Influence of Calcium Chloride on Growth and Alpha-amylose Production for Wild and UV-mutated Strains of Aspergillus Fumigatus

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Abstract

The need for large scale production of alpha-amylases has always been a great area of interest for researchers worldwide and thus, ways to achieve the same through modifications of cultural conditions have been carried out by many. Calcium chloride has been found to influence alpha-amylose production by many microbial strains. The present study explores the effect of calcium chloride on growth and alpha-amylose production for wild type and UV mutated strains of Aspergillus fumigatus NTCC1222 under solid state fermentation where, the fermentation medium was supplemented with variable concentrations of calcium chloride and the amylase activities so obtained were compared with that of unsupplemented fermentation medium. Simultaneously, the growth medium was supplemented with similar concentrations of calcium chloride so as to see the effect of calcium chloride on growth of the fungal strains as well. The study indicated that calcium chloride improved the amylase production for both wild type (348.0 U/mL and 340.5 U/mL at a concentration of 80 and 100 mg/mL, respectively) as well as UV-mutated (361.0 U/mL and 350.5 U/mL at a concentration of 80 and 100 mg/mL, respectively) strains of Aspergillus fumigatus NTCC1222, as compared to unsupplemented fermentation medium (337.5 U/mL). A higher concentration (120 mg/mL) of calcium chloride though slightly reduced the enzyme activity in comparison to the control for both the fungal strains. The growth of both the strains of Aspergillus fumigatus NTCC1222 was found to be slightly decreased in the presence of calcium chloride.

Keywords: Amylase, Calcium chloride, Aspergillus fumigatus.
1. Introduction
Enzymes, owing to their environment friendly nature, are favoured over chemical processes (Mahmoud et al., 1978, Shalini et al., 2009, Shalini et al., 2010, Shalini et al. 2011, Tyagi et al. 2011, Shalini et al., 2013) in industrial applications. Amylases (EC 3.2.1.1) are one of the most widely used industrial enzymes (deSouza and Magalhaes, 2010) that have greatly contribute towards the development of environment friendly products and processes. α-amylases are endo-acting enzymes that catalyze the hydrolysis of α-1,4-O-glycosidic bonds of a starch molecule to breakdown into low molecular weight limit dextrins. They are mostly metallo-enzymes containing Ca$^{+2}$ ions (deSouza and Magalhaes, 2010). Various studies have been done on the optimization of cultural conditions such as, nitrogen source, complex substrates, metal ions and physical, conditions, etc., for enhanced amylase production (Safey and Ammar, 2004, Tokhadze et al., 1975). In the current study, the influence of variable concentration of calcium chloride on growth and amylase production by wild and mutated strains of Aspergillus fumigatus NTCC1222 was studied.

2. Materials and Methods
2.1 Materials
All the chemical reagents and nutrient culture media used were of analytical grade from reputed companies. The substrate (wheat bran) was procured from the local market of Jalandhar, Punjab, India. It was washed with water, squeezed, dried, ground, sieved through fine mesh of pore size 0.5 mm and stored under dry conditions.

2.2 Microorganism
The wild and UV mutated strains of Aspergillus fumigatus NTCC1222 were maintained on wheat bran agar (WBA) plates that contained 2% wheat bran and 2% agar agar. After growth, the fungal cultures were stored at 4°C. For long term preservation, the fungal cultures were inoculated on 15% (v/v) glycerol and subsequently stored at -20°C.

2.3 Influence of calcium chloride
The amylase production by the test fungal strains was done under solid state fermentation (Shalini et al., 2013). 5 g of wheat bran was taken into 250 ml Erlenmeyer flasks. To this, 15 mL of nutrient salt solution (NSS) was added. The NSS contained beef extract 5g/L, NaCl 1 g/L, MgSO$_4$H$_2$O 1 g/L, KH$_2$PO$_4$ 5 g/L and it was further supplemented with variable concentrations (80, 100 and 120 mg/mL) of calcium chloride. The initial pH of the NSS was maintained at 6.0. The flasks were autoclaved and inoculated with two, 5 mm diameter discs, of wild and mutated strains of Aspergillus fumigatus NTCC1222. The flasks were 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. Further, the flasks were rotated on rotary shaker for 10 minutes at 200 rpm. The slurry was then squeezed through 3-4 layers of cheese cloth and the extract was centrifuged at 5000 rpm for 15 minutes. The supernatant (crude
enzyme) was stored at 4°C (Singh et al., 2009). The amylase activity was determined by the method of Miller et al., 1959 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).

2.3 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 Influence of calcium chloride
As Table 1 shows, the amylase activity was 6.5% more for wild type and, 3.01% more for UV-mutated strains of Aspergillus fumigatus NTCC1222 as compared to the control (unsupplemented medium). On the other hand, calcium chloride at a concentration of 120 mg/mL slightly reduced the enzyme activity in comparison to the control for both the fungal strains. The highest amylase activity was observed as 361.0 U/mL for UV-mutated strain in the presence of calcium chloride supplemented medium at a concentration of 80 mg/mL. The wild type strain exhibited a slight loss of (-3.6%) in amylase activity as compared to that of UV-mutated strain at same concentration of calcium chloride. Increase in amylase activity in the presence of calcium chloride has been reported by other researchers as well (Bhardwaj et al., 2012, Unakal et al. 2012). Interestingly, the growth of both the wild type as well as mutated strains of Aspergillus fumigatus NTCC1222 was slightly reduced in the presence of calcium chloride at all concentrations used thereby, indicating that the growth of Aspergillus fumigatus NTCC1222 was not found to improve in the presence of calcium chloride in the presence of calcium chloride though, amylase production was found to improve in its presence.

Table 1: Effect of calcium chloride on growth and amylase production for Aspergillus fumigatus.

<table>
<thead>
<tr>
<th>Microbial strain</th>
<th>Concentration (mg/mL)</th>
<th>Growth Diameter(cm)</th>
<th>Full plate growth</th>
<th>Amylase activity (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>Control</td>
<td>1.996(day-2)</td>
<td>-</td>
<td>337.5±0.9</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.896(day-2)</td>
<td>-</td>
<td>348.0±1.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.945(day-2)</td>
<td>-</td>
<td>340.5±1.8</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.846(day-2)</td>
<td>-</td>
<td>331.9±1.3</td>
</tr>
<tr>
<td>UV-mutated</td>
<td>80</td>
<td>1.560 (day-2)</td>
<td>-</td>
<td>361.0±0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.900 (day-2)</td>
<td>-</td>
<td>350.5±1.2</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.810 (day-2)</td>
<td>-</td>
<td>335.9±1.2</td>
</tr>
</tbody>
</table>
Fermentation conditions:
Wheat Bran: NSS : 1:3
Incubation period, days : 6.0
Temperature, ° C : 37
pH : 6.0

The large scale production of amylase by the test fungal strains can thus utilize a 2-stage fermentation system (growth stage and fermentation stage). The fermentation medium can be supplemented with calcium chloride in the 2nd stage (fermentation stage).

4. Conclusion
As calcium chloride was found to improve amylase production at a concentration of 80 and 100 mg/mL, the fermentation medium for amylase production can be supplemented with calcium chloride for enhanced amylase production for both the wild type as well as UV-mutated strains of Aspergillus fumigatus NTCC1222. As the growth of the fungal strains was slightly reduced for calcium chloride-supplemented medium, a 2-stage fermentation system (growth stage and a fermentation stage) can be used. In such a system, calcium chloride can be added in the fermentation stage for improved amylase production by both the strains of Aspergillus fumigatus NTCC1222.

References

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