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REPORT

Role of metal ions on the catalytic efficiency of dextran hydrolyzing biocatalyst

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Abstract: Hydrothermal spring isolate *Bacillus megaterium* KIBGE-IB31was utilized to produce dextranase. Enzyme was partially purified up to 11.8 fold after dialysis. Different metals ions were tested to explore their behavior with dextranase. It was noticed that cobalt (Co^{+2}), copper (Cu^{+2}), magnesium (Mg^{+2}), manganese (Mn^{+2}), nickle (Ni^{+2}) and zinc (Zn^{+2}) act as activator whilst potassium (K^{+}), sodium (Na^{+}), barium (Ba^{+2}), calcium (Ca^{+2}), mercury (Hg^{+2}), vanadium (V^{+2}), aluminum (Al^{+3}) and ferric (Fe^{+3}) ions display inhibitory action.

Keywords: Dextranase, metal ions, bacillus megaterium, dextran, fermentation.

INTRODUCTION

Dextranase (1,6-α-D-glucan-6-glucanohydrolase, EC 3.2.1.11) hydrolyze α-(1→6)-D-glycoside linkages in dextran (Khalikova *et al.,* 2005). Dextranase not only removes undesirable slime formation, due to dextran, from sugar industry but also release D-glucose and shorter oligosaccharides that are utilized as valuable prebiotic (Lee *et al.,* 2006; Chen *et al.,* 2008). It is also responsible for the development of dental plaque (Kim *et al.,* 2002). Conventionally, dextran is hydrolyzed by chemical treatment, but enzymatic degradation is more efficient and economically feasible. Commercially, filamentous fungi constitute the producer of dextranase (Thitaram *et al.,* 2005) however, prolong incubation period and mycotoxin production by fungal strains limits their application and open the door for exploration of another ideal aspirant. Therefore, in the current study a thermophilic GRAS microbe *Bacillus megaterium* KIBGE-IB31 was utilized for dextranase production and partially purified enzyme was characterized for industrial application.

MATERIALS AND METHODS

Dextranase production and purification

Dextranase was produced from a hydrothermal spring isolate *Bacillus megaterium* KIBGE-IB31 [GenBank: KF241867] by submerged fermentation at 60°C for 24 hours using medium comprising of gL^{-1}: Dextran, 15.0; yeast extract, 1.0; tryptone, 10.0; sodium chloride, 3.0; magnesium sulphate, 0.2 and potassium di hydrogen phosphate, 1.0. Dextranase produced extracellularly in the medium was separated by centrifugation at 40,000 × *g* for 15 minutes at 4°C. The enzyme was precipitated by ammonium sulphate salt (40 %) and the precipitates were solubilized in sodium phosphate buffer (0.1M) of pH 7.0. The soluble precipitates were desalted by PD-10 desalting column (GE Healthcare UK Ltd. Little Chalfont, Buckinghamshire, UK). The resulting partially purified dextranase was then utilized for further studies.

Dextranase assay and total protein

Dextranase activity was estimated by incubating enzyme with 30g L^{-1} dextran (10 kDa) prepared in 0.1M sodium phosphate buffer (pH 7.0) at 50°C for 5.0 minutes. Dextranase activity was inhibited by sodium hydroxide and released reducing sugar was quantified by Nelson-Somogyi method (Nelson, 1944; Somogyi, 1937) using maltotriose as a standard. Enzyme unit is defined as amount of dextranase required to produce 1.0µmol of maltotriose per minute by hydrolyzing dextran under standard assay conditions.

Protein concentration was evaluated by Lowry's method (Lowry *et al.,* 1951) using bovine serum albumin as a standard.

Influence of metal ions

The role of different mono, di and trivalent metal ions on the catalytic efficiency of dextranase was investigated. For this purpose, enzyme solution was mixed with metal ions solutions prepared in different concentration (1.0mM, 5.0mM and 10.0mM) in the ratio of 1:1. Dextranase diluted with buffer in 1:1 ratio was used as control. Metal ions solutions were prepare using their Chloride salts listed in table 1. The mixture was placed at 37°C for 120 minutes. After that sample was taken from the mixture and subjected to estimate dextranase activity. The dextranase activity of all sample were calculated and compared with control.

