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PRODUCTION OF TYPE OF GLUCOSYL TRANSFERASE-DEXTRANSUCRASE AND FRUCTOSE BY USING *LEUCONOSTOC MESENTEROIDES*

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Abstract:-

Extracellular dextransucrase is one of the glucosyl transferase produced by Lactic acid bacteria belonging to genera Leuconostoc,Streptococcus,Lactobacillus. When the strain of Lecunostoc mesenteroides is grown on sucrose rich media the production of extracellular enzyme dextransucrase is induced. Sucrose is the only known substrate able to induce this enzyme production.The benefit of this research work is to produce dextransucrase which is used to synthesize glucooligosaccharides, Dextran as well as release of high concentration of fructose helpful for production of low calorie sugar.

Key words:- *Dextran sucrase, glucooligosaccharides, Leuconostoc mesenteroides*

Introduction:-

Extracellular glucosyltransferase are mostly produced by Lactic acid bacteria belonging to genera *Leuconostoc,Streptococcus*. Glucosyltransferase can be classified on the basis of structure of glucan they synthesize, e.g. dextransurase which synthesize dextran. When the strain of *Leuconostoc mesenteroides* grown in sucrose rich media the production of an extracellular enzyme dextransucrase is induced. Sucrose is the only substrate able of inducing this enzyme production. Dextransucrase uses sucrose as a substrate to produce dextran and fructose as follows.

$$n(C_{12}H_{12}O_{11}) \xrightarrow{\text{Enzyme-dextransucrase}} (C_6H_{10}O_5)n + C_6H_{12}O_6$$

Sucrose — Dextran — Fructose

The optimum temperature for production of dextransucrase is 37°. C and the optimum pH is 5.0 at which enzyme is more active and transform sucrose to dextran and fructose. The objective of this work is to determine the optimum pH & temp, for the production of dextransucrase and fructose.

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terials and Methods:- i) Strain and culture media:-

The strain used in this work is *Lecunostoc mesenteroides* NCIM 2073 obtained from National laboratory Pune .One litre of standard medium consists of 20gm of sucrose, 10gm of meat extract , peptone, 5.0gm of yeast extract, 1ml of tween 80, 2.0 gm of dipotassium hydrogen phosphate and triammonium citrate, 5.0 gm of sodium acetate, 200 gm of magnesium sulphate , 50 mg of manganese sulphate , pH was adjusted to 6.0 (DeMan , Rogaso and Sharpe's medium-MRS) and phosphate with additional salt sterilized separately. The culture medium broth was inoculated with 24 hrs old inoculum and incubated on a rotator shaker at 37°C . The culture was stopped at the end of the growth when the pH reached value 5.0 – 5.6. After centrifugation the supernatant is used as a enzyme source which is further purified by Ammonium sulphate fractionation.

ii) Dextransucrase assay:-

Dextransucrase activity was assayed by measuring the amount of sucrose consumed. One dextransucrase unit was defined as the amount of enzyme that converts 2 mg of sucrose under ideal reaction conditions at 37°C temperature , pH = 5.0. The liquid supernatant (0.5 ml) was mixed with 1 ml of 20% sucrose solution with prefixed pH 5.0 and then incubated at 37°C for 10 min . The fructose produced was confirmed quantitatively by resorcinol method . Experimental product formation for /at various operating conditions was studied . Enzyme activity was carried out for sucrose concentration in the range of 40 to 400 mg ,temperature of 0°C,20°C, 30°C,37°C, 40°C, 50°C, 80°C, 100°C,pH range 3.6 , 4.0 , 4.6 , 4.8 ,5.0 , 5.6 . The optimum temperature was found to be 37°C and pH 5.0 at which there is more enzyme activity with more concentration of fructose produced .

iii) Effect of temperature :-

Generally the temperature used in the enzyme activity is from 0° to 100°C. The enzyme activity incrases with increase in temperature and highest at optimum temperature , decreases with further increase in temperature. High concentration of fructose was observed at 37°C .

Temperature	Fructose (O.D by resorcinol method.)
0	2.69
20	2.72
30	2.78
37	2.80
40	2.75
50	2.65
80	2.58
100	2.53

