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Production of extracellular tannase by *Penicillium notatum* NCIM 923 and optimization of various process parameters

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Extracellular tannase producing efficiency was investigated with 3 different strains of mould. *Aspergillus riger* NCIM 612, *Aspergillus oryzae* NCIM 645 and *Penicillium notatum* NCIM 923 by submerged fermentation. Then with the most efficient tannase producer solid state fermentation was carried out using wheat bran as the substrate. Varlous environmental parameters like time, temperature, agitation, hydration, particle size and pH showed a marked effect on tannase production. Among the different strains used *Penicillium notatum* NCIM 923 was the most efficient tannase producer. Maximum enzyme was produced on day 4 of fermentation carried out at 30°C in stationary condition and at pH 5.

Key words : Tannase enzyme, *Penicillium notatum*, solid state fermentation, wheat bran

INTRODUCTION

The enzyne tannase (tannin acyl hydrolase, EC 3.1.1.20) catalyzes the hydrolysis of ester and depside bonds in hydrolysable tannins, releasing glucose and gallic acid (Dyokeroff and Ambruster, 1933). Most of the reported tannase producing organisms are fungi (Van de Lagemaat and Pyle, 2005; Sabu *et al.*, 2005; Ramirez- Coronel *et al.*, 2003; Lekha and Lonsane, 1997) but they are also produced by yeast (Aoki *et al.*, 1977), bacteria (Vaquero *et al.*,2004; Mondal and Pati, 2000; Osawa *et al.*, 2000 and Deschamps *et al.*, 1983) and plants (Nicholas *et al.*, 1997).

The commercial application of tannase includes preparation of cold water soluble tea (Agbo and Spradlin, 1995), acorn wine (Chae and Yu, 1983) and gallic acid (Pourrat *et al.*,1985). Gallic acid is an important intermediary in the synthesis of the antibacterial drug Trimethoprim (Bajpai and Patil, 1996). Gallic acid is also a substrate for the chemical or enzymatic synthesis of propylgallate, a potent antioxidant. Tannase also has potential applications in the clarification of beer (Masschelelein and Batum, I981) and fruit juices (Canteralli *et al.*,1989), manufacture of coffee flavoured soft drinks (Suzuki, 1973), improvement in the flavour of grape wine and as an analyical probe for determining the structures of naturally occurring gallic acid esters (Seth and Chand, 2000). Tannins which are present in nutritionally important forage trees, shrubs, legumes etc. can impair the digestive process of rumens by complexing with secreted enzymes and endogenous proteins. Pretreatment with tannase can reduce the antinutritional effects of tannins in animal feed (Lekha and Lonsane, 1997). The enzyme can be used to reduce the concentration of tannic acid in tannery effluent thereby reducing the pollution level of tannery wastewater (Murugan *et al.*, 2007). Several studies have reported interesting advantages between the tannase produced by solid state fermentation in relation with that produced by submerged fermentation (Lekha and Lonsane, 1994; Chaterjee *et al.*, 1996; Aguilar *et al.*, 2001; Rana and Bhat, 2005). Till date there are no reports available in the literature on the production of tannase through solid state as well as submerged fermentation by *Penicillium notatum*

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NCIM 923.The endeavour of the present investigation is to select the most efficient tannase producer among *Aspergillus niger* NCIM 612, *Aspergillus oryzae* NCIM 645 and *Penicillium notatum* NCIM 923 and optimization of various process parameters with the most efficient tannase producer to enhance its production. Our aim is also to produce tannase from a very cheap substrate to minimize the cost of such an important commercial enzyme.

Selection of a suitable mould strain, the process of fermentation and the optimum time for production of tannase

Both submerged and solid state fermentation were caried out with the 3 strains of mould for up to 7 days and enzyme assay was done after every 24 hrs interval to select the efficient tannase producer.

The following environmental parameters were optimized by solid state fermentation and incubation time of 96 hrs with *P. notatum* NCIM 923 only.

