P-063 **Immobilization of tannase from *Paecilomyces variotii* by ionic gelation**

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INTRODUCTION

Tannin acyl hydrolases, commonly referred to as tannases (E.C. 3.1.1.20), are inducible enzymes produced by fungi, yeast and bacteria. Tannases have mostly been characterized by their activity on complex polyphenolics, and are able to hydrolyze the "ester" bond (galloyl ester of an alcohol moiety) and the "depside" bond (galloyl ester of gallic acid) of substrates such as tannic acid, epicatechin gallate, epigallocatechin gallate producing glucose and potential agent antioxidants (Mahendram 2006; Battestin 2008).

The major concern in an enzymatic process is the instability of the enzyme under repetitive or prolonged use and inhibition by high substrate and product concentration. Immobilization is a very effective alternative in overcoming problems of instability and repetitive use of enzymes. Entrapment method of immobilization is advantageous over other methods as they do not involve chemical modification of the enzyme.

The objective of this work was to encapsulate the tannase enzyme by ionic gelation using different carriers.

MATERIALS AND METHODS

Tannase production

The *Paecilomyces variotii* microorganism was isolated in a previous study (Macedo 2005) and used for tannase enzyme production according to Battestin (2008).

Ionic gelation

The following carriers have been used to ionic gelation: sodium alginate (viscosity 30 cps), sodium alginate from brown algae (viscosity 250cps), gellan gum (2%) and low-methoxyl pectin. In 10 ml of the media were mixed with 25 mg of *P. variotii* tannase. The mixture was dropped into calcium chloride ($CaCl_2$) - (0.55M) using a peristaltic pump to obtain equal size polymeric beads (20mm) of calcium alginate. Finally, these beads were washed with distilled water to remove excess calcium ions and the unbound enzyme and utilized for tannic acid hydrolysis (Mohapatra 2007).

Hydrolysis of tannic acid using encapsulated tannase

The encapsulated tannase beads were added to 5 mL of 0.2% tannic acid (pH 5.5) solution in glass recipients and incubated for 2h at 60°C on a rotary shaker at 140 rpm. Reaction samples were taken for analysis of hydrolysis of tannic acid. A colorimetric assay was used to determine the tannic acid hydrolysis, based on measuring the residual tannic acid content after the enzymatic reaction (Mondal et al. 2001).

The control experiments were also performed using immobilized beads without tannase. To test the stability of immobilized tannase, the beads were used 3 times for the hydrolysis reaction. After each reaction, the beads were collected, washed with distilled water and used for subsequent reaction experiments with fresh tannic acid (substrate). After every reaction cycle, was analyzed the tannic acid degradation

Encapsulation efficiency (EE)

The percentage of protein encapsulated was expressed with respect to the amount of protein remaining in the capsules and total protein (equation 1). In order to dissolve the capsules they were stirred in 0.2M sodium phosphate buffer for over 12h, then centrifuged at 10000 rpm for 20 min. The concentration of protein in the supernatant fluid was measured by the Bradford method (1976).

$$\text{EE (\%)} = \frac{\text{encapsulated protein}}{\text{initial protein}} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

Tannase enzyme from was encapsulated by ionic gelation on various carriers including: sodium alginate, sodium alginate from brown algae, gelan gum and pectin. Figure 1 shows the encapsulated enzyme in different carriers and Table 1 shows the data of the hydrolysis of tannic acid after three consecutive uses of the encapsulated tannase. Table 2 shows the EE o tannase in different carriers.

The beads of encapsulated tannase formed with alginate, independent of the source of it, were better than those produced with pectin and gellan gum, as the spherical shape, mechanical strength, uniform size and smooth surface of the capsules (Fig. 1).

Hydrolysis of tannic acid by the enzyme encapsulated in sodium alginate was much higher compared to other carriers tested. The increase in hydrolysis of tannic acid, to the first use, with the enzyme encapsulated in sodium alginate was 225, 18 and 44% compared with the encap-

sulation of sodium alginate from brown algae, gellan gum and pectin respectively (Table 1). Regarding the reuse of the enzyme encapsulated in sodium alginate, it is possible to verify only that the enzyme lost activity (44%) after its third reuse in the hydrolysis of tannic acid.

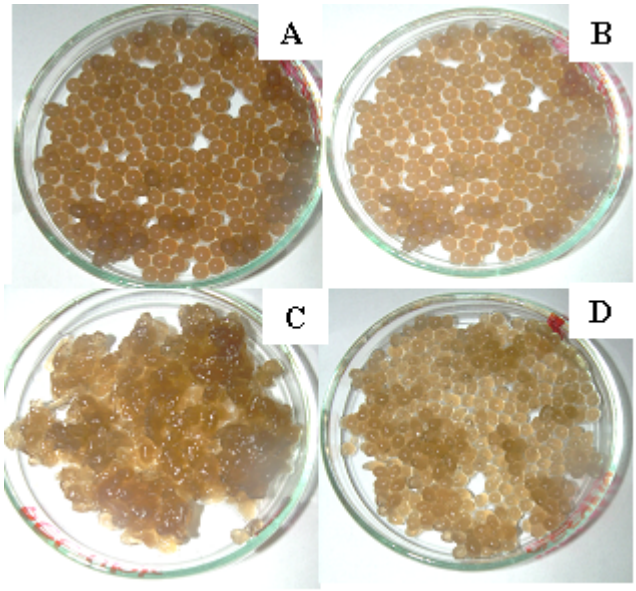

Figure 1 : Beads of encapsulated tannase into sodium alginate (A), sodium alginate from brown algae (B), pectin (C) e gellan gum (D)

The formation, the mechanical and structural properties of beads depend upon different parameters such as the composition and concentration, the presence of impurities of the polysaccharide, nature and concentration of the gelling ion as well as the production process conditions (Chan 2009).

Table 1 : Hydrolysis of tannic acid by encapsulated enzyme in different carriers

Carriers	Tanninc acid hydrolysis (mg/mg capsule)		
	Use 1	Use 2	Use 3
1	0.13 ± 0.02	0.13 ± 0.01	0.10 ± 0.02
2	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01
3	0.11 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
4	0.09 ± 0.02	0.02 ± 0.01	0.01 ± 0.00

1 - Alginate ; 2 - Alginate from brown algae ; 3 - Gellan gum ; 4 - Pectin
Results are presented as the mean (n= 3) ± SD

Table 2 : Encapsulation efficiency of tannase

Carriers	*Encapsulation efficiency (%)*
Alginate	13.99 ± 2.65
Alginate from brown algae	15.48 ± 3.29
Gellan gum	12.48 ± 1.24
Pectin	56.63 ± 6.37

Results are presented as the mean (n= 3) ± SD

The best encapsulation efficiency was obtained with pectin (57%). To alginate, alginate from brown algae and gellan gum the results were similar around 15%.

Evaluating only the EE (Table 2) it could be concluded that the best support for immobilizing the tannase was pectin, however, only a good EE isn't enough, it is also necessary that the capsule has a good mechanical strength and stability in the reuse. Figure 1 shows that the capsules obtained by pectin were very soft and prone to a very intense diffusion of both substrate and enzyme as well as to the breakup. Data from the hydrolysis of tannic acid (Table 1) also shows that pectin capsules containing tannase were effective only on first use with a hydrolysis of 0.09 decreasing to 0.02 and 0.01mg/mg capsule in the second and third use, respectively.

CONCLUSION

Thus we can conclude that the ionic gelation using polysaccharides as support are effective in the encapsulation of tannase and that among the polysaccharides studied, alginate was the most promising.

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