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Production of Dextran from *Leuconostoc mesenteroid*

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A B S T R A C T

Dextran can be produce by a wide range of gram positive and gram negative bacteria e.g. *Leuconostoc mesenteriods* and *Streptococcus mutant*. In the present study dextran was produced from *Leuconostoc mesenteriods*. *L. mesenteriods* was isolated from idli batter identified by different biochemical parameters. Sucrose media was used for dextran production. Dextran was also produced using beet juice and compared with sucrose media. Optimum dextran production was considered according to the optimum pH, temperature and substrate concentration. The optimum pH ranged between 6.5 and 7.0. Maximum dextran yield was obtained when 10% sucrose concentration was used. Optimum temperature was observed 26^{o}C. Production of dextran was more in natural medium (beet juice) than synthetic medium (Sucrose broth). Thus, dextran production on the laboratory scale has successfully done.

Introduction

Dextran is a group of high molecular mass of polysaccharide of D-glucose polymer in which the glucose units are joined chiefly through α-1,6 glycosidic linkages, other through α -1,4 or α – 1,3 glycosidic linkage s and its ratio varies depending on the strain.

Dextran is a bacterial polysaccharide, which is commercially available, and it is used as drugs, especially as blood plasma volume expander. Dextran has found industrial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer (Bhavani and Nisha, 2010). Cross-linked dextran is known as Sephadex, which is widely used for the separation and purification of protein (Pirouzmand *et al.*, 2017). In food industry dextran is currently used as thickener for jam and ice cream. It prevents crystallization of sugar, improves moisture retention, and maintains flavour and appearance of various food items (Ahmad *et al.*, 2015).

Leuconostoc mesenteroides, a hetero-fermentative lactic acid bacterium is particularly well adapted to sugary niches and consequently possesses a wide spectrum of biocatalytic properties useful in carbohydrate modifications.

Leuconostoc mesenteroides and its enzymes can be used to produce carbohydrates and derivatives as diverse as dextran (biopolymer), fructose, mannitol (polyol), leucrose (noncariogenic disaccharide), glucose-1-phosphate, and many others (Kim and Robyt, 1998).

In the present study, dextran was isolated by using *Leuconostoc mesenteroides* strain. Natural (beet juice) and synthetic media was used for the production of dextran. Dextran yield was compared from both medium.

Materials and Methods

Isolation of *Leuconostoc mesenteriods*

Bacterial culture was isolated from idli batter using enrichment media technique (Mukherjee *et al.*, 1965). Sample was inoculated in a broth medium containing sucrose, tryptone, yeast extract and K_2HPO_4. pH was adjusted at 7.0 and autoclaved. Sodium azide (0.005%) was added aseptically to the medium for selective isolation of dextran producing *Leuconostocs* species.

Inoculated broth was incubated for 24 h at 25^{o}C. After the incubation, the microorganism in this medium was plate cultured on MRS agar, which contained tween 80 for increased growth of *Leuconostoc* by providing oleic acid. The plates were incubated at 37 ^{o}C for 24-28 h. After that the microorganism were transferred to Macconkey agar for the cultural characterization of the *Leuconostoc mesenteriods*.

Leuconostoc mesenteriods isolates and its inoculums

Defined medium was used for growth of *Leuconostoc mesenteriods* isolated and inoculum was prepared. 10ml of sterile sucrose broth was inoculated by loopful of growing culture of *L. mesenteriods*. Culture tube was incubated at 26^{o}C for 24 h.

Production of dextran

After 24 h incubation, 10 ml of inoculums was transferred into 90 ml of sterile broth in aseptic condition. Then, again incubated at 26^{o}C for 24-30 h for dextran production. Isolated colonies of *Leuconostoc mesenteriods* maintained on MRS medium. Sucrose medium was used for L. mesenteriods for a dextran production (Subathra Devi *et al.*, 2014). It was Incubated at 25-30^{o}C for 24-48 h. After that equal volume of Chilled ethanol was added in the broth. The liquid was centrifuged at 10000 g for 15 min. Precipitate was collected and purified. Dried powder was dextran. Various parameters checked during production. The pH of the broth was checked and noted as observation during production. The effect of different concentration of sucrose was studied in range from 5% to 20%. The change is substrate concentration and production were noted as observation. Dextran production was determined at different temperature from 26^{o}C -37^{o}C.

Production of dextran from beet juice

Preparation of juice

The sample of beet (red) washed with water and made a juice in juicer. The raw beet juice was mixed with calcium hydroxide for carbonation. Calcium carbonate was produced as precipitate at the bottom. Juice was filtered and liquid was used for dextran production.