Optimization of fermentation temperature

The fermentation was done at 20°C,30°C, and 40°C to know the optimum fermentation temperafure.

Effect of hydration on fermentation

Substrate was hydrated by adding waste material and water in the ratio of 1:0.5, 1:1, 1:2, 1;3,1:4 and enzyme assay was done to optimize the hydration ratio on enzyme production.

Effect of agitation on fermentation

The fermentation was done in static condition as well as at an agitation rate of 50 rpm and l0O rpm and enzyme assay was done.

Effect of particle size on fermentation

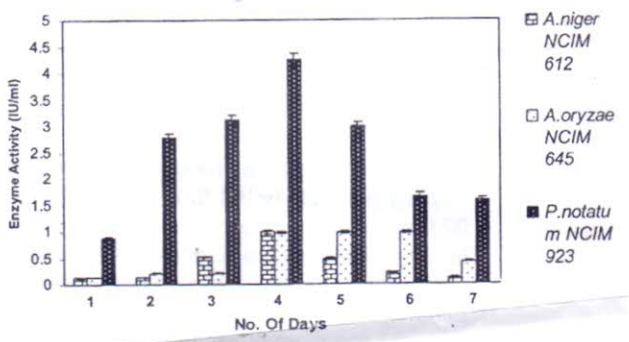
The fermentation was carried out using wheat bran of two different particle sizes- coarse and fine to optimize the effect of particle size on enzyme production.

Effect of pH on fermentation

The fermentation was carried out by hydrating the substrate with citrate phosphate buffer of pH 3-8 to optimize the pH on enzyme production.

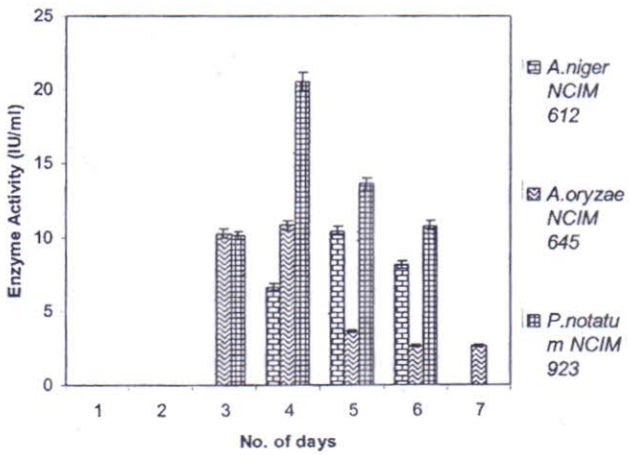
RESULTS AND DISCUSSION

Production of tannase was studied with 3 different strains of mould using submerged and solid state fermentation. Fig. 1 shows that better tannase production was with *Penicillium notatum* NCIM 923 in submerged (SmF) as well as in solid state fermentation (SSF) on all the days compared to *Aspergillus niger* NCIM 912 or *Aspergillus oryzae* NCIM 945. Reports are there that tannase is produced mainly by moulds. (Sabu *et al.*, 2005; Ramirez-coronel *et al.*,2003; Lekha and Lonsane, 1997).Tannase production has been tested with a number of *Aspergillus* and *Penicillium* strains in SmF (Batra and Saxena, 2004) where *Aspergillus niger* gave moderate tannase activity while *Aspergillus oryzae* showed no tannase activity, but there are no reports on the production of tannase by *Penicillium notatum* NCIM 923.The other *Penicillium* strains tested by Batra and Saxena showed



Error bars indicate standard deviation from hiplicate determinations. Data are mean of three independent readings with significance of P<0.05.

Fig. 1 : Production of tannase by 3 different strains of mould by solid state fermentation



Error bars indicate standard deviation from hiplicate determinations. Data are mean of three independent readings with significance of P<0.05.

