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Purification and characterization of invertase from *Aspergillus fumigatus* and *Penicillium brevicompactum*

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ABSTRACT

Invertase was isolated from selected soil micro organism namely *A.fumigatus* and *P. brevicompactum*, the enzyme was purified by ammonium sulfate precipitation and Ion Exchange chromatography. The purification fold was 7.85 for *A.fumigatus* and 6.35 for *P.brevicompactum*. The enzyme showed maximum activity at pH 6 and optimum temperature was 50° C at a substrate concentration of 1.0 gm. The activiy of the enzyme was inhibited by divalent metal ion Zn^{2+} where as activated by Na^{+}. Immobilisation by sodium alginate method increased the stability of the enzyme.

Key words: Invertase, *Aspergillus fumigatus* & *Penicillium brevicompactum.*

INTRODUCTION

Invertases or β d- fructofuranosidase (EC No. 3.2.1.26) are special kind of enzymes that catalyze the hydrolyses of sucrose. The enzyme cleaves α-1-4 glycosidic linkage between α-D-glucose and β-d-fructose molecules of sucrose by hydrolyse producing monosaccharides such as glucose and fructose. β d- fructofuranosidase are extracellular as well as intracellular enzymes (Mahmoud, 2007). Invertase also hydrolyses bête fructans such as raffinose into simple sugars (Baig *et al* 2003). In contrary to most other enzymes, invertase exhibits relatively high activity over a broad range of pH (3.5 – 5.5), with optimum near 4.5. The enzyme activity reaches a maximum at about 55 C (Fontana *et al.* 1992).

Invert syrup production by microbial invertase is not widespread because of ease in chemical hydrolysis and high price of the enzyme. It is also used in the production of calf feed preparation, assimilation of alcohol in fortified wines and in manufactural inverted sugars as food for honeybees. (Chou and Jasovsky, 1993)

In this present study we report the purification and biochemical characterization of thermo stable extracellular invertase produced by filamentous fungi *A.fumigatus* and *P.brevicompactum* by using sugarcane bagasse as substrate. We have studied in detail the effects of various conditions affecting the extracellular invertase activity.

MATERIAL AND METHODS

Isolation and Enumeration of Fungi From Soil

A large number of fungi of different groups are found in soil. They constitute the major place among soil microorganisms .A small amount of soil sample was collected from four corners and centre of the sugarcane field at pollachi by making a 'V' shaped pit.They were mixed to make one lot. 10g of the soil sample was weighed and then the sample was serially diluted with sterile distilled water. 1.0 ml of the diluted soil suspension was transferred aseptically into the PDA Agar plates (Supplemented with aureomycin antibiotic each 30 mg /L). Gently rotate the plate so as to spread the suspensions on the medium. The plates were incubated at 30° C for 4-5 days

Fermentation technique

Production of β–fructofuranosidase was carried out by shake flake technique using 250 ml Erlenmeyer flasks. Fifty ml of fermentation medium was transferred to each Erlenmeyer flasks. The cotton-plugged flasks were autoclaved at 15 lbs/inch^2 pressure for 15 minutes and cooled in room temperature. It was then inoculated with 24 hours old culture of *A.fumigatus* and *P.brevicompactum* and incubated at the optimum temperature 30^{0}C. At the 4th day of incubation the biomass, invertase activity and protein content was estimated. Flasks were then incubated in an orbitol shaker at 30º C temperature. (Montiel-Gonzalez *et al.* 2002)

Purification of Invertase

All steps of enzyme purification were carried out at 4^{o}C. The crude enzyme was purified by the method proposed by Dahot *et al.* 1996 in a stepwise process.

Step I- Ammonium Sulphate precipitation

Solid Ammonium Sulphate was slowly added to crude extract with gentle stirring and the proteins that precipitsted at 30 % saturation was collected by centrifugation for 30 minutes at 15,000rpm. The precipitate was dissolved in 10 mM Tris Hcl buffer and dialysed against distilled water over night.

