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Effect of carbon compounds and metal ions on the production of tannase by *Penicillium notatum* NCIM 923 utilizing agri-horticultural wastes by solid state fermentation

SASWATI GAYEN AND UMA GHOSH*
Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata 700 032, West Bengal

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In the present investigation tannase enzyme was produced from a suitable combination of marigold flower wastes and wheat bran using *Penicillium notatum* NCIM 923 and the effect of different carbon sources and metal salts on the production of enzyme was studied. It was evident that *Penicillium notatum* NCIM 923 produced maximum activity when 0.7% glucose was used during enzyme production as a carbon source. Among the various metal salts used maximum enzyme activity was obtained with 0.04% calcium lactate. So, tannase enzyme which has applications in various industries was obtained in high percentage and with more or less free of cost using marigold flower and wheat bran and by adding 0.7% glucose as a carbon source and 0.04% calcium lactate as a source of metal salt.

Key words: Tannase enzyme, *Penicillium notatum*, marigold wastes, wheat bran, carbon sources, metal salts

INTRODUCTION

Solid-state fermentation has emerged as a potential technology for the production of microbial products such as feed, fuel, food, industrial chemicals, pharmaceutical products and most importantly enzymes. Utilization of agri-horticultural residues as substrates in solid state fermentation processes provides an alternative avenue and value-addition to these otherwise under or non-utilized residues thereby solving their disposal problems and minimizing environmental pollution.(Bose *et al.*, 2006).

Tannin acyl hydrolase [E.C.3.11.20] commonly referred to as tannase is a commercially important enzyme, produced mainly by fungi (Van de Lagemaat and Pyle, 2005; Sabu *et al.*, 2005; Ramirez-Coronel *et al.*, 2003; Lekha and Lonsane, 1997) but also 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). Tannase catalyses the hydrolysis reaction of the ester bonds present in the hydrolysable tannins and gallic acid esters.

E-mail: ughoshftbe@yahoo.co.in.

At the moment acute water pollution is being created by dumping of various chemical effluents from different types of industries situated near the water bodies. One such industry is the leather or tannery industry which releases dangerous pollutants like polyphenols into the water bodies thus creating intense water pollution. Tannase which can be produced almost free of cost from agri-horticultural wastes can be used for the removal of these pollutants prior to the release of the effluents into the water bodies (Murugan *et al.*, 2007).

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, 1981) 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 analytical probe for determining the structures of naturally occurring gallic acid es-

ters (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 industrial applications of tannase have not been fully exploited because of its high cost. So our present aim is to produce tannase enzyme almost free of cost from a suitable combination of marigold flower waste and wheat bran utilizing *Penicillium notatum* NCIM 923 and to study the effects of carbon compounds and metal salts on the production of the enzyme.

MATERIALS AND METHODS

Penicillium notatum NCIM 923 was grown on Czapek Dox agar medium and subcultured monthly and stored at 4°C. 25 g of wheat bran and marigold flower wastes in the ratio 4:1 were taken in 500 ml conical flasks and to it 25 ml of distilled water was added and mixed well. The flask was autoclaved at 121°C for 15 min and inoculated with 5x 10^7 spores per ml of *Penicillium notatum* NCIM 923 and incubated at 30°C for 4 days for solid state fermentation (Gayen and Ghosh, 2008).

After fermentation 50 ml of distilled water was added to the flasks and kept for 2 hrs at 90 rpm in an incubator shaker (Sambros) and filtered through cheese cloth. The filtrate was centrifuged at 10,000 rpm for 30 mins. The centrifugate obtained was the crude enzyme.

Tannase activity was estimated by a protein precipitation method (Iibuchi *et al.*, 1966).The reaction mixture contained 1ml 1% tannic acid (in citrate phosphate buffer, pH 5.0), 2 ml of citrate phosphate buffer (pH 5.0) and 1 ml of the culture filtrate. The mixture was incubated at 37°C for 30 mins in a water bath. The reaction was stopped by adding 4 ml 2% BSA solution. In the control BSA was added in the incubation mixture prior to incubation. All tubes were left for 20 mins at room temperature to precipitate residual tannins and were centrifuged at 3000 xg for 20 mins. The tannase activity in the supernatant was estimated after appropriate dilution and reading O.D. at 260 nm (this wavelength corresponds to the optimal absorption of gallic acid) against double distilled water as blank. One enzyme unit is the amount of enzyme that liberates 1µ mol gallic acid per ml per min under standard assay conditions.

