Asian Journal of Bio Science, **Vol. 3 No. 2 : 275-278 (October, 2008)**

Immobilization of tannase for commercial use in food industries

VINOD CHHOKAR*, SHWETA KATIYAR, VIKAS BENIWAL, ANIL KUMAR AND J.S.RANA
Department of Bio and Nano Technology, Guru Jambheshwar University of Science and Technology, HISAR (Haryana) INDIA

(Accepted : July, 2008)

Immobilization of tannase from *Aspergillus heteromorphus* was studied on DEAE-Sephadex A-50, Chitin and Ca-alginate. Among the three methods studied, DEAE-Sephadex A-50 was observed the best support to immobilize *A. heteromorphus* tannase with 91.5% immobilization. On the other hand Ca-alginate and Chitin supported 79.3% and 34.5% immobilization, respectively. When the immobilized enzyme was studied for its repeated use, a constant decline in the enzyme activity was observed. However, at the end of seventh cycle, operational stability of the immobilized enzyme was 88 %, 61.7 % and 55.7 % for Chitin, Ca-alginate and DEAE-Cellulose, respectively.

Key words : Tannase, Immobilization, Food industries, *Aspergillus heteromophus.*

INTRODUCTION

Tannin acyl hydrolase (E.C.3.1.1.20) commonly called as tannase, is a hydrolytic enzyme that acts on tannins (Lekha and Lonsane, 1997). Tannins are water soluble polyphenolic compounds with different molecular mass, which form hydrogen bonds in solution that results in the formation of tannin protein complexes and they are thus considered effective anti nutritional compounds. Tannase catalyse the hydrolysis of ester and depside linkages in hydrolysable tannins such as tannic acid, releasing gallic acid and glucose. (Haworth *et al.*,1985). The products of hydrolysis are glucose and gallic acid. Gallic acid is used in the synthesis of propyl gallate, which is mainly used as antioxidant in fats and oils, as well as in beverages. Tannase is also extensively used for the preparation of instant tea, acorn wine, coffee-flavoured soft drinks, clarification of beer and fruit juices, and detannification of foods (Rodríguez *et al.*, 2008; Aguilar *et al.*, 2007 and Lekha and Lonsane, 1997).

Despite the several advantages of tannase in food industries, the commercial use has been limited because (a) most of them are relatively unstable; (b) the cost of enzyme isolation and purification is still very high, and (c) it is technically expensive to recover active enzyme from the reaction mixture after completion of the catalytic process (Kennedy *et al.*, 1987 and Lekha and Lonsane, 1997).These problems can be circumvented by immobilizing the enzyme. Owing to this, the objective of the present study was to immobilize tannase from *Aspergillus heteromorphus*.

MATERIALS AND METHODS

Microorganism and maintenance of culture:
A tannase producing fungus was isolated from the soil of G.J.U.S.&T. campus and identified as *Aspergillus heteromorphus*. The strain was sub-cultured at an interval of 4–5 weeks and maintained routinely on potato dextrose agar (PDA) slants. Freshly grown slant cultures (30^0C) were then used for further work or stored at 4^0C.

Preparation of spore inoculum:
Fungal spore inoculum was prepared by adding 2.5 ml of sterile distilled water containing 0.1 % Tween 80 to a fully sporulated culture. The spores were dislodged using a sterile inoculation loop under strict aseptic conditions and the number of spores in the suspension was determined using the Neubauer chamber. The volume of 1 mL of the prepared spore suspension was used as the inoculum, with concentration of $5x10^9$ spores.

Fermentation of medium:
For the fermentation process, a 250 mL Erlenmeyer flask with 50 ml of Czapek Dox minimal medium (Bradoo *et al.*, 1996) containing (g/L): $NaNO_3$, 6; KH_2PO_4, 1.52; KCl, 0.52; $MgSO_4$. $7H_2O$, 0.52; $FeSO_4$. $7H_2O$, 0.01; $ZnSO_4$. $7H_2O$, 0.01 was employed. The medium was adjusted to pH 5.0 and then sterilized at 121°C for 15 min. Tannic acid solution was prepared separately and the solution was adjusted to pH 4.5 with 0.1 M NaOH, then sterilized by filtering through a sterile membrane (pore size 0.2 mm) and added to the sterilized medium afterward to adjust the final tannic acid concentration of 1%. Flasks were incubated at 30^0C in an incubator shaker at 150

* Author for correspondence.