Step II- Ion Exchange Chromatography

10 ml of pooled enzyme was loaded on to a DEAE cellulose chromatographic column (1cm X 10cm) equilibrated with tris Hcl buffer, 100 mM, pH 7.5. The enzyme was eluted with a linear salt concentration gradient (NaCl 250-1500mM) in the same buffer.10 ml of fractions were collected at the flow rate range of 20ml/hr.

Characterization of Invertase Activity

The characterization of commercial processes but also for the meaningful determined by measuring the enzyme activity by varying the single parameter such as pH, temperature and substrate concentration of the medium keeping the remaining parameter unaltered. The effect of metal ions and immobilization on invertase activity is also determined. (Arruda *et al.* 1999; Meena and Raja, 2003)

Protein Estimation

Quantitative estimation of the protein content of individual fraction obtained after different steps was done by the method of Lowry *et al.* 1956

Enzyme Assay

β-Fructofuranosidase assay was determined by measuring the reducing sugars released by the hydrolysis of sucrose. The reaction was carried out at 30^{0}C for 5 minutes. The reducing sugars released in the reaction mixture were assayed by DNS method. The cell free extract obtained after centrifugation is used as the enzyme source for determining the crude enzyme activity. (Al-Bakir *et al.* 2007)

Assay Mixture

The determination invertase activity was carried out of at 30^{0}C in a mixture of 1.0ml of 0.02M acetate buffer (pH 6), 1.0 ml of 0.03 M sucrose solution and 0.1 ml of cell free extract.

The mixture was incubated at room temperature (-30^{0}C) for 5 minutes after making up the volume of the mixture to 4.0 ml with distilled water. The hydrolysis was stopped by adding 2.0 ml of DNSA reagents and then invertase activity was assayed as described using glucose or fructose as a standard. (Al-Bakir *et al.* 2007)

Enzyme Units

One unit of invertase (IU) was defined as the amount of enzyme which liberated / mg of product / minute /ml under the assay condition. (Cairns *et al.* 2006)

RESULTS AND DISCUSSION

The crude invertase enzyme from the *Aspergillus fumigatus* and *Penicillium brevicompactum* was purified by ammonium sulphate precipitation, dialysis and DEAE cellulose chromatography. The purified enzyme had low protein content when compared to the crude enzyme. The specific activity of the enzyme was increased after purification.

Invertase from *A.fumigatus* and *P.brevicompactum* was successively purified by DEAE cellulose column chromatography. Table 1

and 2 shows the elution profile of ion-exchange chromatography of crude *Aspergillus fumigatus* and *Penicillium brevicompactum* extracts on CEAE cellulose column.

The crude extract of the enzyme from *Aspergillus fumigatus* had 9970 enzyme units with a specific activity of 27.0 units mg^{-1}. The crude extract of the enzyme from *Penicillium brevicompactum* had 9940 enzyme units with a specific activity of 31.0 units mg^{-1}.

The crude extract of *Aspergillus fumigatus* and *Penicillium brevicompactum* was subjected to 30% ammonium sulphate precipitation. The precipitates had 7012 units and 6360 respectivily. The specific activity of *Aspergillus fumigatus* and *Penicillium brevicompactum* was 44.50 units mg^{-1} protein with 1.65 fold increases and 47.25 units mg^{-1} protein with 1.52 fold increases.

Similar observation was obtained by Santiago *et al.* 1967, the enzyme purified by using

Table 1: Purification of invertase from *Aspergillus fumigatus*

Description	Invertase Activity (Units)	Total protein (mg\ml)	Specific Activity Units/mg	Purification fold
Crude extract	9970	369.45	27	1
30% Ammonium sulphate saturation	7012.50	157.58	44.50	1.65
DEAE Cellulose Column Chromatography	424.25	2.01	212	7.85

Table 2: Purification of invertase from *Penicillium brevicompactum*

Description	Invertase Activity (Units)	Total protein (mg\ml)	Specific Activity Units/mg	Purification fold
Crude extract	9940	320.64	31	1
30% Ammonium sulphate saturation	6360	134.60	47.25	1.52
DEAE Cellulose Column Chromatography	393.75	1.99	197.50	6.35

Table 3: Effect of immobilization on invertase

Enzyme Source	Enzyme Sample	Invertase Activity (IU\ml)
Aspergillus fumigatus	Crude extract	15.21
	Immobilized	23.49
Penicillium purpogenum	Crude extract	14.95
	Immobilized	21.65

column chromatography produced from Saccharomyces strain. This was also supported by Shaheen I and Haq Nawaz Bhatti (2007), the enzyme purified by using column chromatography produced from newly isolated *Fusarium spp.,* under solid state fermentation.