The fermentation was carried out using 1% concentration of different carbon compounds like glucose, lactose, maltose, sucrose and starch. The fermentation was also carried out using different concentrations of maltose, glucose and starch as carbon sources, i.e. 0.1%, 0.3%, 0.5%, 0.7%, 0.9%, 1%, 1.3% and 1.5%.

The fermentation was carried out using 0.1% concentration of various metal salts like potassium sulphate, copper sulphate, sodium benzoate, cobalt sulphate, lead acetate, stroncium acetate and calcium lactate. The fermentation was also carried out using 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% and 0.1% concentration of potassium sulphate, copper sulphate and calcium lactate.

Appropriate controls using wheat bran and marigold flower waste moistened with only distilled water were kept for all the experiments.

RESULTS AND DISCUSSION

Various carbon compounds like glucose, sucrose, lactose, maltose and starch at 1% concentration were tested for their effect on tannase production (Fig. 1-5). The result showed that glucose and maltose gave elevated level of tannase production. When starch was used tannase production was elevated by only 0.4% than control and lactose and sucrose inhibited tannase production. Using the carbon compounds which gave highest tannase production further work was carried out at various concentrations from 0.1%- 1%.Maximum enzyme production was obtained with 0.7% concentration of maltose followed by 0.5% and 1.3%. 0.1% and 0.3% concentration of maltose gave no enzyme activity. In case of glucose maximum enzyme production was obtained with 0.7% concentration followed by 0.5% and 0.9%. Least activity was obtained with 0.1% and 0.3% concentration of glucose. Using starch as a carbon compound maximum enzyme production was obtained with 1.3% concentration followed by 0.7 % and 0.9%. Least activity was obtained with 0.1% and no activity was obtained with 0.3% concentration of starch.

When a comparative study was done with the 3 highest activity giving carbon compounds (0.7% maltose, 0.7% glucose and 1.3% starch) maximum enzyme production was obtained with 0.7% glucose followed by 0.7% maltose and 1.3% starch. Sabu *et al.*, (2006) studied the effect of additional carbon sources on tannase production by *Lactobacillus* sp. ASR-S1 and found that starch, lactose, glucose, maltose and sucrose all had inhibitory effect on tannase production. Effect of additional carbon sources on tannase production by any *Penicillium* sp. has not been reported.

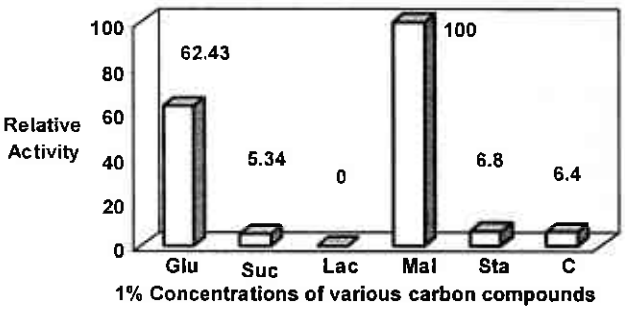

Fig. 1: Effect of various carbon compounds on enzyme production.

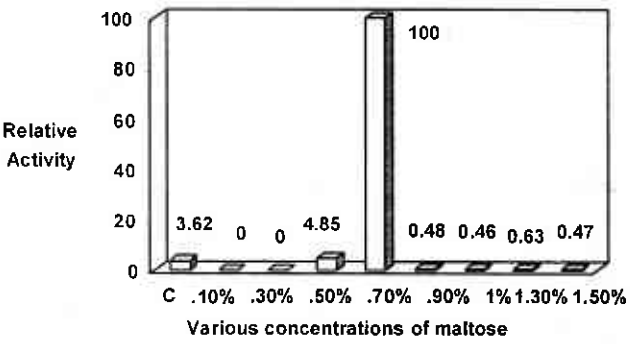

Fig. 2: Effect of various concentrations of maltose on enzyme production.

