, Streptococcus mutants Weissella confusa etc. It has a wide range of applications in the food, pharmaceuticals 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 this study, attempts were made to investigate the influence of various parameters such as pH, temperature, incubation period, agitation speed, aeration and inoculum size for dextran production by Weissella confusa in 4% sucrose medium. The optimum pH, temperature, incubation period, agitation, aeration and inoculum size for dextran production were found to be 6.5, 35^{0}C, 48 hours, 150 rpm, 1.5: 5 (medium to head space ratio), and 5% respectively. Dextran was recovered from broth by alcohol precipitation and this purified dextran was subjected to HPLC analysis and the results indicated that it was a low molecular weight dextran. Thus the studies indicates that low molecular weight dextran producer identified genetically as Weissella confusa can be used for commercial production of medically and industrially important dextran.***
Keywords: Dextran, pH, temperature, inoculum size, Weissella confusa.

I. INTRODUCTION

Dextran is a bacterial exopolysaccharide [1], 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 [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 may be influenced by composition of media and various process parameters [11]. The environmental factors or process parameters significantly influence product production in any commercial production. The present study was aimed to optimize the various environmental factors influencing the maximum production of dextran by *Weissella confusa*. The parameters studied were pH, temperature, incubation period, agitation speed, aeration and inoculum size.

II. MATERIALS AND METHODS

A. *ISOLATION OF DEXTRAN PRODUCER WEISSELLA CONFUSA*

Bacterial culture under study was isolated from idli batter/black gram soaked water, using enrichment culture technique. 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.

B. *FERMENTATION*

Both 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

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optimum factor. The flasks were incubated for 48 hours broth samples collected from different flasks and tested for dextran production 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.

C. EFFECT OF VARIOUS PARAMETERS ON DEXTRAN PRODUCTION

The process parameters like pH, temperature, incubation period, agitation and aeration were carried out over a range. The range of pH was from 5.5 – 7.0 which was adjusted with 1N KOH and 1N H_2SO4. Care was taken to adjust the desired pH both in pre sterilized and post sterilized medium. Temperature was tested from 4^0c – 40^0c. Incubation period was tested from 24 – 96 hours. Agitation varied from 100 – 200 with a difference of 50 rpm (revolution per minute). Aeration, the most important factor was studied taking volume of medium to air ratio in a range varying from 1:5 to 3:5. Inoculum size was tested from 1% to 10% while 1% inoculum had 10^6 CFU/ml. While studying one parameter's variation the others were all kept at a constant level. The flasks were incubated for 96 hours. The fermented broth was sampled assayed every 24 hours until the maximum dextran production [5].

D. RECOVERY

Dextran was recovered from broth by alcohol precipitation, dried under vacuum over $CaCl_2$ at 30^0C and weighed [14]. Product was assayed and found to contain glucose polymer (Dextran) by using anthrone method. 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].

III. RESULTS

The best potential of a strain is realized only under the best regulated process regime. The dextran production was optimized under different environmental conditions. Influence of environmental factors on dextran production by the newly isolated *Weissella confuse* was studied taking into consideration the effect of pH, temperature, incubation period, agitation speed, aeration and inoculum size over a range. A gradual increase in dextran production was observed with an increase in the factor under study and decreased when the factor was increased beyond the optimum level. The optimum pH of the culture medium obtained was pH 6.5 with production yield of 1.2 g/100ml (Fig-1). The optimum incubation temperature was observed at 48 hrs with peak dextran yield of 1.3g/100ml (Fig-2). The Optimum agitation speed was 150 rpm with a maximum dextran yield of 1.3g/100ml (Fig-3). The optimized aeration ratio was 1.5: 5 medium to air ratio with a maximum yield of 1.2g/100ml (Fig-4). The optimum inoculum size which showed peak production was 5% and dextran yield was 1.3g/100ml (Fig-5).