The purified enzyme of *Aspergillus fumigatus* & *Penicillium brevicompactum* incubated with acetate buffer with varying pH 3.0 to 8.0. The enzymes show maximum activity at pH 6. (Figure 1) This data obtained was supported by Hoi-Seon-Lee *et al.* 1996, the purified invertase activtity reaches maximum at pH 6.5 produced from carrot. This was also supported by Kiel *et al.* 1977, the optimum pH for the invertase activity is 6.0 produced from *Actinomyces viscosus*.

Figure 2 shows the effect of temperature on the enzyme activity was studied by incubating the enzyme mixtures obtained from *Aspergillus fumigatus* and *Penicillium brevicompactum* at varying temperatures within the range of 20^{0}C to 80^{0}C. The optimum temperatures for the enzyme activity for both organisms were found to be at the temperature of 50^{0}C.

This result obtained was also supported by Kuramitsu, 1973, the invertase activity reaches maximum at the temperature of 47^{0}C produced from cariogenic streptococcus mutants. This was also supported by Weerasooriya *et al.* 2003, the optimum temperature for the invertase activity was 37^{0}C obtained from the flowers of *Madhuca longifolia*.

The effect of substrate concentration on the enzyme activity was studied by incubating the purified enzymes from *Aspergillus fumigatus* and *Penicillium brevicompactum* at varying concentrations of sucrose ranging from 0.5% to

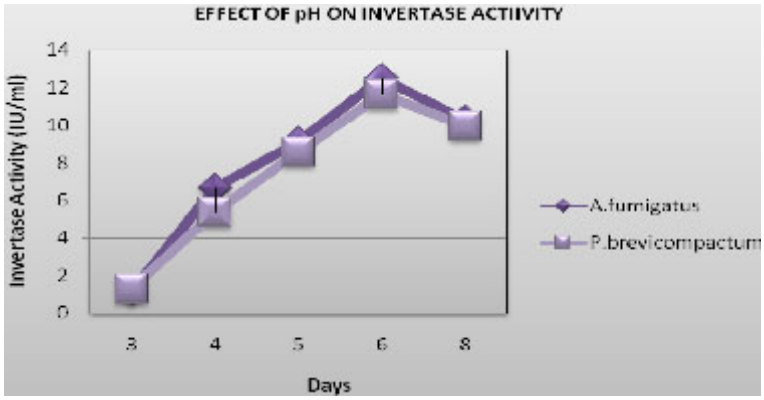


Fig. 1: Effect of pH on invertase activity

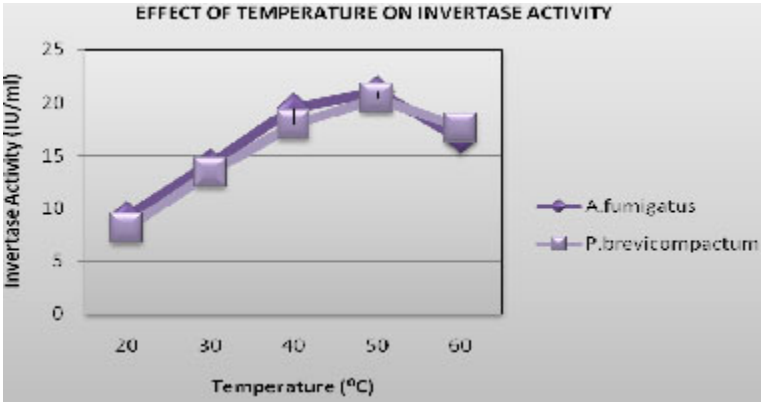


Fig. 2: Effect of temperature on invertase activity

2.5% under standard assay conditions. The invertase from *Aspergillus fumigatus* enzyme activity reaches maximum at the substrate concentration of 1g/100ml and invertase activity of *Penicillium brevicompactum* enzyme reaches maximum at the substrate concentration of 1.5g/100ml (Figure 3).