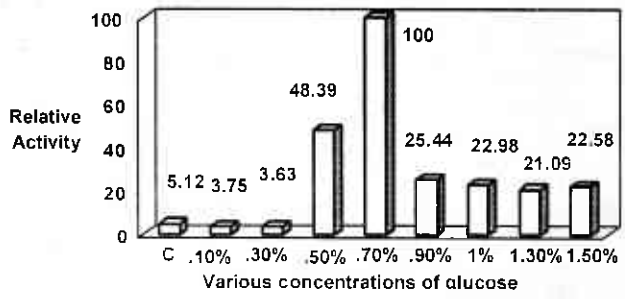

Fig. 3: Effect of various concentrations of glucose on enzyme production.

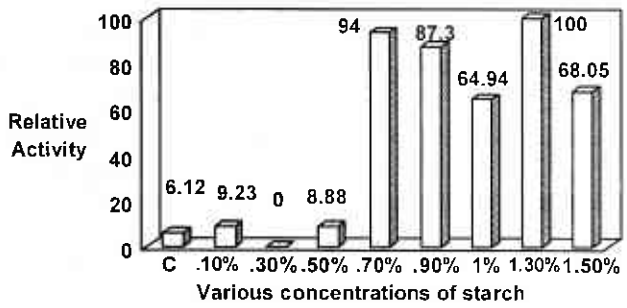

Fig. 4: Effect of various concentrations of starch on enzyme production.

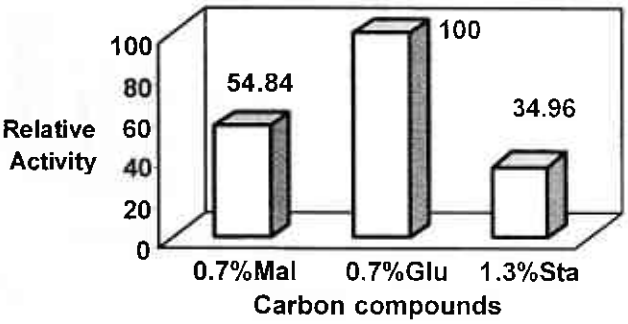

Fig. 5: Comparative study of the 3 highest activity giving carbon compounds on enzyme production.

Various metal ions like potassium sulphate, copper sulphate, sodium benzoate cobalt sulphate, lead acetate, stroncium acetate and calcium lactate at 0.1% concentration were tested for their effect on tannase production (Fig. 6-10). The result showed that among all the metal ions studied copper sulphate, potassium sulphate and calcium lactate gave elevated level of tannase production. Sodium benzoate gave moderate level of increase in tannase production while cobalt sulphate, lead acetate and stroncium acetate showed increased level of tannase production than control but not in appreciable amount. Using the metal ions which gave highest tannase production further work was carried out at various concentrations from 0.01%- 0.1%.Maximum enzyme production was obtained with 0.01% concentration of copper sulphate followed by 0.07 % and 0.05 % concentration of copper sulphate. Least activity was obtained with 0.03 % and 0.02 % concentration of copper sulphate. In case of potassium sulphate maximum enzyme production was obtained with 0.08% concentration followed by 0.03 % and 0.02 % concentration. Least activity was obtained with 0.01 % and 0.05% concentration of potassium sulphate. When calcium lactate was used as a metal salt maximum enzyme production was obtained with 0.04% concentration fol-

lowed by 0.02 % and 0.05 % concentration. Least activity was obtained both with 0.08 % and 0.09% concentration of calcium lactate while 0.01% concentration gave no activity at all. When a comparative study was done with the 3 highest activity giving metal salts (0.01 % $CuS0_4$, 0.04 % calcium lactate and 0.08% K_2S0_4) maximum enzyme activity was obtained with 0.04% calcium lactate followed by 0.08% potassium sulphate and 0.01% copper sulphate.