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Table 1: List of metal ions used in the study

Monovalent ions	Divalent ions	Trivalent ions
Cesium (Cs^{+})	Cobalt (Co^{+2})	Ferric (Fe^{+3})
Potassium (K^{+})	Copper(Cu^{+2})	Aluminum (Al^{+3})
Sodium (Na^{+})	Mercury(Hg^{+2})	
	Magnesium (Mg^{+2})	
	Manganese (Mn^{+2})	
	Nickel (Ni^{+2})	
	Vanadium (V^{+2})	

Table 2: Partial Purification of dextranase from *Bacillus megaterium* KIBGE-IB31

Dextranase Fractions	Total Volume (ml)	Total Protein (mg)	Total Activity (U)	Specific Activity (U mg^{-1})	Fold Purification	Percent Recovery
Crude	100.0	290	46468	160.2	1.0	100
Partially Purified Dextranase	5.0	20	26925	1346.25	8.4	57.9
Dialyzed Dextranase	5.0	15	28415	1894.3	11.8	61.1

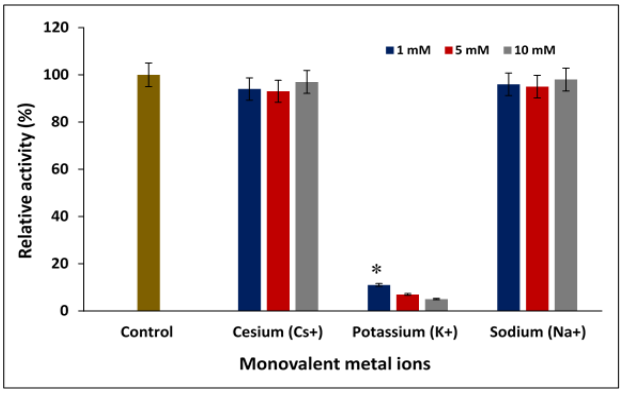

Fig. 1: Influence of monovalent metal ions on the catalytic activity of dextranase (n=3; Standard deviation ± SD: 2%; Tukeys's test, p-value < 0.005).
*shows significant inhibitory effect as compare to control.

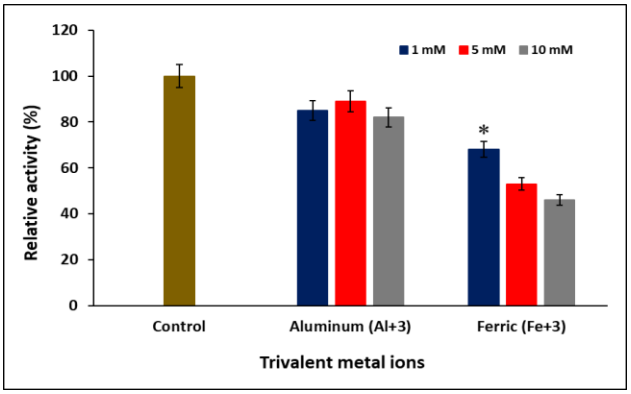

Fig. 3: Influence of trivalent metal ions on the catalytic activity of dextranase (n=3; Standard deviation ± SD: 2%; Tukeys's test, p-value < 0.005).
*shows significant inhibitory effect as compare to control.

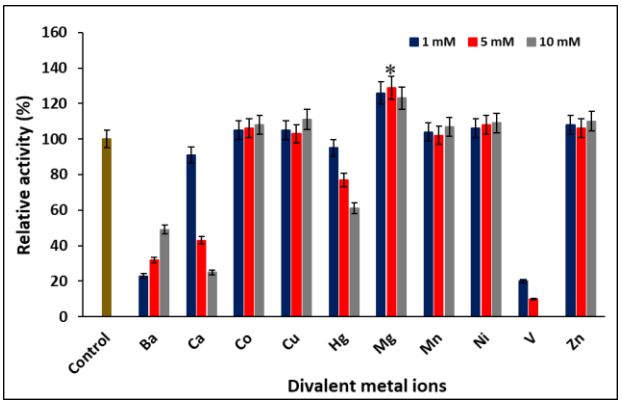

Fig. 2: Influence of divalent metal ions on dextranase activity (n=3; Standard deviation ± SD: 2%; Tukeys's test, p-value <0.005).
*shows significant enhancing effect as compare to control.

