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IMPACT OF MEDIA ON ISOLATION OF DEXTRANASE PRODUCING FUNGAL STRAINS

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ABSTRACT

Six strains of fungi for dextranase activity have been isolated on malt extract agar medium, which contained dextran as sole carbon source. Out of six strains, Strain-1 and Strain-6 could produce suitable dextranase activity and based on higher activity Strain-1 was selected for further studies.

Keywords : *Malt extract Agar, Fungal isolation, Dextranase activity*

INTRODUCTION

It is well known that the presence of *Leuconostoc* and other bacteria with cane juice in sugar factories cause loss of sucrose and result in the formation of jelly like material called dextran a bacterial polysaccharide which, is polymer of glucose. The polysaccharide interferes in sugar manufacturing process in addition to loss of sucrose resulting in heavy loss to sugar factories (Priyanka and Santosh, 2009 & 2010).

Thus for improving factory performance, removal of dextran is essential. Generally dextran levels present in mixed juice vary from 300 to 600 mg/l. In the removal of this polysaccharide material, application of enzyme dextranase produced by bacteria, moulds like *Penicillium* and *Aspergillus sp.,* and yeast is of great importance.

Dextranase [(1-6)-α-D-glucan6-glucanohydrolases or glucanases (E.C. 3.2.1.11)] are the enzyme that specifically hydrolyze the α-(1-6) linkages in dextran. Since many dextrans contain a relatively high concentration of secondary linkages, other than α-(1-6), and enzyme that can break (1-2), (1-3) and (1-4) linkages in dextran, is also included together with true dextranase. The hydrolysis products of dextran by dextranase are glucose (with exodextranase), isomaltose and isomalto-oligosaccahrides. Therefore, these are commonly called by common name as glucanases (E.C. 3.2.1.11). The prefixes show the nature of linkages attacked. For example, enzyme which is specific for

1-6 linkages is known as (1-6)-α-D-glucanase or (1-6)-α-D-glucanohydrolase. When enzyme is specific for other linkage like 1-3 then it is known as (1-3)-α-D-glucanase.

Enzymatic method does not interfere with the sugar manufacturing process (Tilbury 1974). This enzymatic method is quick, easy and can be practised at commercial scale. Tilbury (1971) reported removal of about 68% of dextran in 20 minutes. The enzyme can be used without changing the pH of juice and at a temperature of 50°C. It also helps in treating deteriorated juices. The use of dextranase in sugar mills not only improves the factory performance but also improves the sugar quality (Fulcher, 1974). The excessive elongation of the crystal and viscosity of syrups and molasses may be reduced by the enzymatic decomposition of dextran (Hidi, 1975).

The present investigation deals with the isolation and purification of fungal strain which produce enzyme dextranase.

MATERIALS AND METHODS

Isolation of fungal strains

Isolation of fungal strains which may produce dextranase was done on Malt extract Agar medium having composition (g/l) Dextran 10, Malt extract 20, Peptone 15, Agar 20 and pH-7.0. The enzyme dextranase was obtained from the medium after the removal of fungal mycelium.

Assay of Dextranase Activity

Mycelium free culture filtrate was used as enzyme (extracellular) source after suitable dilution (1:50) with 0.1M acetate buffer pH 5.6. Mycelium was ultrasonicated in a vibronics (v_2) at 20 kc/sec. for 10 minutes at 4°C and centrifuged at 3000rpm (cold). Supernatant was used as (intracellular) enzyme.

Dextranase activity was assayed by the method of Koseric et al. (1973). Reaction mixture containing 2 ml of 2.5 % dextran in acetate buffer (0.1M, pH 5.6) and 1ml of enzyme in a total volume of 3 ml was incubated at 40°C for 20 minutes. Reaction was stopped by adding 3ml of DNS(di nitro salicylic acid) reagent and colours were read in Shimadzu U.V visible Spectrophotometer.

Dextranase Unit

One unit of dextranase is defined as the amount of enzyme, which liberate one μ mole of glucose/ml/minute.

RESULTS AND DISCUSSIONS

The enzyme dextranase (1-6-α-D-glucan6-glucanohydrolases E.C. 3.2.1.11), synthesized by some moulds and bacteria, is of great significance in

the sugar industry as it is capable of degrading the biopolymer dextran formed in the cane juice during its processing for the production of sugar (Madhu et al., 1984).

