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 activity of the enzyme was inhibited by divalent metal ion Zn²⁺ 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 bêê 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)
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/inch² 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°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°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°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 at 30°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.

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
Table 1: Purification of invertase from *Aspergillus fumigatus*

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

Table 2: Purification of invertase from *Penicillium brevicompactum*

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

Table 3: Effect of immobilization on invertase

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Enzyme Sample</th>
<th>Invertase Activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>Crude extract</td>
<td>15.21</td>
</tr>
<tr>
<td></td>
<td>Immobilized</td>
<td>23.49</td>
</tr>
<tr>
<td><em>Penicillium purpogenum</em></td>
<td>Crude extract</td>
<td>14.95</td>
</tr>
<tr>
<td></td>
<td>Immobilized</td>
<td>21.65</td>
</tr>
</tbody>
</table>
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 activity 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 Actinomycetes 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°C to 80°C. The optimum temperatures for the enzyme activity for both organisms were found to be at the temperature of 50°C.

This result obtained was also supported by Kuramitsu, 1973, the invertase activity reaches maximum at the temperature of 47°C produced from cariogenic streptococcus mutants. This was also supported by Weerasooriya et al. 2003, the optimum temperature for the invertase activity was 37°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
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. guttinis* and *F. solani* were also activated by Na$^+$ and Mg$^{2+}$. This 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 to the high cost of enzymes, their instability and irrecoverability. This can be overcome by
immobilization. The invertase activity before and after immobilization 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.

REFERENCES