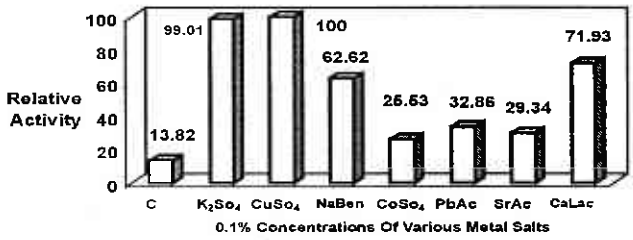

Fig. 6: Effect of metal salts on enzyme production.

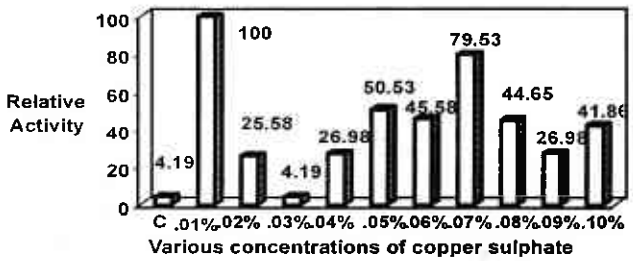

Fig.7: Effect of various concentrations of copper sulphate on enzyme production

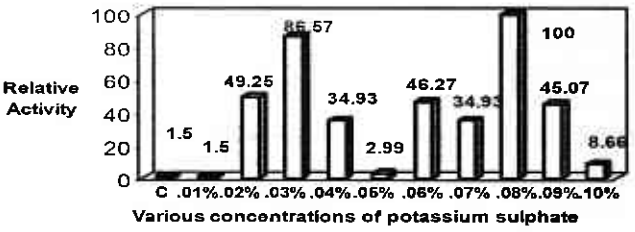

Fig. 8: Effect of various concentrations of potassium sulphate on enzyme production.

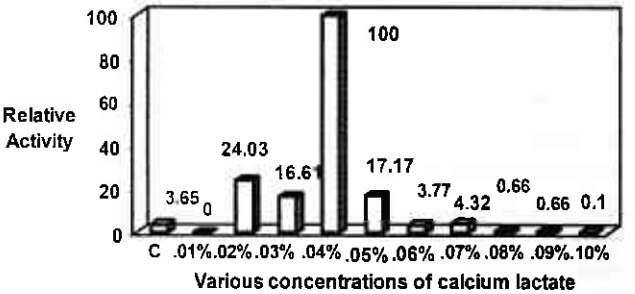

Fig. 9: Effect of various concentrations of calcium lactate on enzyme production.

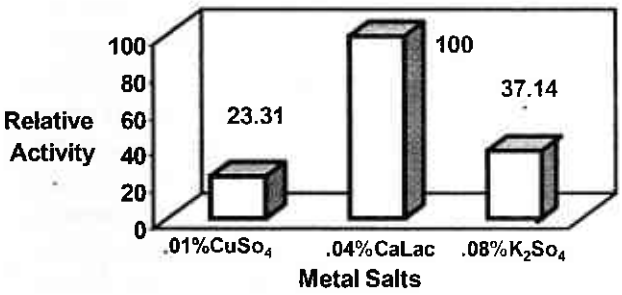

Fig.10: Comparative study of the three highest activity giving metal compounds on enzyme production.

The effect of metal ions on tannase from *Aspergillus awamori* MTCC9299 was studied by (Chhokar *et al.*, 2010) which showed that Mg^{+2}, Mn^{+2}, Ca^{+2}, Na^{+} and K^{+} elevated the tannase activity; on the other hand Cu^{+2}, Fe^{+2} and Co^{+2} inhibited tannase activity. Sabu *et al.*, (2005) also studied effect of metal ions on tannase from *A. niger* ATCC 16620 and found that the addition of metal ions like Zn^{+2}, Mn^{+2}, Cu^{+2}, Ca^{+2}, Mg^{+2} and Fe^{+2} inhibited the enzyme activity. Effect of metal ions on tannase production by any *Penicillium* sp. has not been reported.

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