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QUARTERLY

β-Galactosidase in immobilized cells of gherkin *Cucumis sativus* L.

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Cell suspensions of gherkin (*Cucumis sativus* L.) were permeabilized by Tween-80, and immobilized by glutaraldehyde. β-Galactosidase showed pH optimum at 4.9 and temperature optimum at 58°C. The enzyme catalysed hydrolysis was linear for 3 h with 60–68% conversion of the substrate. The cells characterized by high β-galactosidase activity and stability on long-term storage showed valuable technological properties.

Plant cells were first immobilized by Brodelius *et al.* [1] with alginate. At present the most widely used technique of cell immobilization is the entrapment in agar, agarose, kappa-carrageenan, collagen, alginates, chitosan, polyacrylamide, polyurethane, or cellulose [2, 3]. The spontaneous adhesion or covalent binding of cells to the surface of insoluble carriers was also examined [4, 5]. Calcium pectate gel (CPG) and calcium alginate gel (CAG) were used for the entrapment of permeabilized cells of *Kluyveromyces marxianus* CCY e SY 2 as the source of lactase (β-galactosidase). The activities of β-galactosidase in permeabilized cells entrapped in these hardened gels were stable on storage at 4°C for 2

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Abbreviations: ATDNO, (1-methyldodecyl)-dimethylamine-4-oxide; CAG, calcium alginate gel; CLCTC, chlortetracycline hydrochloride; CPG, calcium pectate gel; βPNG, *p*-nitro-phenyl-β-D-galactopyranoside; TTC, 2,3,5-triphenyltetrazolium chloride.

months [6, 7]. Recently, the use of polyvinylalcohol [8] or glutaraldehyde for cell immobilization has been investigated [7, 9].

β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) catalyses the hydrolysis of terminal β-galactoside linkage of glycosides. The enzyme, is widely distributed in various plant tissues, but its precise role is not well understood. It has been suggested that this enzyme is involved in degradation of plant cell-wall polysaccharides during cell growth, fruit ripening and seed and pollen germination [10–13]. Although β-galactosidase is commonly present in plants immobilized plant cells so far have not been used for preparation of the enzyme. In the present work enzymatic hydrolysis of terminal β-galactosidic linkage of glycosides by gherkin cell suspensions and the cells immobilized by glutaraldehyde was compared.

MATERIAL AND METHODS

Tissue cultures. Long-term callus culture was derived from seedlings of *Cucumis sativus* L. var. Znojmia and continuously subcultured every three weeks on Murashige-Skoog medium [14], supplemented with 2,4-dichlorophenoxyacetic acid (0.5 mg/l), benzylaminopurine (0.2 mg/l), and vitamins, respectively, according to Brown and Lawrence [15] at 24 ±1°C in the dark.

Cell permeabilization. Cell suspensions were filtered through a nylon cloth and 15 g of fresh mass suspended in 50 ml of 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 3000 ml of distilled water and 2000 ml of 0.15 M NaCl solution.

Immobilization. The permeabilized cells were immediately suspended in 50 ml 0.15 M NaCl solution, at slow addition of 5 ml of 25% glutaraldehyde under mild stirring at room temperature for 2 h. Immobilized cells were washed with 2000 ml of distilled water and 2000 ml of 0.15 M NaCl solution and separated by filtration.

Fresh and dry mass. Fresh and dry mass of cell suspensions were determined gravimetrically. For determination of dry mass, samples were dried to constant mass at 105°C.

The influence of temperature and some sugars on enzyme activity. The influence of temperature was tested from 20°C to 100°C.

Storage stability. Stability of β-galactosidase during storage was monitored in the following experiments. The immobilized cells were stored at 4°C in 0.15 M NaCl supplied with following compounds: a – chloramphenicol 50 mg/l, b – chlortetracycline hydrochloride (CLCTC) 50 mg/l, c – (1-methyldodecyl)-dimethylamine-4-oxide (ATDNO) 100 mg/l [16]. These experiments were repeated at least three times.

Glucose utilization. The immobilized cells and cell suspensions were exposed to initial glucose concentration 200 mg/l in the cultivation medium [14, 15] devoid of sucrose. Concentration of glucose was determined by the method of Trinder [17].

The cells immobilized by glutaraldehyde were dried for 24–36 h in thin layer at laboratory temperature and then were stored in tightly closed polyethylene bottles at 4°C or 25°C, respectively. The dry biocatalyst needs to be soaked in water or buffer at least for 1–2 h prior to its application (unpublished).

Enzyme assay. The enzyme assay was performed by the modified method of Simons *et al.* [10] using *p*-nitro-phenyl-β-D-galactopyranoside (βPNG) as a substrate. The reaction mixture contained 0.1 g of wet cells and 0.5 mg βPNG in 2 ml McIlvaine buffer, pH 4.9. The control contained boiled cells. Both mixtures were kept for 20 min to 5 h at 30°C on rotary shaker (80 r.p.m.) and the reaction was stopped by adding of 1 M Na_2CO_3. Substrate conversion was calculated basing on the decrease in substrate concentration following 3 h incubation. The nitrophenol released was determined spectrophotometrically at 420 nm.

The cells were separated from the reaction mixture, dried and the enzyme activity was calculated for 1 g of dry mass [18].

The determination of enzyme activity was repeated at least five times and the enzyme activity is expressed in katals. Protein content was determined by the method of Bradford [19] using bovine serum albumin as a standard.

Cell viability. This was determined by the method of Dixon [20] with 2,3,5-triphenyltetrazolium chloride (TTC) or fluoresceindiacetate and oxygen electrode.