Fig. 2 : Production of tannase by 3 different strains of mould by solid state fermentation

moderate tannase activity. Several studies have reported interesting advantages between tannase produced by solid state fermentation in relation with

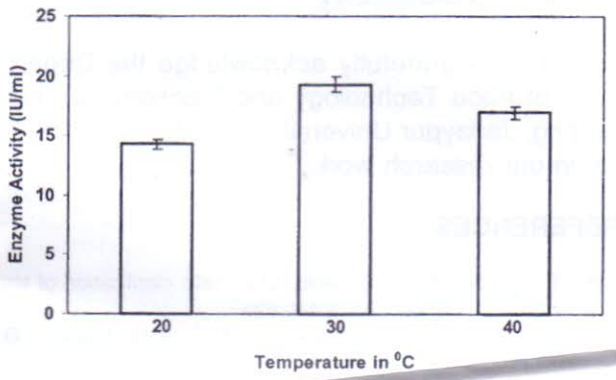


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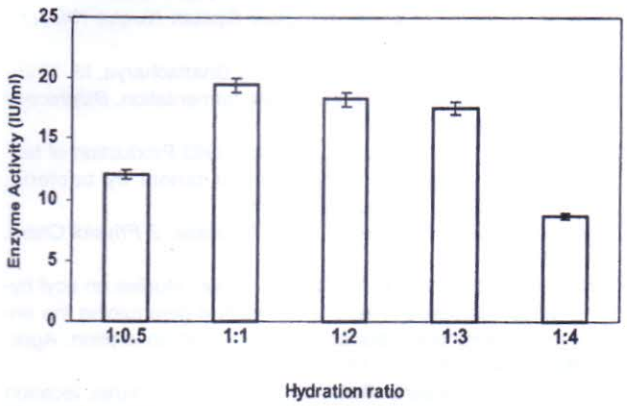
Fig. 3 : Optimum temperature of production of tannase

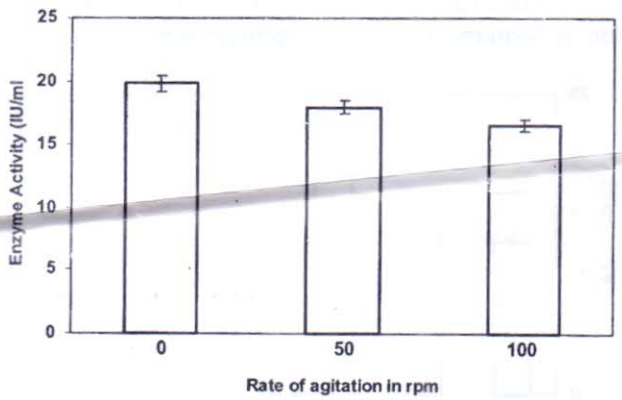
submerged fermentation (Lekha and Lonsane, 1994; Chaterjee *et al.*, 1996; Aguilar *et al.*, 2001; Rana and Bhat, 2005).This may be due to that in SSF microbial cultures are closer to their natural habitat and probably hence their activity is in-



Error bars indicate standard deviation from hiplicate determinations. Data are mean of three independent readings with significance of P<0.05.

Fig. 4 : Optimum hydration of substrate for production of tannase

creased (Pandey, 2003). In our study it is clearly seen that tannase production by *P. notatun* NCIM 923 gave much higher activity in solid state fermentation (20.55±0.146) than in submerged fermentation (4.25 ± 0.155). Highest enzyme production was obtained on day 4 of fermentation. Reports are also there that production of tannase is best in the 3-5 days range (Batra and Saxena,2004; Rajakumar and Nandy 1983; Sharma *et al.*,2007) which is accordance with the result we obtained. Fig. 2 shows the effect of temperature on enzyme production. Enzyme production is much better at 30°C (19.32 ± 0.011) than at 20°C (14.28 ± 0.129) or at 40°C (16.92±0.14). This is accordance with the report by (Batra and Saxena, 2004) that tannase producing *Penicillium* strains grow best at 30° ±1°C.



Error bars indicate standard deviation from hiplicate determinations. Data are mean of three independent readings with significance of P<0.05.

