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BRIEF COMMUNICATION

β-Galactosidase in immobilized cells of *Papaver somniferum*

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Abstract

Cell suspension culture of poppy was permeabilized by Tween 80 and immobilized by glutaraldehyde. β-Galactosidase showed optimum pH 4.8 and temperature of 50 °C. The enzyme hydrolysis was linear during 3 h reaching 68 % conversion. The cells characterized by high β-galactosidase activity and stability in long-term storage showed convenient physico-mechanical properties.

Additional key words: cell immobilization, cell suspension, glutaraldehyde, poppy.

Many matrices from synthetic polymers or biological materials have been used for the immobilization of cells. The most widely used technique of cell immobilization is the entrapment, when the living cells are enclosed into particles of gels: agar, agarose, kappa-karragenan, polyurethane, or cellulose (Tampion and Tampion 1987, Hulst and Tramper 1989). Some biogels, lack open spaces to accomodate new cell growth which then results in their rupture and the release of cells into the medium (Iqbal and Zafar 1994). Their efficiency is also limited by diffusion restrictions (Hulst and Tramper 1989) and decreased ezyme activity (Wada *et al.* 1979). Recently, the use of polyvinylalcohol and glutaraldehyde for cell immobilization has been investigated (Wu and Wisecarver 1992, Hasal *et al.* 1992).

Poppy cells immobilized in calcium alginate retained their biological activity for 6 months. These cells performed the biotransformation of codeinone to codeine with higher biotransformation ratio than in cell suspensions (Furuya *et al.* 1984).

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β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) catalyses the hydrolysis of terminal β-galactosidic linkage of glycosides. The enzyme is widely distributed in various plant tissues, the precise role of the enzyme is not well understood. It has been suggested that it is involved in the degradation of plant cell-wall polysaccharides in relation to cell growth, fruit ripening and seed germination (Simons *et al.* 1989, Sekimata *et al.* 1989).

The enzymatic hydrolysis of terminal β-galactosidic linkage of glycosides by poppy suspensions, as well as immobilized cells by glutaraldehyde was studied.

Long-term tissue culture was derived from seedlings of *Papaver somniferum* L. cv. Amarín by Dr. K. Erdelský (Department of Plant Physiology, Comenius University, Bratislava) and was cultivated as described previously (Stano *et al.* 1995).

Cell suspension was filtered through a nylon cloth and 15 g of fresh mass was suspended in 50 cm^3 of 6 % Tween 80 in 0.15 M NaCl solution. Permeabilization proceeded for 3 h under moderate stirring at 20 °C. The cells were filtered off and washed with 3 000 cm^3 of distilled water and 1 500 cm^3 of 0.15 M NaCl solution.

The permeabilized cells were immediatelly suspended in 50 cm^3 of 0.15 M NaCl solution and 5 cm^3 of 25 % glutaraldehyde solution was added slowly under mild stirring at laboratory temperature. The immobilized cells were washed with 2 000 cm^3 of distilled water and 2 000 cm^3 of 0. 15 M NaCl solution and separated by filtration.

Fresh and dry masses of living and immobilized cells were determined gravimetrically. For determination of dry mass, samples were dried to the constant mass at 100 °C.

The influence of temperature on enzymatic activity was tested in the range from 20 to 100 °C. The effect of glucose, fructose, galactose and gluconelactone on the activity of β-galactosidase in suspension culture and immobilized cells was tested in the following concentrations: l, 5, 10 and 20 mM, respectively.

The ezyme activity was determined by a modified method of Simons *et al.* (1989) using *p*-nitrophenyl-β-D-galactopyranoside (PNG) as substrate. The cells were separated from the reaction mixture, dried and enzyme activity was calculated per dry mass unit. The enzyme activity is expressed in katals. Protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Cell viability was determined by vital staining according to Dixon (1991) with 2,3,5-tri-phenyltetrazolium chloride (TTC) and fluorescein diacetate and by respiratory activity, measured with oxygen electrode.

Immobilized cells (cells enclosed in a polymer matrix) are cultivated in similar way as suspension cultures (Furuya *et al.* 1984, Tampion and Tampion 1987, Hulst and Tramper 1989). However, the microscopic investigation of cells immobilized by glutaraldehyde compared with cell suspensions showed evident morphological changes. The most striking is the thinning of cell walls after permeabilization by Tween 80. Important is also appearance of cell plasmolysis and the little aggregation of cells occuring during immobilization. The cells immobilized by glutaraldehyde are non viable.

It was shown that biotransformation ratio of cells entraped in beds is usually comparable with their viability. Furuya *et al.* (1984) observed that the biotransformation ratio of codeinone to codeine by fresh immobilized cells of *Papaver somniferum* was 70 %, and after 30 d under optimal conditions it decreased to 42 %. The immobilization of cells has important advantages, *e.g.*, the possibility of continuous flow-through arrangement, possibility of maintaining high cell densities, easy separation of products from biocatalyst, considerable prolongation of the biocatalyst half life, physical protection from shear forces, prevention of cell aggregation, stimulation of the secondary metabolite production compared with a free cell suspension, and the preservation of the activity of multifunctional enzyme system (Hulst and Tramper 1989, Tampion and Tampion 1987). This may be useful for the biosynthesis of some plant secondary metabolites, or their precursors. In the cells immobilized by crosslinking using bifunctional reagents, *e.g.*, glutaraldehyde, L-3,4-dihydroxyphenylalanine-decarboxylase and tyrosine decarboxylase activities reached after 3 months still high values (Stano *et al.* 1994). However, it is probable that the multifunctional enzyme system is not fully conserved. In the case of immobilization by glutaraldehyde the permeabilization led to substantial loss of proteins, while enzyme activity showed a moderate decrease (Table 1). The immobilized cells had pH optimum at 4.8 as viable cells. The immobilized cells did not show a minor peak as it was shown by α-galactosidase (unpublished results). PNG hydrolysis was linear for 3 h reaching 75 % of conversion, than almost stops. The temperature optimum for immobilized cells and suspensions was 50 °C and for the partially purified ezyme 40 °C. These value was lower than for α-galactosidase. The inhibitory effect of 0.1 - 0.5 mM *p*-chlormercuribenzoic acid can be eliminated with 5 - 10 mM cysteine, dithiothreitol, or 2-mercaptoethanol (Budík 1992). These results indicate that the SH groups are essential for the ezyme activities of α- and β-galactosidase.

