Biotechnology Letters Vol.2 No.1, 29-34,

IMMOBILIZED BACTERIAL CELLS CONTAINING A THERMOSTABLE β-GALACTOSIDASE

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SUMMARY: A constitutive β-galactosidase has been localized in the cytosol of thermoacidophilic bacterium *Caldariella acidophila*. Cells have been entrapped in polyacrylamide gel with full retention of enzymic activity; no activity decrease is observed after 8 months of storage. Enzyme properties in entrapped cells are similar to those of the free enzyme. A 73% hydrolysis of lactose has been achieved in a continuous system on a 2 ml entrapped cell column operating at 70° C; half life in these conditions is 30 days.

Introduction

In recent years, immobilization of microbial cells and organelles meets with growing interest for potential applications of such a new type of biocatalyst in industrial technology (Chibata et al., 1974; Broun et al., 1978; Casas et al., 1979; Couderc and Barratti, 1979; Fukui and Thomas, 1979; Linko et al., 1979). Entrapping cells of thermophilic procaryotes offers even further possibility with regard to immobilization procedures and industrial applications. In fact, high stability of enzymes from thermophilic microrganisms towards high temperature, organic solvents and other denaturants is widely recognized (Ljungdahl and Sherod, 1976; Zuber, 1976; Amelunxen and Murdock, 1978; De Rosa et al., 1979).

In this paper we report preliminary data on immobilization of cells of *Caldariella acidophila*, an extreme thermophilic bacterium having a constitutive β-galactosidase (EC 3.2.1.23) activity.

Experimental procedure

Bacterial growth. *Caldariella acidophila*, strain MT-4, has been isolated from an acidic hot spring in Agnano, Napoli (De Rosa et al., 1975). Bacteria are grown in a standard medium (De Rosa et al., 1975) at 87° C, pH 3, in a 25 l fermentor (Terzano) with paddle agitation and an air stream of 3 l/min. Cells are recovered in the logarithmic phase of growth and washed twice with 0.01 M Tris-HCl buffer, pH 7.5. Cells can be stored at -20° C for months without any significant decrease of enzymic activity; however, frozen cells show a complete loss of vitality.

Crude homogenate is prepared by grinding for 10 min at full speed in an Omnimixer (Sorvall) a suspension of cells (10 g) and glass powder (20 g, 100-200 mesh) in 20 ml of the Tris-HCl buffer; a clear supernatant is obtained by centrifugation at 37,000 x *g* for 1 h.

Enzyme assay. Reaction mixture for β-galactosidase assay contains, in a final volume of 1 ml, 0.02 M sodium cacodylate buffer, pH 5.0, 1.5 mM p-nitrophenyl-β-galactopyranoside (Serva) and either 50 mg of wet polyacrylamide-entrapped cells (PEC) or 0.05 ml of a homogeneous cell suspension (1 g wet cells in 10 ml of the cacodylate buffer). After 5 min of incubation at 80° C, the reaction is stopped by cooling and adding 4 ml of 0.25 M Na_2CO_3; finally, after centrifugation for 10 min at 9,000 x *g*, p-nitrophenate is determined at 420 nm. One unit of β-galactosidase is the amount of enzyme that produces 1 μmol of p-nitrophenate in 1 min under the described conditions. When lactose is used as substrate, 0.1 M lactose substitutes for p-nitrophenyl-β-galactopyranoside in the reaction mixture. After stopping the reaction by cooling, the produced glucose is determined by the glucose oxidase-peroxidase assay (Boehringer Mannheim).

Cell immobilization. Ten g of wet cells are suspended in 20 ml of the Tris-HCl buffer and added with 7.5 g of acrylamide (Calbiochem), 0.4 g of bisacrylamide and 0.2 ml of 5% (w/v) potassium persulphate. Nitrogen gas is bubbled through the mixture which is then allowed to stand at

30-35° C for polymerization. The gel is granulated by a 5 min treatment in an Omnimixer at the highest speed and the granules are poured into a column and washed for two days with tap water.

Results and discussion

A thermostable β-galactosidase activity has been localized in the cytosol of the extreme thermoacidophilic bacterium *Caldariella acidophila*. The enzyme is constitutive as no increase of β-galactosidase activity is observed when standard culture medium is supplemented with lactose.

Enzymic activity is also found in intact cell suspensions but, in this case, only one tenth of the activity present in crude homogenate is revealed (see Table) because of permeability features of intact cell membrane. A strong increase in β-galactosidase activity of cell suspensions is observed when cells are permeabilized with an organic solvent treatment which disorganizes cell membrane structure without causing any apparent lysis of *C. acidophila* cells.

Cell entrapment in polyacrylamide gel results in a sharp increase of β-galactosidase activity as compared with intact free cells (see Table). Such an increase is probably related, as for the acetone treatment, to membrane permeabilization effects ascribed in this case to acrylamide monomers in the polymerization mixture. No significant cellular lysis has been observed by direct microscopic observation of unpolymerized cell suspensions. Acetone treatment of PEC does not cause any marked change of β-galactosidase activity.

PEC can be optimally stored in a wet form at 4° C; up to date, after 8 months of storage in these conditions, no decrease of the enzymic activity has been observed. Either lyophilization or heat dehydration at 80° C of PEC give rise to a 30-40% loss of enzymic activity; moreover, half life of lyophilized or dehydrated PEC, stored at 4° C, is about one month.

Temperature and pH optima for β-galactosidase activity of both entrapped cells and free enzyme are 90° C and 5.0, respectively. Half life of PEC in the cacodylate buffer at 70 and 85° C is 10 and 2 days, respectively; such figures are of the same order of magnitude of those obtained with free enzyme. The K_m of PEC for lactose is 44 mM in the standard assay conditions.

Preliminary data obtained by passing at a flow rate of 1 ml/h lactose (10 mM) through a PEC column (0.7 x 5.2 cm) thermostated at 70° C show a 73% hydrolysis of the substrate. Under the above described conditions, half life of PEC in the continuous system is about three times higher (30 days) than that observed in the absence of the substrate. TLC analysis of column eluate reveales the presence of lactose, glucose and galactose only.

To evaluate the potentiality of PEC for industrial processing of milk, commercial milk has been passed through the column obtaining a 9% hydrolysis of milk lactose; this value is very similar to that obtained by passing 116 mM lactose, which is the extimated concentration of this disaccharide in the natural product. Studies are in progress to optimize conditions for lactose hydrolysis in natural products by scaling up our experimental model.

Main drawbacks in industrial processing of milk and related products for lactose hydrolysis are represented by i) microbial contamination and ii) poisoning of the immobilized catalyst by fats. Preliminary data reported in this paper show that *C. acidophila* cells entrapped in an insoluble lattice may represent a valuable system to overcome the above cited disadvantages as i) the operating temperature strongly prevents microbial growth and ii) poisoning by fats can be removed by washing the resin with an organic solvent.

T A B L E

Activity of β-galactosidase in differently treated cells

Sample	Enzymic units* per g of wet cells
Crude homogenate	11.3
Intact cells	1.1
Acetone-treated cells**	12.2
Polyacrylamide-entrapped cells (PEC)	9.4

*Assays are performed with p-nitrophenyl-β-galactopyranoside as substrate.

**One gram of wet cells are stirred for 1 h in 10 ml of acetone; after centrifugation, cells are resuspended in 25 ml of the cacodylate buffer.

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