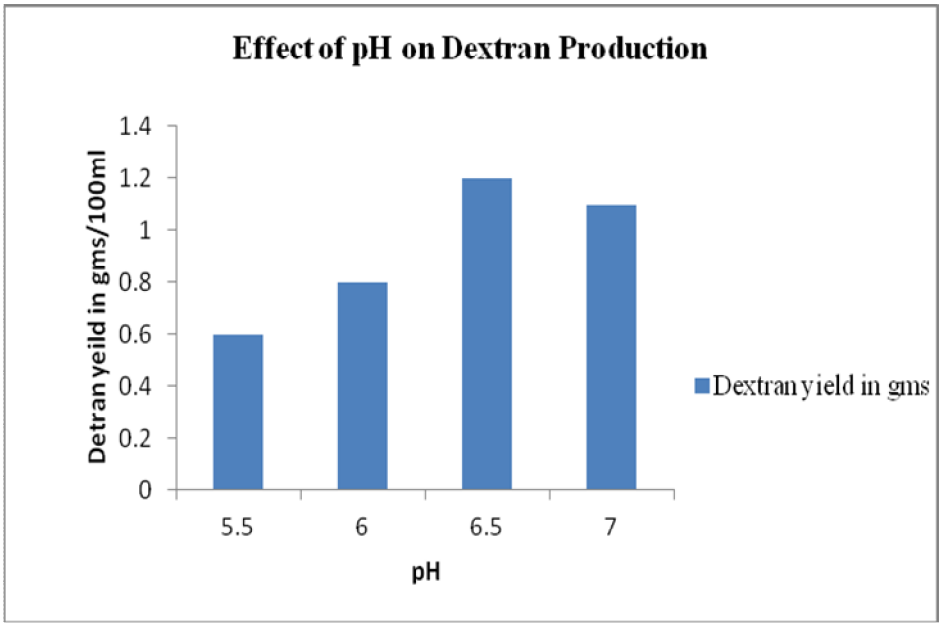

Fig-1: Effect of pH on dextran production by *Weissella confusa*

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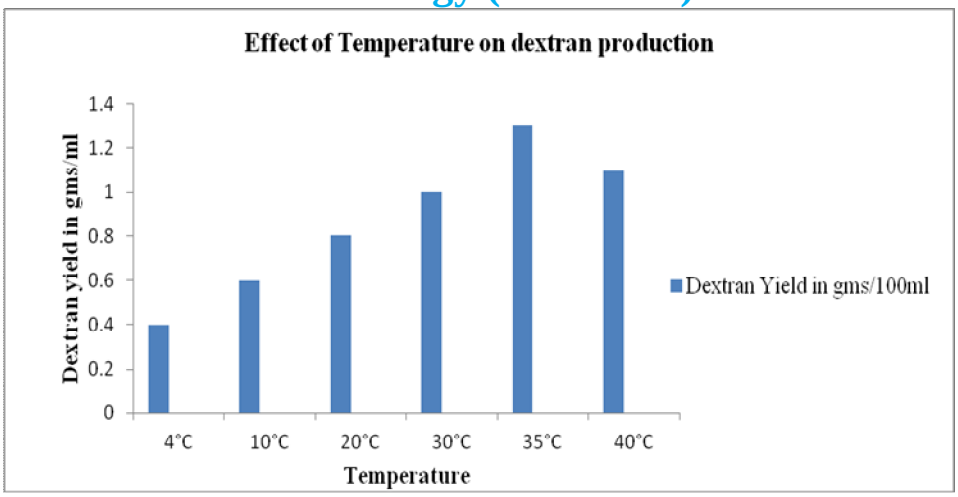

