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Utilization of agri-horticultural wastes for production of tannase enzyme using *Penicillium notatum* NCIM 923 by solid state fermentation

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Wastes are responsible for a major economic loss besides creating environmental pollution and disposal problem. So utilization of wastes is selected to reduce these problems as well as to produce the enzyme almost free of cost. Tannase enzyme can be used in the reduction of tannin present in tannery wastewater thereby reducing the pollution level of water bodies. It can be used in the clarification of fruit juices, preparation of cold water soluble instant tea, reduction of chill haze formation in beer, production of coffee flavored soft drinks and reduction of antinutritional effects of tannin in animal feed. The aim of this work was to produce tannase from various solid wastes by solid-state fermentation technique and the organism of choice being *Penicillium notatum* NCIM 923. Among the various solid wastes used a combination of wheat bran and marigold flower produced maximum tannase activity.

Key words: Marigold, *Penicillium notatum*, tannase, solid-state fermentation, wastes, wheat-bran.

INTRODUCTION

Wastes are the rejected materials, which have very less value in human society. Due to natural fermentation, it releases hazardous gases in the environment, which are also responsible for environmental pollution. In day-to-day life wastage is more than utilization. Thus wastes are also responsible for a major economic loss besides environmental pollution (Bose *et al.*, 2006). Bio-conversion of these wastes to a commercially important enzyme like tannase not only provides useful economic products to solve the disposal problem but also minimize the environmental pollution hazards.

The enzyme 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). The product, gallic acid (3, 4, 5-trihydroxy benzoic acid), is of great importance in food and pharmaceutical industries (Pourrat *et al.*, 1985). Gallic acid is mostly utilized in the pharmaceutical industry for manufacture of trimethoxy benzaldehyde, which is used in the production of a broad-spectrum antibiotic, trimethoprim (Bajpai and Patil, 1996). 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). Tannase enzyme has also been used in the prevention of phenol-induced mediarization in wine (Koichi and Tokuji, 1972), manufacture of coffee-flavoured soft drinks (Suzuki, 1973), clarification of fruit juices (Canteralli *et al.*, 1989), stabilization of malt polyphenol (Giovanelli, 1989), preparation of instant tea (Agbo and Spradlin, 1995), reduction of chill haze formation in beer (Masschelein and Batum, 1981) and reduction of antinutritional effects of tannins in animal feed (Lekha and Lonsane, 1997). Bacteria (Deschamps *et al.*, 1980, 1983; Kumar *et al.*, 1999, Mondal and Pati, 2000), yeast (Aoki *et al.*, 1976), and filamentous fungi are known tannase producers (Bradoo *et al.*, 1996). The present paper deals with the utilization of various waste materials by *Penicillium notatum*, which is an effective tannase

producer to produce the tannase enzyme and characterization of various process parameters to produce maximum tannase activity.

MATERIALS AND METHODS

Organism used

Penicillium notatum NCIM 923 was grown on Czapekdox agar medium and subcultured monthly and stored at 4°C.

Solid-State fermentation

25 g of various wastes materials like wheat bran, vegetable waste, tea leaves waste, baggasse, mixed flower (china rose, marigold, dopatti) wastes and marigold flower wastes were taken in 500 ml conical flasks and to it 25 ml of distilled water was added and mixed well. The flasks were autoclaved at 121°C and inoculated with 5×10^7 spores per ml and incubated at 30°C for fermentation.

Extraction of enzyme

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 cheesecloth. The filtrate was centrifuged at 10,000 rpm for 30 mins. The centrifugate obtained was the crude enzyme.

Enzyme assay

Tannase activity was estimated by a protein precipitation method (Iibuchi *et al.*, 1966).

Enzyme characterization

Optimization of number of days

The fermentation was carried on up to 7 days and enzyme assay was done after every 24 hrs interval to get maximum enzyme activity.

Production of enzyme using various substrates

The fermentation was carried using various substrates to see which substrate produce maximum enzyme activity.

Optimization of various combinations of waste materials

The fermentation was carried on using various combinations of wheat bran and marigold in different ratios (1:9, 1:4, 2:7, 2:3, 1:1, 3:2, 7:3, 4:1, 9:1) to obtain maximum production of enzyme.

Optimization of fermentation temperature

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

Effect of agitation on fermentation

The fermentation was done in static condition as well as at an agitation rate of 50 rpm and 100 rpm and enzyme assay was done.

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.

RESULTS AND DISCUSSION

Optimization of number of days

On optimization of number of days it was found that maximum tannase activity was obtained on the 4th day of fermentation (Fig. 1). About 66.73% of tannase activity was obtained on the 4th day. The next highest activity was obtained on day 3 followed by day 6 and 5. No activity was obtained on 1st, 2nd and 7th day of fermentation.

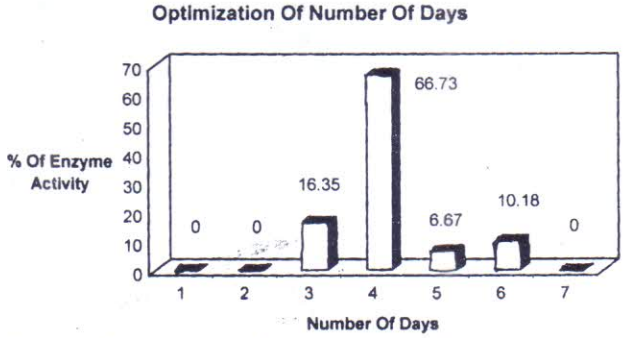

Fig. 1 : Optimization of number of days for production of tannase enzyme using wheat bran as the substrate.