Table 1. β-galactosidase activity and specific activity in cell suspensions and immobilized cells.

Cells	Protein [mg g^{-1}(d.m.)]	Activity [nkat g^{-1}(d.m.)]	Specific activity [nkat g^{-1}(protein)]
Cell suspension	45.2 ± 0.25	4.2 ± 0.21	0.093
Permeabilized cells	14.8 ± 0.20	3.1 ± 0.20	0.209
Immobilized cells	14.7 ± 0.25	3.2 ± 0.25	0.217

Storage stability of immobilized cells (at 4 °C) were tested during half year. In the case of β-galactosidase a more marked decrease of activity after immobilization (68 % of original activity) was observed compared with the activity loss of tyrosine and DOPA decarboxylase (Stano *et al.* 1995). However, after a 4 - 6 month storage at 4 °C in 0.15 M NaCl and 0.02 % sodium azide β-galactosidase activity increased to 70 - 88 % of the original activity (Table 2).

According to the results of Machová (1984) and Budík (1991) glucose and galactose inhibited the activity of the α- and β-galactosidase in a moderate way. The

same effect by glucose and galactose has been ascertained in living poppy cells (Budík 1991). This fact could be an explanation of the moderate decrease of the activity during immobilization and activation resulting from the time-dependent dissociation of the possible inhibitors (monosaccharides or others) by the enzyme.

Table 2. Storage stability of immobilized cells.

Material	Relative β-galactosidase activity [%]
Cell suspension	100
Inactivated cells	0
Fresh immobilized cells	68
Inactivated immobilized cells	0
Immobilized cells after 1 month	66
Immobilized cells after 3 month	72
Immobilized cells after 4 month	78
Immobilized cells after 5 month	83
Immobilized cells after 6 month	88

The immobilization of poppy cells by glutaraldehyde and their storage in 0.15 M NaCl with 0.02 % sodium azide solution seems to be a very convenient method for long-term preservation of different catalysts. However, the conservation of the multifunctional enzyme systems of cell needs futher study. The results obtained indicate the possibility to use immobilized α- and β-galactosidases in the biotechnology of certain saccharides (raffinose, stachyose and galactans).

References

Budík, D.: α-galactosidase in poppy cell suspension (*Papaver somnifrum* L.). - Thesis. Comenius University, Bratislava 1992.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Dixon, R.A.: Isolation and maintenance of callus and cell suspension cultures. - In: Dixon, R.A. (ed.): Plant Cell Culture. A Practical Approach. Pp. 1-20. IRL Press, Oxford, Washington 1991.

Furuya, T., Yoshikawa, T., Taira, M.: Biotransfonnation of codeinone to codeine by immobilized cells of *Papaver somniferum*. - Phytochemistry **23**: 999- 1001, 1984.

Hasal, P., Vojtíšek, V., Čejková, A., Kleczek, P., Kofroňová, 0.: An immobilized whole yeast cell biocatalyst for enzymatic sucrose hydrolysis. - Enzyme Microbiol. Technol. **14**: 221-229, 1992.

Hulst, A.C., Tramper, J.: Immobilized plant cells: A literature survey. - Enzyme Microbiol. Technol. **11**: 546-558, 1989.

Iqbal, M., Zafar, S.I.: Petiolar felt-sheath of palm: A new matrix for fungal immobilization. - Biotechnol. Technol. **8**: 755-758, 1994.

Machová, B.: α-Galactosidase in gherkin seedlings. - Thesis. Comenius University, Bratislava 1984.

Sekimata, M., Ogura, K., Tsumuraya, Y., Hashimoto, Y., Yamamoto, S.: A β-galactosidase from radish (*Raphanus sativus* L.) seeds. - Plant Physiol. **90**: 567-574, 1989.

Simons, G., Giannakouros, T., Georgatsos, J.G.: Plant β-galactosidases: Purification by affinity chromatography and properties. - Phytochemistry **28**: 2587-2592, 1989.

Stano, J., Nemec, P., Weissová, K., Kovács, P., Kákoniová, D., Lišková, D.: Decarboxylation of L-tyrosine and L-DOPA by immobilized cells of *Papaver somniferum*. - Phytochemistry **38**: 859-860, 1995.

Tampion, J., Tampion, M.D.: Immobilized Cells: Principles and Applications. - Cambridge University Press. Cambridge 1987.

Wada, M., Kato, J., Chibata, I.: A new immobilization cells. Immobilized growing cells using carrageenan gel and their properties. - Eur. J. appl. Microbiol. Biotechnol. **8**: 241 -247, 1979.

Wu, K.Y.A., Wisecarver, K.D.: Cell immobilization using PVA crosslinked with boric acid. - Biotechnol. Bioeng. **39**: 447-449, 1992.