Fig-2: Effect of temperature on dextran production by *Weissella confusa*

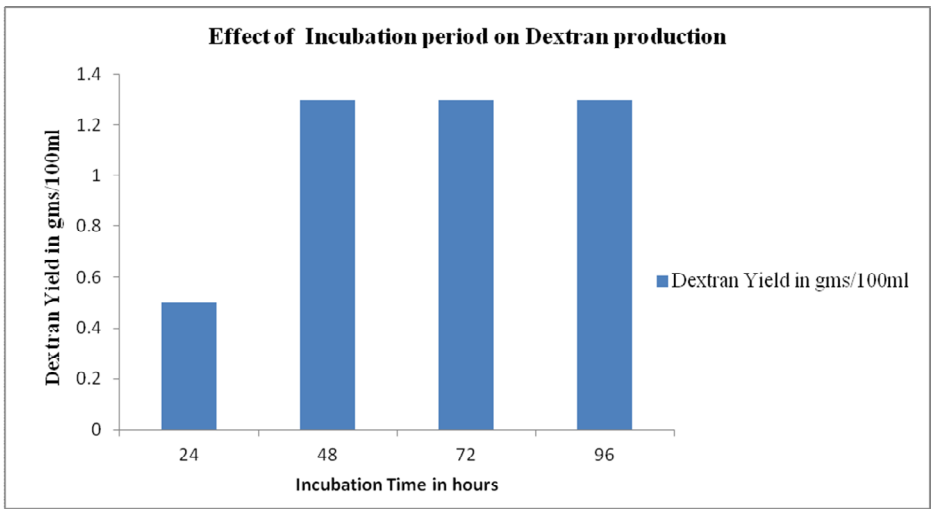

Fig-3: Effect of incubation period on dextran production by *Weissella confusa*

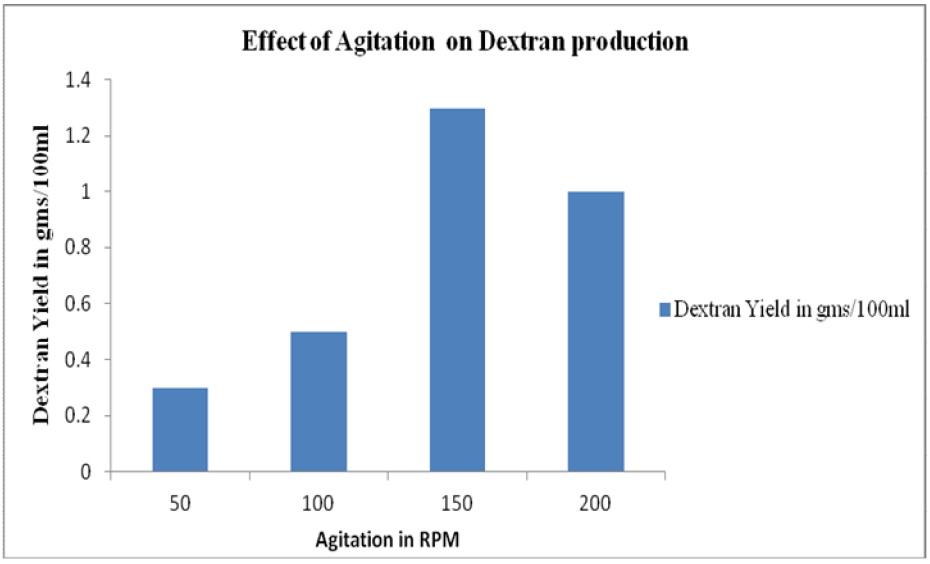

Fig-4: Effect of agitation rate on dextran yield by *Weissella confusa*

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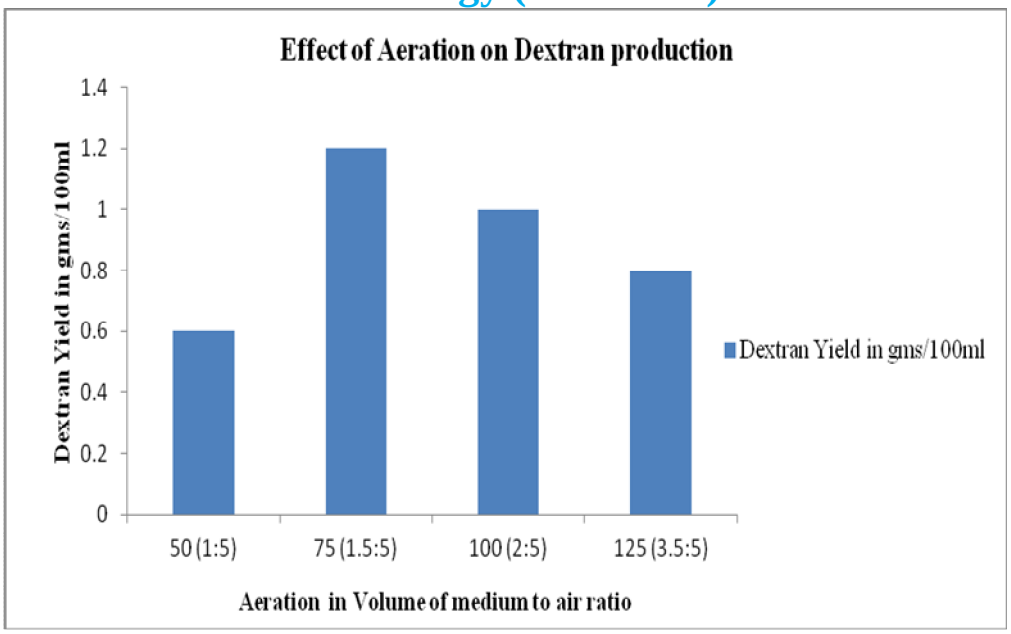

Fig-5: Effect of aeration on dextran production by *Weissella confusa*

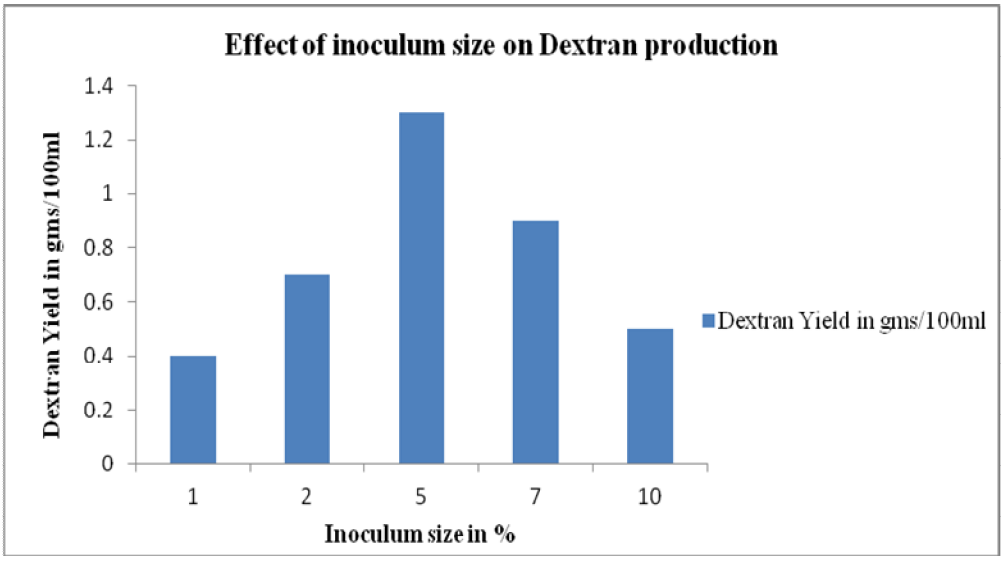

Fig-6: Effect of inoculum size on dextran production by *Weissella confusa*

IV. CONCLUSIONS

A potential dextran producer was isolated and identified by microscopic, cultural, biochemical and by 16s-rRNA sequencing as *Weissella confusa*. The isolate produced low molecular dextran in sucrose medium under optimal environmental conditions like pH of 6.5, temperature of incubation 35^0C, incubation period of 48 hours, an agitation of 150 RPM, aeration of 1.5:5 ratio and inoculum size of 5%. These optimized environmental factors could be used for commercial production of low molecular weight dextran that can be used for clinical purpose.