STATISTICAL ANALYSIS

All the experiments were performed in triplicate and represented as mean ± standard deviation. One way analysis of variance and Tukey's multiple range tests using SPSS software (version 17.0) were applied for analyzing data.

RESULTS

Dextranase produced from *B. megaterium* KIBGE-IB31 was partially purified and it was noted that fold purification of dextranase was increase to 8.4-fold after precipitation while it was further increased to 11.8-fold after performing dialysis. The dextranase was seemed to recover up to 61% after following above mentioned steps. Brief description of partial purification is presented in table 2.

This partially purified dextranase was then used to investigate metal ions effect on dextranase activity. Monovalent, divalent and trivalent metal ions solutions were prepared in different concentration (1.0, 5.0 and 10 mM) and incubated with dextranase at pH 7.0 for 2.0 hours at 37°C.

By studying the influence of monovalent ions, it was evaluated that potassium highly inhibit dextranase activity even at very low concentration (1.0mM) and only 11% activity was observed at this concentration in comparison with control. Whilst sodium and cesium ions did not exert any significant effect on the activity of dextranase even at 10mM concentration. Sodium as well as cesium ions both showed 98% and 97% activity respectively at 10mM (fig. 1).

Different divalent cations were used to observe their influence on dextranase activity. It was evaluated that the activity of dextranase is enhanced 1.26 times even at low concentration (1.0mM) of magnesium ions. Cobalt, copper, manganese, nickel and zinc were appeared to act as activator of dextranase in contrast Barium, calcium, mercury and vanadium observed to behave as inhibitor ions. Vanadium completely inhibits activity at 10Mm while at 1.0Mm reduced approximately 80% activity as compared with control (fig. 2).

Trivalent metal ions, aluminum and ferric ions showed inhibition of dextranase even at low concentration and it increase with the increase in their concentration. Ferric reduced the activity of dextranase 64% at 10mM concentration while slight inhibition was observed by aluminum cations and dextranase retained 82% activity at the same concentration (fig. 3).

DISCUSSION

Among monovalent cations potassium found to be strong inhibitor of dextranase while on the other side sodium and cesium did not affect the enzyme activity. In another study on dextranase sodium as well as potassium both seemed to be the activator of dextranase (Igarashi *et al.*, 1992).

By investigating the behavior of divalent ions it was noticed that magnesium at very low concentration proficiently enhanced the dextranase activity. The same activation effect of magnesium on dextranase was also observed on dextranase purified from *Hypocrea lixii* F1002 and *Arthrobacter oxydans* KQ11 (Wang *et al.*, 2014; Wu *et al.,* 2011). In contrast, in some studies it was noted that magnesium ions did not exert significant effect on dextranase isolated *Thermoanaerobacter pseudethanolicus* and *Chaetomium erraticum* (Park *et al.*, 2012; Virgen-Ortíz *et al.,* 2015). The slight activating behavior of divalent metal ions was also noticed by cobalt, copper, manganese, nickle and zinc. The same metal ions except copper also displayed activating action on dextranase purified from *Lipomyces starkey* (Chen *et al.,* 2008). However, Barium, calcium, mercury and vanadium showed pronounced inhibition with increase in metal ions concentration.

In this study, aluminum and ferric ions were recognized to be the strong inhibitor of dextranase. These two trivalent ions are also previously reported to be the inhibitor of dextranase by Chen *et al.*, in 2008.
The findings of the current study are in contrast to the outcomes of the other study on dextranase where copper exhibited activation while magnesium and manganese showed inhibitory effect on the activity of dextranase (Bhatia *et al.,* 2016).

From the literature survey it has been assessed that cofactor is not essential for the activity of dextranase however; some divalent metal ions like calcium, cobalt, copper, magnesium and manganese behave as activator in some studies. On the other side, in most of the cases mercury and copper while in some cases zinc and ferric ions considered to be the inhibitor of dextranase (Schomburg and Salzmann, 1991).

CONCLUSIONS

Dextranase from thermophilic *B. megaterium* KIBGE-IB31 was partially purified. Role of different metal ions was evaluated on the catalytic activity of dextranase. It was determined that none of cation was essential for the activity however, some act as activator and other behave as inhibitor on the efficiency of dextranase.

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