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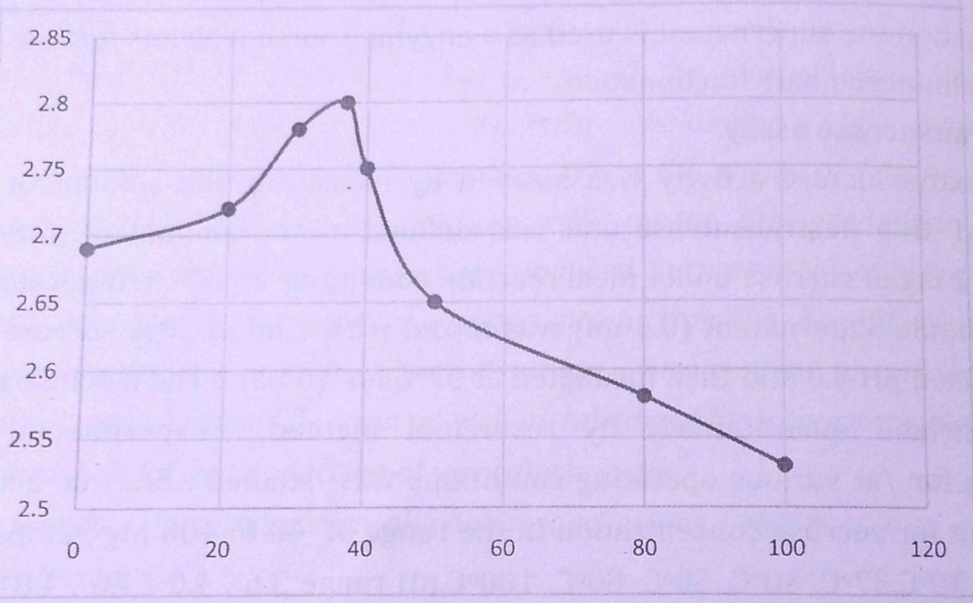
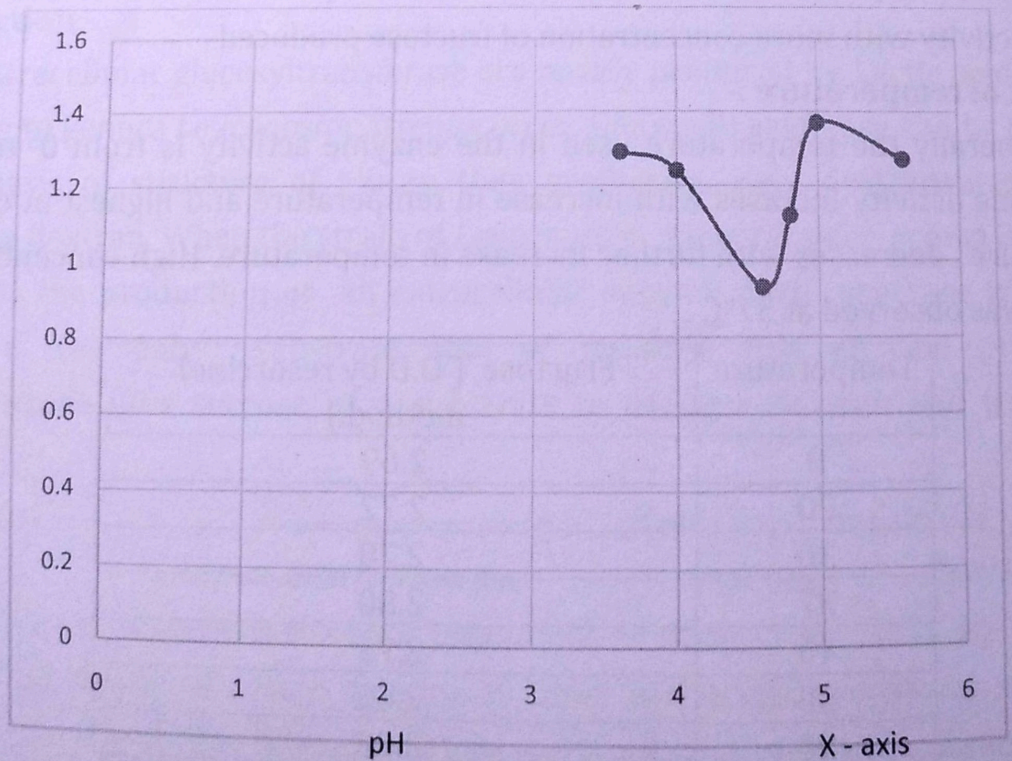
iv)Effect of pH:- The enzyme activity increases with increase in pH and optimum at 5.0 beyond this there is decrease in enzyme activity .

pH	Fructose (O.D by resorcinol method.)
3.6	1.29
4.0	1.24.
4.6	0.93
4.8	1.12
5.0	1.37
5.6	1.27



Temperature optima of Dextransucrase optimum is 37.C



pH Optima of Dextransucrase the optimum pH-5.0

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v)Effect of substrate concentration on enzyme activity:- Higher concentration of sucrose produces more fructose and Further increase in substrate conc. there is no effect on enzyme activity because saturation takes place and constant readings are obtained. which obeys the Hyperbolic curve of Enzyme catalyzed reactions.

Sucrose	mg of sucrose	Buffer (in ml)	Enzyme (ml)		Resorcinol reagent (in ml)	5:1 HCl (in ml)		O.D at 530 nm
0.2	40	1.0	0.5	Incubate	1	5	Keep	0.48
0.4	80	1.0	0.5	the	1	5	The	0.74
0.6	120	1.0	0.5	set	1	5	set	0.96
0.8	160	1.0	0.5	at 37.C	1	5	in	0.98
1.0	200	1.0	0.5	For	1	5	Boiling	1.20
1.2	240	1.0	0.5	10 min	1	5		1.25
1.4	280	1.0	0.5		1	5	Water bath	1.30
1.6	320	1.0	0.5		1	5	for 10	1.34
1.8	360	1.0	0.5		1	5	Min	1.35
2.0	400	1.0	0.5		1	5		1.35

Result & Conclusion:-

Fructose released after enzyme activity can be assayed by reacting the fructose present in the reaction mixture after enzyme action on Sucrose . The released fructose react with Resorcinol to furfural derivative a brown color complex the intensity of color is directly proportional to conc. Of fructose present. O.D.is measured at 530 nm.

We have described the production and purification of extracellular dextransucrase enzyme from *Lecunostoc mesenteroides* . as a result of the preformed study active producer of dextransucrase were found. The enzyme activity is higher at pH 5.0 . The enzyme shows highest activity at temp 37.C.

Applications:-

1. The strain *Lecunostoc mesenteroides* is used for synthesis of dextran which can be used for chromatographic material, blood volume expander, in food industry.
2. The enzyme dextransucrase from strain *Lecunostoc mesenteroides* is used for synthesis of glucooligosaccharides presently marketed for human nutritional and dermocosmetic applications.

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3. The enzyme dextransurase from strain *Lecunostoc mesenteroides* is used for production of fructose used in food industry as a low calorie sugar.

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