V. ACKNOWLEDGMENT

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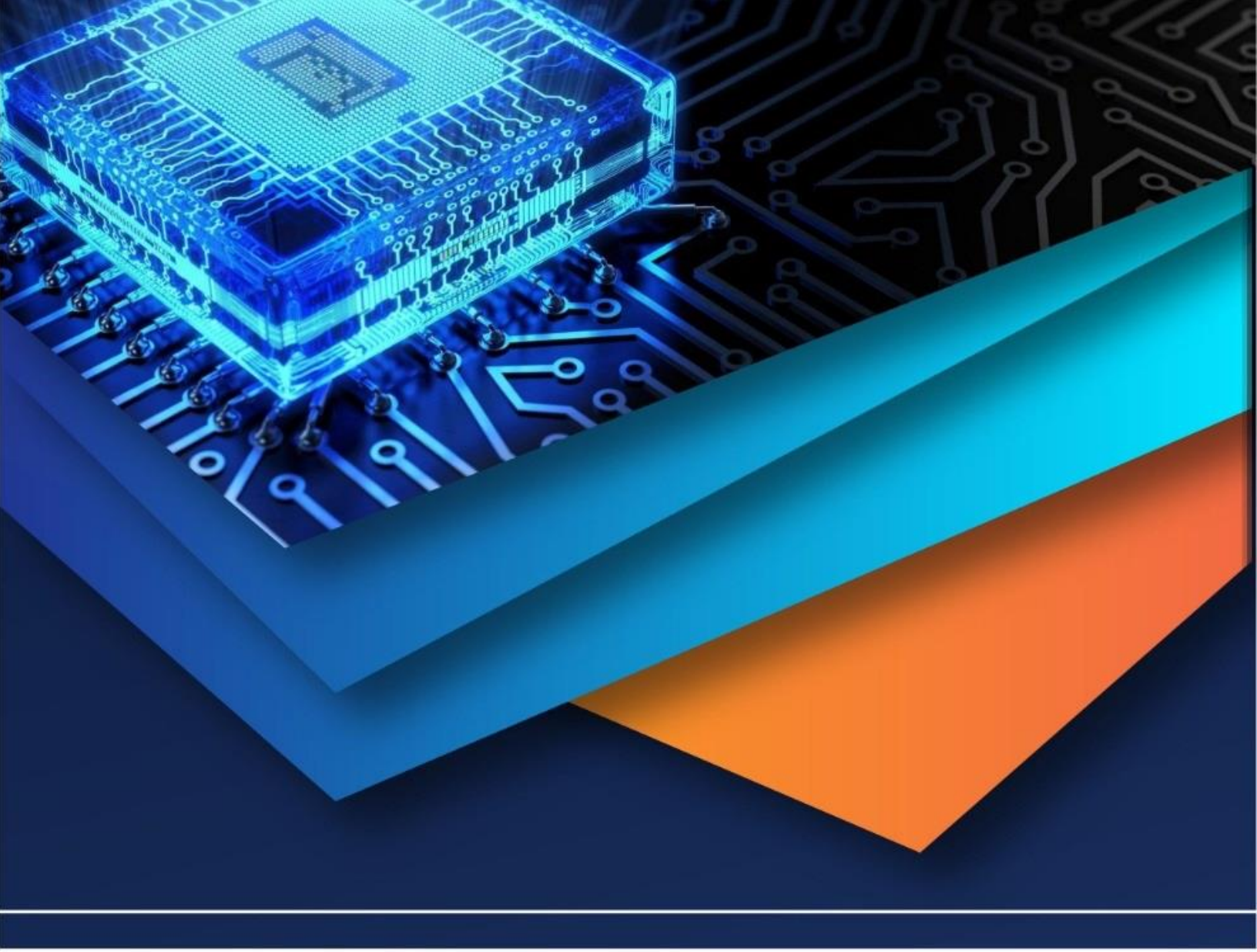

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