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Short Communication: Polystyrene—an inert carrier for L-glutaminase production by marine *Vibrio costicola* under solid-state fermentation

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Polystyrene beads, impregnated with mineral salts/glutamine medium as inert support, were used to produce L-glutaminase from *Vibrio costicola* by solid-state fermentation. Maximum enzyme yield, 88 U/g substrate, was after 36 h. Glucose at 10 g/kg enhanced the enzyme yield by 66%. The support system allowed glutaminase to be recovered with higher specific activity and lower viscosity than when a wheat-bran system was used.

Key words: L-Glutaminase, inert support, marine bacteria, polystyrene, solid-state fermentation.

Solid-state fermentation (SSF) processes using conventional, nutritionally-rich substrates such as wheat bran have certain inherent problems which could largely be overcome by the use of nutritionally inert supports (Zhu *et al.* 1994). The objective of the present study was to evaluate the use of polystyrene as an inert solid support for the production of L-glutaminase (L-glutamine amidohydrolase; EC, 3.5.1.2), a potent antileukaemic drug and a flavour-enhancing agent of fermented foods, by marine *Vibrio costicola* under SSF.

Materials and Methods

Microorganism

Vibrio costicola ACMR 267 (Renu & Chandrasekaran 1992) was grown in a medium containing (g/l): KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; $NaNO_3$, 0.1; $CaCl_2$, 0.1; trisodium citrate dihydrate, 0.1; glucose, 5; NaCl, 10; and L-glutamine, 10; pH 7.0. The inoculum consisted of cells from a 24-h-old culture, washed twice and resuspended in sterile saline.

Solid Substrate

Expanded polystyrene [Poly (1-phenylethylene)], a commercially available insulating and packaging material, was used as the inert solid support. Beads of 2 to 3 mm diam. were pretreated by autoclaving at 121°C for 15 min, which caused the beads to collapse to about one third of their original size (Brydson 1982).

SSF on Polystyrene or Wheat Bran

A 5-g sample of pretreated beads or wheat bran, 1.0 to 1.5 mm in diam., in a 250-ml Erlenmeyer flask, was moistened with 5 ml mineral salts medium containing 10 g L-glutamine/kg (Renu & Chandrasekaran 1992), autoclaved for 1 h, cooled to room temperature, inoculated with 2 ml of the prepared inoculum and incubated at 37°C for 72 h. No free water was present after inoculation. The pooled extractant from two extractions, each with 5 vol. 0.1 M phosphate buffer, pH 7.0, was filtered through dampened cheese cloth and centrifuged at 10,000 × *g* for 30 min at 4°C. The supernatant was assayed for protein (Lowry's method) and glutaminase (Imada *et al.* 1973). One unit of glutaminase was defined as the amount of enzyme that liberated 1 μmol ammonia under optimal assay conditions. Enzyme activity was expressed as U/g dry substrate (gds). Viscosity was measured using an Ostwald viscometer. The effect of glucose and yeast extract as additional nutrients, either alone (10 g/kg) or in combination (5 g each/kg) on enzyme yield was also tested.

Biomass Estimation

Biomass was estimated in terms of cell protein (Herbert *et al.* 1971) and as dry weight of the cell pellet after enzyme extraction, fresh cells being dried at 105°C overnight.

All experiments were carried out in triplicate and mean values are presented. Appropriate control flasks were also maintained.

Results and Discussion

L-Glutaminase with much higher specific activity could be recovered using polystyrene as support than when using wheat bran (Table 1). Enzyme synthesis was induced by L-glutamine, wheat bran without glutamine failing to support enzyme production (data not shown). The extract obtained from the polystyrene support was also much less viscous (mean viscosity 0.966 Ns/m²) than that from wheat bran

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Table 1. L-Glutaminase production by *V. costicola* during SSF on polystyrene or wheat bran.

Time (h)	Polystyrene		Wheat bran	
	Yield (U/gds)	Sp. act (U/mg protein)	Yield (U/gds)	Sp. act (U/mg protein)
0	Nil	Nil	Nil	Nil
12	35	5.5	56	0.4
24	69	4.2	111	0.8
36	88	4.4	114	0.8
48	56	3.9	92	0.7
60	43	3.8	70	0.5
72	39	3.7	67	0.5

gds—g dry substrate.

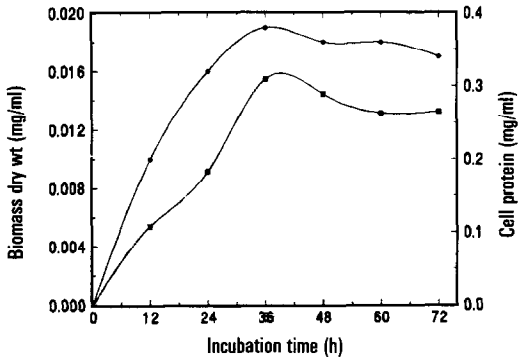

Figure 1. Biomass obtained during L-glutaminase production by *V. costicola* under SSF using polystyrene as inert support. ●—Biomass dry wt; ■—cell protein.

(2.072 Ns/m²) and was free of undesired proteins. The wheat bran extract contained amylase (200 to 400 U/gds) and cellulase (filter paper activity 1.0 to 1.6 U/gds) besides glutaminase.

Incorporation of glucose as additional carbon source not only enhanced enzyme yield, from 69 to 115 U/gds (a 66% increase) after 24 h, but also increased maximal yield (from 88 to 115 U/gds) and decreased the incubation time for maximal enzyme yield (from 36 to 24 h). The increase in enzyme yield was probably the result of the more rapid growth achieved when glucose was available as an additional carbon source. Addition of yeast extract had no significant effect on any parameter measured (data not shown).

Whereas the bacterial growth profile is almost impossible to monitor when conventional substrates are used, biomass, in terms of cell protein and dry weight, could be easily measured using polystyrene as support (Figure 1).

Although wheat bran is cheaper than polystyrene, the latter could be more economical for the production of enzymes because of higher specific activity, purity and cheaper downstream processing, as seen here in glutaminase production. Maximal recovery of the leachate (92% to 96%) and reusability of beads are added advantages of using polystyrene for SSF. We conclude that polystyrene could be a suitable inert solid support for the economic production of L-glutaminase by marine bacteria.

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