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Effect of Toluene on Fungal Growth and Amylase Production-A Step towards Exploration of Enzymes for Industrial Applications

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Abstract

Large scale production of enzymes is one of the most important areas of study today. This is due to increase in interest in enzymes for their environment friendly nature. Conventional, chemical-based industries are under tremendous pressure to incorporate microbial systems including, microbial enzymes, for reducing the pollution load. Amylases along with other enzymes, play a crucial role in providing environment-compatible solutions to conventional chemical-based industries. Numerous studies have been carried out on the effect of various regulatory molecules on amylase production by microorganisms. These studies provide important information on the influence of different substances on amylase production which further helps to improve the enzyme production for industrial applications. Organic solvents like, toluene, have been used to purify amylases and hence, these solvents might influence the enzyme activity during purification. Thus, checking the influence of toluene on amylase production is an important parameter for determining the compatibility of these organic solvents with amylases for efficient enzyme recovery. Though a few studies have been carried out to check the influence on toluene on amylase activity during purification yet, studies on the influence of toluene on amylase production and microbial growth are rare. In the current study, toluene was added (0.25-0.75%) to the fungal growth medium and amylase production medium to check its influence on microbial growth and amylase production for Aspergillus fumigatus NTCC1222. The study showed that amylase production

decreased in the presence of toluene at all concentrations used. Higher was the toluene concentration, more was the loss in amylase activity. The amylase activity was 291.1 U/mL at a toluene concentration of 0.25% as compared to unsupplemented fermentation medium (340.9 U/mL). Interestingly, toluene significantly favoured the growth of Aspergillus fumigatus NTCC1222 at all concentration of toluene used, with 0.5% the most preferable toluene concentration. The study suggests that though, toluene depressed amylase activity yet due to its prominent positive effect on the growth, it can be used as a supplement to the culture medium used for the growth of Aspergillus fumigatus NTCC1222.

Keywords: Environment, Amylase, Toluene, Aspergillus fumigates.

1. Introduction

Nowadays, the use of enzymes is making the development of environment compatible processes possible (Mahmoud, 1978, Shalini et al., 2009, Shalini et al., 2010, Shalini et al. 2011, Tyagi et al. 2011, Shalini et al., 2013a) which is adding environment-friendliness to conventional chemical processes. Though, a number of enzymes are being tested for numerous industrial applications yet, the search of newer, better sources of enzymes is a never-ending process. Alpha-amylases are one of the most widely used industrial enzymes (deSouza and Magalhaes, 2010) and fungi like, *Aspergillus* sp., are one of the most attractive sources of amylases. *Aspergillus fumigatus* has been found to the least explored as compared to other *Aspergillus* sp. like, *Aspergillus flavus* (Khoo et al., 2004), *Aspergillus niger* (Hernández et al., 2006), *Aspergillus oryzae* (Morkeberg, et al., 1995, Monfort et al., 1996), *Aspergillus nidulans* (Monfort et al., 1996), etc. In the current study, the influence of toluene, an organic solvent, used for amylase purification, on growth and amylase production for *Aspergillus fumigatus* NTCC1222 (Shalini et al. 2013b, Shalini et al. 2013c, Shalini et al. 2013d, Shalini et al. 2013e) was studied.

2. Materials and Methods

2.1 Effect of toluene on growth and amylase production for Aspergillus fumigatus

The influence of toluene on growth was checked by growing the test fungus on wheat bran agar medium (2% w/v wheat bran of 0.5 mm particle size, 2% w/v agar agar and variable concentrations of toluene, 0.25%-0.75%) for 6 days at 37^{o}C. The growth was measured by measuring the diameter of the fungal colony. Simultaneously, control growth medium (without toluene supplementation) was inoculated with the test fungal strain and the growth was compared with that for toluene-supplemented growth medium. For determining the effect of toluene on amylase production, the fungus was inoculated (two, 5 mm diameter fungal discs) into autoclaved, 250 ml Erlenmeyer

flasks, containing 5 g of wheat bran and 15 mL of nutrient salt solution (NSS) (Shalini et al., 2013). 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 (Shalini *et al.*, 2009). The amylase activity was then determined (Miller *et al*., 1959). 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 6.0 (U/mL).