RESULTS

Microscopic examination of the cells immobilized by glutaraldehyde compared to cell suspensions showed evident morphological differences. The most striking was thinning of cell walls after permeabilization with Tween-80. Noteworthy was also the appearance of cell plasmolysis and some aggregation of the cells ocurring during immobilization. It was observed that the cells immobilized by glutaraldehyde did not utilize glucose (Fig. 1) and were not viable as they do not show respiratory activity and are not stained with fluorescein, or 2,3,5-triphenyltetrazolium chloride.

Permeabilization and immobilization by glutaraldehyde led to substantial loss of proteins, with a concomitant increase of specific enzyme activity (Table 1).

Enzyme hydrolysis of βPNG was linear within 3 h reaching 60–68% of substrate conversion, than practically stops. β-Galactosidase in the immobilized cells as in the viable cells showed pH optimum at 4.9. The temperature optimum of the immobilized cells and the cells in suspension was at 58°C.

As illustrated in Table 2, the activity of the enzyme in gherkin cells immobilized with glutaraldehyde (in 0.15 M NaCl with all preservatives tested) during 6 months storage is still relatively high.

DISCUSSION

Immobilized cells (cells enclosed in a polymer matrix) are cultivated in a similar way as suspension cultures [2, 3, 21].

Sucrose is probably the most widely used carbon source in plant tissue cultures. Its utilization is followed by a rapid initial inversion and sequential phases of glucose and fructose consumption. Glucose and fructose are present in the media in roughly equal amounts after the first few days of inoculation, but the cells did not consume fructose until glucose is present [22]. Figure 1 shows the results of glucose utilization by cell suspensions and by glutaraldehyde immobilized cells. In contrast to the cells immobilized in the alginate gels [22] the glutaraldehyde crosslinked cells did not utilize glucose (Fig. 1).

Similar properties as by β-galactosidase immobilized in gherkin were reported for β-galactosidase isolated from winter rape [12], poppy [23], and ginseng [24].

The inhibitory effect of 0.1–0.5 mM *p*-chlormercuribenzoic acid on α-galactosidase can be

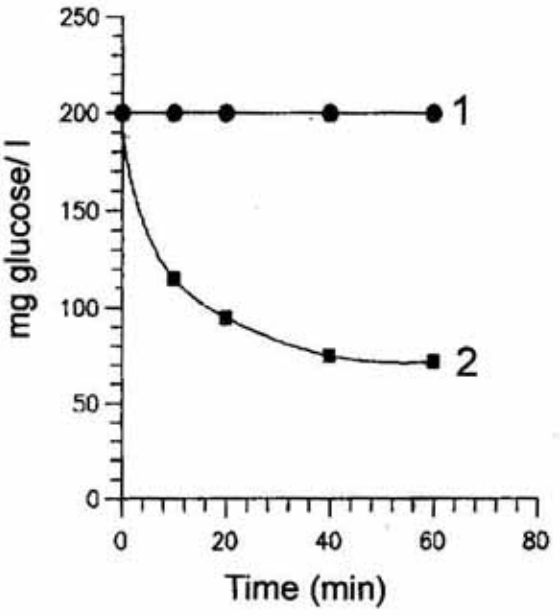

Figure 1. Time course of glucose utilization in: 1, cells immobilized by glutaraldehyde; 2, cells suspensions.

eliminated with 5-10 mM cysteine, dithiothreitol or 2-mercaptoethanol [25]. These results indicate that the SH- groups are essential for the enzyme activities of both α- and β-galactosidase [26].

with the enzyme. The tested preservatives do not influence the enzyme activity.

The immobilization costs are very low, and no special equipment is needed. Aeration, agitation and the kind of cultivation medium

Table 1. β-Galactosidase activity in cells suspension and in immobilized cells of gherkin

Cells	Protein (mg/g dry mass)	Activity (nkat/g dry mass)	Specific activity (pkat/mg protein)
Suspension	45.3 ± 0.66	4.6 ± 0.31	0.101
Permeabilized	14.7 ± 0.70	3.2 ± 0.23	0.217
Immobilized	14.5 ± 0.64	3.1 ± 0.23	0.213

β-Galactosidase in the immobilized gherkin cells were inhibited by galactose and glucose similarly as the partially purified enzyme preparation [26].

As illustrated in Table 2, the activity of the enzyme in gherkin cells immobilized by glu-

have no influence on biotransformational potential of glutaraldehyde immobilized cells. Immobilization of the cells makes enzyme isolation unnecessary, whereas the specific enzyme activity of biocatalysts remains quite high [9]. The cells immobilized by glutaralde-

Table 2. Stability of β-galactosidase in the immobilized gherkin cells on storage

Conservance	Original activity in suspension culture (%)				
	0 month	1 month	2 months	3 months	6 months
None	68	-	-	-	-
CLCTC (50 mg/l)	66	68	70	79	91
ATDNO (100 mg/l)	66	69	71	81	93
Chloramphenicol (50 mg/l)	66	71	73	83	95
Sodium azide (200 mg/l)	64	71	74	85	98
Frozen in 0.15 M NaCl	65	70	75	84	99

CLCTC, chlortetracycline hydrochloride; ATDNO, (1-methyldodecyl)-dimethylamine-4-oxide; original activity = enzyme activity (100%) in cell suspension without immobilization.

taraldehyde (in 0.15 M NaCl with all preservatives tested) during 6 months' storage is still relatively high. The same phenomenon (an increase of α- and β-galactosidase during storage was observed in the immobilized cells of poppy and ginseng [23-25] as well as in other plants (unpublished). The observed increase in the activity on storage remains unclear. It might be due to a gradual dissociation of inhibitory compounds originally interacting

hyde (by crosslinking) compared with the cells immobilized by entrapment in beads (alginate, carrageenan or other matrices) bring some important advantages.

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