The activity of the enzyme produced by the *Cladosporium sp.,* was subjected to alteration with varying substrate concentration. The result revealed that 1.0 ml of enzyme can hydrolyse a maximum of 0.4gm of substrate. (Gogoi *et al.* 1998.)

The purified enzymes of *Aspergillus fumigatus* & *Penicillium brevicompactum* were incubated with different cations. Invertase activity was stimulated by $Cacl_2$ and NaCl. The elevated concentrations of Mg^{2+}, Zn^{2+} and Cu^{2+} drastically inhibited the invertase activity. Zn^{2+} was found to be the potent inhibitor of invertase (Figure 4).

This was also supported by Kestwal *et al.* 2008, the invertase activity was stimulated by low concentrations of $Mncl_2$ and NaCl from A.ochraceus. invertase from R.gultinis and F.solani were also activated by Na^{+} and Mg^{2+}. Thia was also supported by FernandoPrado *et al.* 1985, the elevated concentration of Ba^{2+}, Cu^{2+}, NH^{4+} and Zn^{2+} inhibits the invertase activity from Ricinus communis.

Immobilization enhances the invertase activity from *Aspergillus fumigatus* and *Penicillium brevicompactum.* The use of free enzyme in industrial application has been limited, mainly due o the high cost of enzymes, their instability and irrecoverability. This can be overcome by

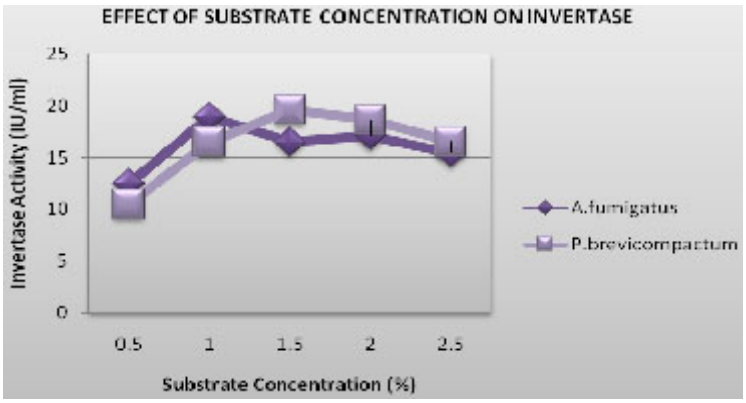
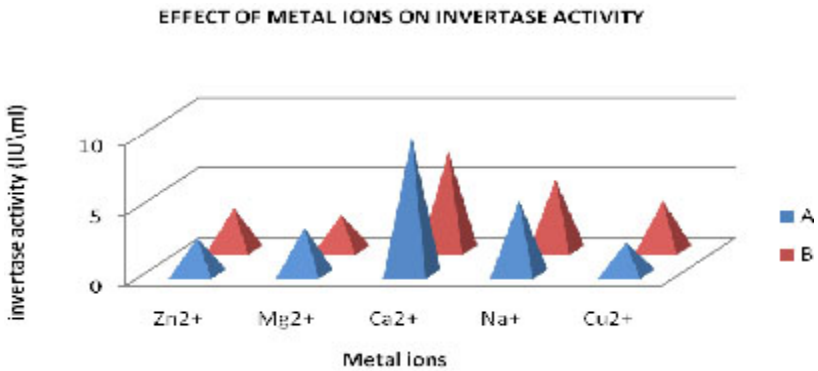


Fig. 3: Effect of substrate concentration on invertase activity



A - *A.fumigatus* B - *P.brevicompactum*

Fig. 4: Effect of metal ions on invertase activity

immobilization. The invertase activity before and after immobolization is given in table 3.

CONCLUSION

From the present study, we could see that parameters like pH, temperature, substrate concentration, metal ions and immobilization had different effect in the enzyme characterization after purification. Hence purified form of the enzyme Invertase from the two fungal strains can be used in food industries.

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