Sorbitol: An Enhancer of Growth and Alpha-amylase Production for Aspergillus Fumigatus NTCC1222 Using Wheat Bran as Substrate

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Abstract

Amylase, one of the most important industrial enzymes has attracted great attention from researchers worldwide due to their significant commercial value across various industries. Its large scale production hence, is one of the most important fermentation processes. The amylase production is influenced by a number of factors. Though, a few studies have reported the influence of sorbitol on amylase production by many microorganisms yet, the same has not been much discussed for Aspergillus fumigatus – an important source of amylases. The present study aims to study the effect of sorbitol on growth and amylase production for indigenously isolated fungal strain, Aspergillus fumigatus NTCC1222 – with promising applications in textile desizing. Aspergillus fumigatus NTCC1222 was subjected to solid state fermentation where, the basal fermentation medium was supplemented with 0.25-0.75% concentration of sorbitol. Simultaneously, the growth medium was supplemented with similar concentrations of sorbitol. It was found that sorbitol enhanced amylase activity at sorbitol concentrations of 0.25, 0.30, 0.35, 0.45 and 0.50%, in comparison to unsupplemented fermentation medium while a higher concentration leads to slight loss in amylase activity. The highest improvement (520.2 U/mL) was observed for 0.25% sorbitol concentration as compared to unsupplemented fermentation medium (336.7 U/mL). Interestingly, sorbitol increased the fungal growth at all concentrations. Higher was the sorbitol concentration, better was the growth. For industrial production of amylases by the test fungus,
sorbitol can be used as a growth as well as amylase production enhancer which surely increases industrial value of the test fungal strain as an amylase source.

**Keywords**: alpha-amylase, inducers, inhibitors, sorbitol, Aspergillus fumigates.

1. **Introduction**
Microbial alpha amylases are considered to be one of the most important industrial enzymes (Couto *et al.*, 2006, Shalini *et al.*, 2009, Shalini *et al.*, 2010, Shalini *et al.*, 2011, Tyagi *et al.*, 2011, Shalini *et al.*, 2013, Mukherji *et al.*, 2009). *Bacillus* sp. (Nurmatov *et al.* 2001, Dey *et al.* 2002) and *Aspergillus* sp. (Goto *et al.* 1998, Gigras *et al.* 2002) are considered to be the main microbial sources for amylase production. The morphology of *Aspergillus* sp. allows it to efficiently colonize and penetrate the solid substrate (Hernandez *et al.*, 2006). Thus, keeping in view the importance of *Aspergillus* sp. as a source of amylase, the influence of sorbitol on the production of alpha amylase by *Aspergillus fumigatus* NTCC1222 under solid state fermentation, was investigated.

2. **Materials and Methods**

2.1 **Materials**
All the chemical reagents and nutrient culture media used were of analytical grade and procured from reputed companies (Himedia Pvt. Ltd. India and LobaChemie Pvt. Ltd., India). Wheat bran was procured from the local market of Jalandhar, Punjab, India. It was washed with water, squeezed, dried, ground and sieved through fine mesh of pore size 0.5 mm to obtain powdered form of the substrate. The powdered substrate was then kept in polyethylene bags under dry conditions for future use.

2.2 **Microorganism**
The test fungal strain (*Aspergillus fumigatus* NTCC1222) was procured from laboratory of Microbiology, Department of Biotechnology and Biosciences, Lovely Professional University, Punjab, India. It was maintained on potato dextrose agar (PDA) plates and subsequently stored at 4°C. For long term preservation, the test fungal strain was inoculated on 15% (v/v) glycerol stocks and subsequently stored at -20°C.