Fig. 5 : Optimum aeration/agitationfor production of tannase

Certain quantity of water is essential for new cell synthesis. Initial moisture content of the solid substrate is an important factor which dictates the growth of the organism and enzyme production. Fig.3 shows the effect of hydration on enzyme production. Result showed that hydration ratio of 1:1 is optimum for tannase production. A much better activity of 19.48 ± 0.348 was obtained when the substrate was hydrated with distilled water in the ratio 1:1. Enzyme production decreased when hydration ratio was increased or decreased from 1:1.

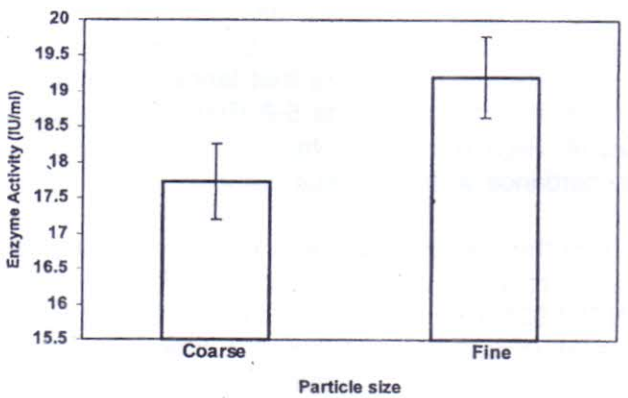


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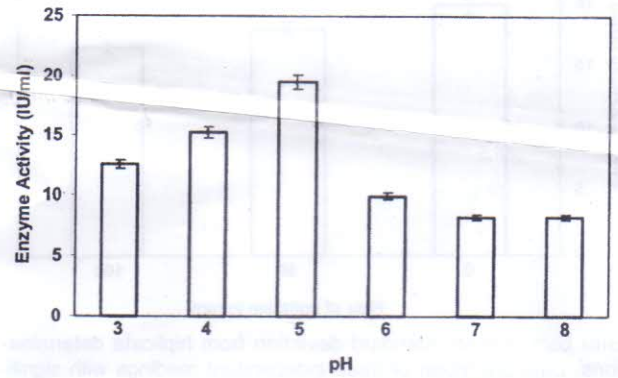
Fig. 6 : Optimum particle size of substrate for production of tannase

Fig. 4 shows the effect of agitation on enzyme production. Enzyme production is better under static condition (19.8±0.218) and decreases correspondingly as agitation rate increases. For solid state fermentation agitation is harmful for the growth of the mould strain because its extensive hyphal network tends to disrupt resulting in lower enzyme production. Aeration is facilitated by spaces between substrate particles and no additional aeration is required by the microorganisms.



Error bars indicate standard deviation from hiplicate determinations. Data are mean of three independent readings with significance of P<0.05.

Fig. 7 : Optimum pH for production of tannase

Fig. 5 shows the effect of particle size on enzyme production. Fine particle sized substrate gave better enrpe production (19.8±0.218) than coarse sized substrate (17.72±0.269). Mycelial mat were able to form effectively in fine particle sized substrate than in coarse particle sized substrate and consequently yield was also better in fine particle sized substrate.

Fig. 6 shows the effect of pH on tannase production. Enzyme production increased with pH, reached its maximum at pH 5 (19.52±0.261) and again reduced. Reports are there that tannase production is better in the pH range 5-6 (Batra and Saxena, 2004; Rajakumar and Nandy, 1983) which is in accordance with the result obtained here.

From the above results it is evident that tannase was produced efficiently by *Penicillium notatum* NCIM 923 than *Aspergillus niger* NCIM 612 or *Aspergillus oryzae* NCIM 645 in submerged as well as in solid state fermentation and yield was much better under SSF than in SmF. Yields were better when fermentation was done at 30°C, under stationary condition, hydration ratio of 1:1, with fine particle sized substrate and hydration of substrate with buffer of pH 5. So, tannase erzyme, which has applications in various industries, was obtained in high percentage and with more or less free of cost using wheat bran as substrate.

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