Dextran production

Loopful of *Leuconostoc mesenteriods* culture was inoculated in 10 ml of beet juice. The tube was incubated at 26^{o}C for 24 h. After that equal volume of Chilled ethanol was added in the broth. The liquid was centrifuged at 10000 g for 15 min. Precipitated dextran was collected and purified. Purified dextran was dried at 30˚C.

Results and Discussion

Leuconostoc mesenteriods is a well known dextran producing bacteria (Munir *et al.*, 2019). *Leuconostoc mesenteriods* was isolated from idli batter, in this experiment. Medium composition is of critical importance for maximum production of dextran. In the present study, several parameters considered such as pH, substrate utilization and temperature.

Effect of pH has been studied which that the pH increases production of dextran decreases. The optimum pH ranged between 6.5 - 7.0. Santos have studied the effect of pH on dextran sucrose activity and dextran production by *L. mesenteriods* and found that dextran production was obtained at pH 5.5 (Santos *et al.,* 2000). During this experiment, the pH increase, the production of dextran decreases.

The effect of different concentration of sucrose was studied from 5% to 20%. It was also observed that dextran production was affect by the different concentration of sucrose. Maximum dextran yield was obtained when 10% sucrose concentration was used in the fermentation medium. Present study indicates, concentration of substrate (sucrose) decreases as the cell mass increases. Maximum dextarn yield was obtained when 10% sucrose concentration was used in the fermentation medium. Higher concentration of sucrose in the fermentation medium had an inhibitory effect, known as substrate inhibitory effect, which decreased dextran production

Dextran production was determined at different temperature from 26˚c to 37˚c, and the maximum dextran production by *Leuconostoc mesenteriods* was achieved at 26˚c. as the temperature increased or decreased in dextran production was noticed. Production of dextran was more in natural medium (beet juice) than synthetic mediu (sucrose broth) at the same temperature and other conditions, which was used for synthetic medium.

The optimum temperature required for production ranges between 25˚C to 30˚C, the range of temperature during my study was about 26˚C to 37˚C for 24 h. When the incubation temperature was high, the temperature did not favour cell multiplication, and lead towards less enzyme and dextran production as compared to optimum temperature i.e.26˚C.

Production of dextran was more in natural medium (Beet juice) than synthetic medium (Sucrose broth) at the same temperature and other conditions, which was used for synthetic medium.

Confirmation of dextran was done by solubility. Dextran is readily soluble in water i.e. in distilled water form a clear, stable solution. It showed that, the production of dextran on different media containing different salt composition. Dextran was also soluble in ethylene glycol. Dextran was insoluble in monohydric alcohol, for e.g. methanol, ethanol. Thus all this parameters significantly affect the production of dextran (Table 1–5).

Table.1 Isolation and characterization of *Leuconostoc mesenteriods*

Sr,no	Cultural and biochemical test	Observation
1	Gram staining	-gram positive -coccobacili with rounded ends.
2	Motility	-Motile
3	Growth an MRS agar at 37˚C	-pale yellow colourer, smooth slimy entire marginated convex colonies.
4	On MacConkey agar	-Lactose fermenting pink coloured colonies.
5	Biochemical test:- Indole MR VP Citrate	 -ve -ve +ve -ve
6	Carbohydrate fermentation Glucose Lactose Mannitol	(acid and gas) +ve +ve -ve

Table.2 Effect of pH on dextran production

Sr. no	pH of medium	Dextran yield in g
1	5.5	0.54
2	7.0	1.22
3	8.5	0.82

Table.3 Effect of substrate concentration

Sr. no	Sucrose concentration in medium (%)	Dextran yield in g
1	5	0.85
2	10	0.96
3	15	0.70
4	20	0.55

Table.4 Effect of temperature on dextran production

Sr. no	Temperature (^{o}C)	Dextran yield in gram (g)
1	26	1.20
2	30	0.92
3	37	0.76

Table.5 Production of dextran from beet juice:

Sr. no	Beet juice	Dextran yield in (g)
1	Pure juice	2.94
2	Diluted form of juice	2.20

It is concluded in this experiment, substrate concentration that is sucrose indicated proper growth of organism in the fermentation medium. If the substrate concentration is maintained properly, high production of dextran can be achieved at optimum pH and temperature. The potency of produced dextran compared with standard dextran and its solubility in distilled water, it can be concluded that the precipitation and purification of dextran on the laboratory scale successfully done.

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