2.3 **Effect of sorbitol on growth and amylase production for Aspergillus fumigatus NTCC1222**
The amylase production was carried out under solid state fermentation (SSF) using optimum incubation period (6 days), pH (6), temperature (37°C), nitrogen source (beef extract), substrate to moistening agent ratio of 1:3 (Bali, 2011). 5 g of wheat bran was taken into 250 ml Erlenmeyer flasks. To this, 15 mL of nutrient salt solution, NSS was
added so as to maintain a substrate to moistening agent ratio of 1:3. The basal NSS contained beef extract 5g/L, NaCl 1 g/L, MgSO₄, H₂O 1 g/L, KH₂PO₄ 5 g/L and it was supplemented with variable concentration (0.25-0.75%) of sorbitol. The flasks were autoclaved at 121°C at 15 psi pressure for 15 minutes, cooled and inoculated with two 5 mm diameter discs of test fungus grown on wheat bran agar medium (2% wheat bran and 2% agar agar). The flasks were subsequently incubated at 37°C for 6 days. 15 mL of distilled water was added to each Erlenmeyer flasks and the contents were crushed with a glass rod. The flasks were placed on rotary shaker for 10 minutes at 200 rpm. The slurry, so obtained, was squeezed through 3-4 layers of cheese cloth and the extract was centrifuged at 5000 rpm for 15 minutes. The supernatant was treated as crude enzyme (Singh et al., 2009). The activity of enzyme was determined in terms of reducing sugars released (Miller et al., 1959). The reducing sugars released by enzymatic hydrolysis were determined at 540 nm using glucose as standard. One enzyme unit was defined as the amount of enzyme that hydrolyzed 1 mg of starch (0.1% w/v) in min at 37 °C and pH 5.0 (U/mL).

2.4 Statistical analysis
All experiments were carried out in triplicates. The results for enzyme activity were mean ‘±’ standard deviation (SD) of the values and those for microbial growth were reported as an average of the values.

3. Results and Discussion
3.1 Effect of sorbitol on growth and amylase production for Aspergillus fumigatus NTCC1222
As reported in Table 1, sorbitol was found to improve amylase activity upto 0.50% of sorbitol. The highest increase in enzyme activity (35.27%) was observed in the presence of 0.25% sorbitol. As the concentration of sorbitol increased from 0.25%, a gradual decrease in amylase activity was recorded. Still, the amylase activity was higher than control (unsupplemented fermentation medium) till a sorbitol concentration of 0.50%. The amylase activity was higher by 4.48% than control at a concentration of 0.50%. Even at a concentration of 0.75%, sorbitol decreased enzyme activity of 14.52% only. The improvement in amylase activity of microbial strain in the presence of sorbitol has been observed by others too (Srivastava and Baruah, 1986, Demirkan, 2011, Monga et al., 2011) Interesting, the growth of test fungus improved in the presence of sorbitol at all concentrations, with the highest improvement at a concentration of 0.25% (35.27% increase in growth).
Table 1: Effect of sorbitol on growth and amylase production for test fungus.

<table>
<thead>
<tr>
<th>Effector</th>
<th>Concentration (%)</th>
<th>Growth</th>
<th>Diameter (cm)</th>
<th>Full plate growth</th>
<th>Amylase activity (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>3.143</td>
<td>-</td>
<td>336.7±1.3</td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td></td>
<td>3.254 (day-3)</td>
<td>Received till 4th day of incubation</td>
<td>520.2±1.4</td>
</tr>
<tr>
<td>0.30</td>
<td></td>
<td></td>
<td>3.270 (day-2)</td>
<td>-</td>
<td>441.2±1.2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.35</td>
<td></td>
<td>3.310 (day-2)</td>
<td>-</td>
<td>439.1±0.6</td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td></td>
<td>3.312 (day-2)</td>
<td>-</td>
<td>380.2±1.1</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td></td>
<td>3.340 (day-3)</td>
<td>Received till 4th day of incubation</td>
<td>352.5±0.9</td>
</tr>
<tr>
<td></td>
<td>0.55</td>
<td></td>
<td>3.400 (day-2)</td>
<td>-</td>
<td>309.1±1.8</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td></td>
<td>3.490 (day-2)</td>
<td>-</td>
<td>291.5±1.0</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td></td>
<td>3.546 (day-3)</td>
<td>Received till 4th day of incubation</td>
<td>287.8±0.7</td>
</tr>
</tbody>
</table>

Fermentation conditions:
- Wheat bran: NSS : 1:3
- Incubation period, days : 6.0
- Temperature, °C : 37
- pH : 6.0

4. Conclusion
Sorbitol was found to enhance growth and alpha amylase activity for *Aspergillus fumigatus* NTCC1222 under SSF. Thus, it can be used as potential inducers for the production of amylase by *Aspergillus fumigatus* NTCC1222 as well as increase in biomass of the said fungal strain for industrial applications.

References