Production of enzyme using various substrates

Fermentation was done using various substrates like wheat bran, vegetable waste, tea leaves extract, baggasse, mixed flower waste and marigold flower

waste. Highest activity of 36% (Fig. 2) was obtained when marigold flower waste was used as substrate whereas mixed flower waste gave 19.4% of activity. Wheat bran gave 12.9% of activity.

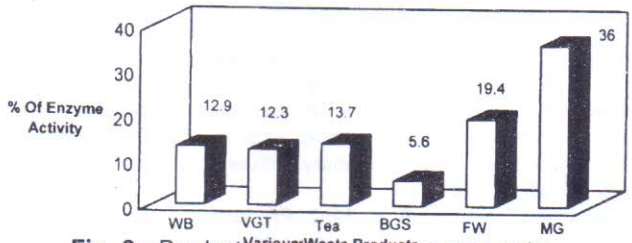

Fig. 2 : Production of tannase enzyme using various substrates.

Utilization of various combinations of waste materials

Fermentation was done using various combinations of wheat bran and marigold to study the effect of combinations of substrates on production of enzyme. About 22.08% of tannase activity was produced when wheat bran and marigold was used in the ratio of 4:1 (Fig. 3) and 14.53% of activity was obtained when the ratios were 9:1 and 1:4.

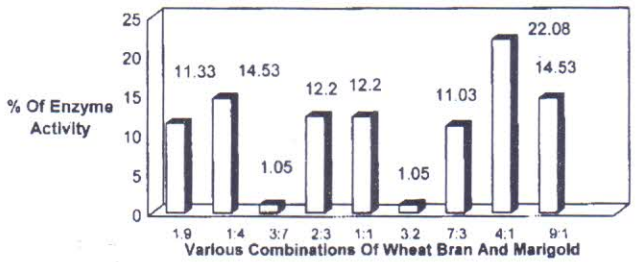

Fig. 3 : Utilization of various combinations of wheat bran and marigold for production of tannase enzyme.

Optimization of fermentation temperature

Fermentation was done at 20°C, 30°C and 40°C. Maximum activity of 56.03% was obtained when fermentation was done at 30°C (Fig. 4). At 20°C 40.17% of activity was obtained. At 40°C the activity was the lowest of about 3.81%.

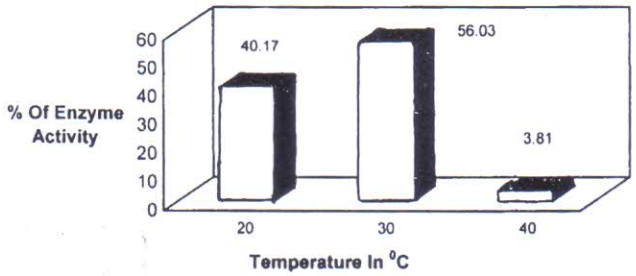

Fig. 4 : Optimization of fermentation temperature for production of tannase enzyme.

Effect of agitation on fermentation

Fermentation was done at static condition as well as in shaking condition of 50 rpm and 100 rpm respectively. Maximum production of enzyme was obtained when fermentation was done under stationary condition (Fig. 5). Production of enzyme decreased in agitation rate of 50 rpm and 100 rpm respectively.

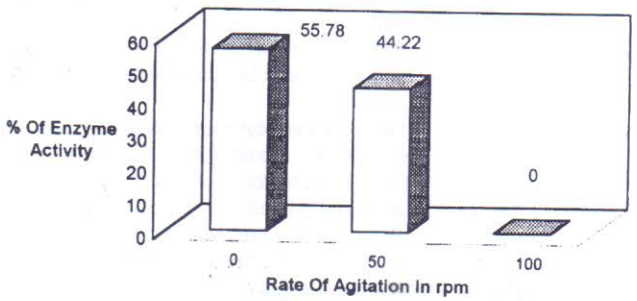

Fig. 5 : Effect of agitation on production of tannase enzyme.

Effect of hydration on fermentation

Maximum activity of 43.16% was obtained when the hydration ratio was 1:1 (Fig. 6). Hydration ratios of 1:3 and 1:4 gave more or less same percentage of activity of 28.76 and 28.06. But no activity was obtained when hydration ratios of 1:0.5 and 1:2 were used.

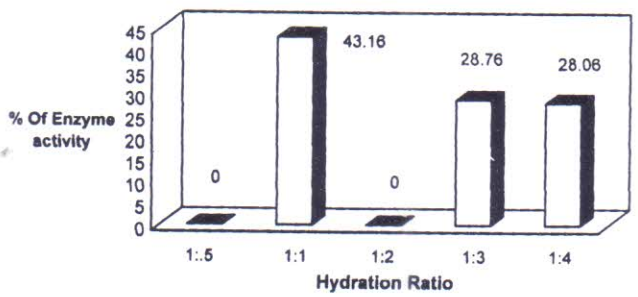

Fig. 6 : Effect of hydration on production of tannase enzyme.

From the above results it is evident that *Penicillium notatum* NCIM 923 produced highest activity on day 4 when wheat bran and marigold are used in a ratio of 4:1. Yields will be better if fermentation is done at 30°C, under stationary condition and in hydration ratio of 1:1. So, tannase enzyme, which has applications in various industries, was obtained in high percentage and with more or less free of cost. So, marigold flower and wheat bran can be used effectively to produce tannase enzyme.

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