2.2 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 toluene on growth and amylase production for Aspergillus fumigatus

Though toluene was found to influence the amylase activity as well as growth of the test fungus (Figure 1) yet, its effect on amylase activity was in contrast to its effect on fungal growth. While the amylase activity decreased in the presence of toluene at all concentration used, the growth of test fungus improved in the presence of toluene.

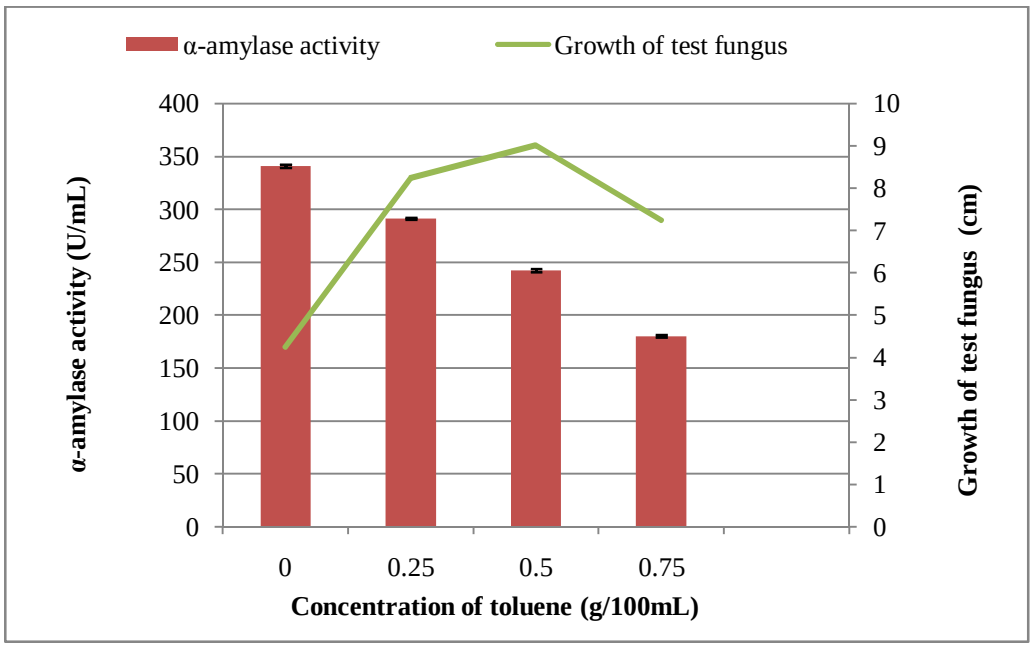

Fig. 1: Effect of toluene on growth of Aspergillus fumigatus.

Higher was the concentration of toluene higher was the loss in amylase activity, with a loss of 47.11% at a concentration of 0.75 % toluene. At a concentration of 0.25 and 0.50%, the amylase activity was lesser by 14.60 and 28.92%, respectively, as compared to the unsupplemented fermentation medium. Thus, toluene was found to have a negative effect on the amylase production for *Aspergillus fumigatus* NTCC1222 under optimized conditions of SSF. The growth of the fungus on the other hand, was found to improve in the presence of toluene to a significant extent. The best growth was observed for growth medium supplemented with 0.50% of toluene (52.83% increase in growth as compared to unsupplemented fermentation medium).

4. Conclusion

The study suggests that though, toluene depressed amylase activity yet due to its prominent positive effect on the growth, it can be used as a growth supplement and added to medium used for the growth of *Aspergillus fumigatus* NTCC1222.

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