For the production of enzyme dextranase, fungal strains were isolated on different media viz. Potato Dextrose Agar (PDA), Yeast extract dextrose agar (YEPD agar) and Malt extract agar (MEA). The composition of various media is presented in table-1.

The isolation of dextranase producing fungi was carried out by keeping the plates open for a few days and then replating on fresh media after separating fungal colonies. It is observed from table-2 that fungal colonies grew well on all media used, but best growth was obtained on malt extract agar medium, which contained dextran as sole carbon source. The selected strains onto this medium were screened for production of extracellular dextranase.

Out of ten fungal strains isolated on MEA medium, only six strains showed extracellular dextranase where little activity of endocellular dextranase was also seen. Based on the activity of dextranase six strains were grow on MEA medium with 1% dextran as substrate by first inoculating the spores in 10 ml of liquid medium in culture tubes to give 10^4/ml spore suspension. The cultures were grown on a wrist shaker for 48 h and then transferred to 100 ml of fresh medium in 250 ml Erlenmeyer flask for 4-5 days at 30 ± 1°C on a rotary shaker. The fermented broth was assayed for dextranase activity and reducing sugars.

The extracellular dextranase & endocellular dextranase activity of six isolated fungal strains is presented in table-3. It is seen that the extracellular enzyme activity of Strain-1, Strain-2, Strain-3, Strain-4, Strain-5 & Strain-6 were (Units ml^{-1}), 35.5, 30.0, 25.2, 25.5, 28.2 &32.5 respectively. The endocellular dextranase activities were (Units ml^{-1}) 14.5, 12.1, 6.5, 4.2, 10.2 and 12.8 respectively. The Strain-1 produce maximum extracellular and endocellular dextranase activity of 35.5 & 14.5 while slightly less activity was observed with Strain-6 which produce an extracellular activity of 32.5, the value with Strain-4 being 25.5. Since Strain-1 produced consistent activity of dextranase, Therefore Strain-1 was selected for further studies.

Carbon source is an important for dextranase production, hence the Strain-1 was grown on various carbon sources to see and their effect on dextranase activity (table-4). It is seen that maximum activity of dextranase was recorded when dextran was used as sole carbon source. Higher molecular weight dextran produced higher activities. The dextran produced by Leuconostac *mesenteroides* was comparatively better substrate than purified preperations of dextran procured from market.

Table-1 : Composition of growth media for isolation of fungal strains

Ingredients	Potato Dextrose Agar	Yeast extract Agar	Malt extract Agar
Malt extract	---	---	2%
Yeast extract	---	1%	---
Dextran	---	---	1%
Peptone	---	1%	1.5%
Dextrose	2%	----	---
Potato	30%	---	---
Agar	2%	2%	2%
pH	Neutral	5.2	7

Table-2 : Charecterstics of fungi on different media

Media	Fungal growth	Dextranase activity (Units ml^{-1})
PDA	Present	Low activity
YEA	Present	Very low activity
MEA	Good growth Present	High activity

Table-3 : Dextranase activity of isolated strains on MEA media

Strains	Extracellular enzyme activity (Units ml^{-1})	Endocellular enzyme activity (Units ml^{-1})
Strain-1	35.5	14.5
Strain-2	30.0	12.1
Strain-3	25.2	6.5
Strain-4	15.5	4.2
Strain-5	28.2	10.2
Strain-6	32.5	12.8

Table-4 : Effect of different carbon sources on the production of dextranase

S.No.	Carbon source	Concentration (%)	Dextranase activity (Units ml^{-1})
1.	Dextran (MW 40×10^6)	1	35.5
2.	Dextran (MW 275000)	1	20.1
3.	Dextran (MW 75000)	1	15.5
4.	Dextran (MW17500)	1	10.1
5.	Cellulose powder	1	0
6.	Starch	1	0
7.	Levan	1	0
8.	D-glucose	1	0
9.	D-fructose	1	0
10.	Sucrose	1	0
11.	Maltose	1	0
12.	Dextran (Leuconostoc)	1	36.5
13.	Sephadex G-50	1	0

It can thus be concluded that the best growth of fungi was obtained on malt extract agar medium, which contained dextran as carbon source. Out of six isolated fungal strains, strain-1 and Strain-6 produced dextranase activity at satisfactory levels and the maximum activity was seen in case of Strain-1 which was chosen for subsequent studies. The maximum activity of dextranase was seen when dextran was used as sole carbon source. Higher molecular weight dextran produced higher activities.

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