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Activity of β–galactosidase in immobilized cells of tomato

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SUMMARY. The cell suspension culture of tomato was permeabilized by Tween 80 and hexadecyltrimethylammonium bromide, respectively, and immobilized by glutaraldehyde. β–Galactosidase showed a pH optimum at 4.6 and a temperature optimum at 50 °C. The enzyme hydrolysis was linear within 3.5 h and reached 65 % conversion. Good storage stability was achieved in a solution of 0.15 mol.l^{-1} NaCl supplemented with a chloramphenicol, (1-methyldodecyl)-dimethylamin-4-oxide (ATDNO), and chlortetracycline hydrochloride (CLCTC) respectively. The cells were characterized by a high enzyme activity, stability in long term storage and showed convenient physico-mechanical properties.

KEYWORDS: immobilization, permeabilization, β–galactosidase, tomato

Immobilization techniques have had a great impact on technology. In the last decades, several methods for fixation of biocatalysts were developed. Enzymes, living or non-living microorganisms, animal and plant cells, as well as combined systems, have been bound within or to carrier materials [1-6]. Immobilization of the cells or enzymes represents an effective way of highly efficient enzyme catalysts important for biotransformation process [7]. Biological or synthetic materials have been used for the immobilization of cells. The most widely used technique of cell immobilization is the entrapment, when living cells are enclosed into particles of the gels, e.g. agar,

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agarose, κ–carrageenan, collagen, chitosan, polyacrylamide, polyurethane, or cellulose [1,8]. The spontaneous adhesion or covalent binding of cells to the surface of insoluble carriers was also examined [6-10]. Recently, the application of polyvinylalcohol and glutaraldehyde [11,12], or Tween 80 and glutaraldehyde [2] for cell immobilization have been applied.

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, however, its role is not well understood. The enzyme is supposed to be involved in the degradation of plant cell wall polysaccharides in relation to the cell growth, fruit ripening and the seeds and pollen germination [13-16]. Although β–galactosidase is generally present in plants, this source has not been used previously for the cell immobilization. The enzyme hydrolysis of the terminal β–galactosidic linkage of glycosides by tomato cell suspensions, as well as by cells immobilized by glutaraldehyde was studied in this paper.

Materials and methods

Tissue cultures

Long-term callus culture was derived from seedlings of tomato (*Lycopersicum esculentum* Mill.) and continuosly subcultured every week on Murashige-Skoog medium as was described by Blanáriková et al. [17].

Cell permeabilization

Cell suspensions were filtered through a nylon cloth and 15 g of fresh mass suspended in 50 ml of 5 % Tween 80 and 0.1 % hexadecyltrimethylammonium bromide in 0.15 mol.l^{-1} NaCl solution, respectively. Permeabilization proceeded for 3.5 h under moderate stirring at 20 °C. The cells were filtered off, washed with 2000 ml of distilled water and 3000 ml of 0.15 mol.l^{-1} NaCl solution, and separated by filtration.

Cell immobilization

The permeabilized cells were immediatelly suspended in 50 ml of 0.15 mol.l^{-1} NaCl solution, and 5 ml of 25 % glutaraldehyde solution was added slowly under gentle stirring for a period of 2.5 h at laboratory temperature. The immobilized cells were then separated and washed with 2000 ml of distilled water and then with 2500 ml of 0.15 mol.l^{-1} NaCl solution, and were stored in 0.15 mol.l^{-1} NaCl solution with a preservative at 4 °C and/or -20°C.

Determination of wet and dry mass

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

Influence of temperature

The effect of temperature on enzyme activity was tested in the range from 20 to 100 °C.

Storage stability

Stability of β–galactosidase during the storage was monitored in the following experiments. The immobilized cells were stored at 4 °C in 0.15 mol.l^{-1} NaCl with the addition of following compounds alternatively:

a) chloramphenicol 50 mg.l^{-1},
b) chlortetracycline hydrochloride (CLCTC) 50 mg.l^{-1},
c) (1-methyldodecyl)-dimethylamine-4-oxide (ATDNO) 100 mg.l^{-1} [18].

Glucose utilization

The immobilized cells and cell suspensions were exposed to initial glucose concentration 200 mg.l^{-1} in the cultivation media [17] without the presence of sucrose. The concentration of glucose was determined by the method of Trinder [19].

Enzyme assay

The enzyme assay was performed by a modified method of Simons et al. [18] using *p*-nitro-phenyl-β–D-galactopyranoside (β–PNG) as a substrate. The reaction mixture contained 0.1 g of wet cells and 0.5 mg β–PNG in 2 ml of McIlvaine buffer of pH 4.6. The control contained boiled cells only. Both mixtures were kept in a period ranging from 20 min to 5 h at 30 °C at rotary shaking (80 rpm) and the reaction was terminated by the addition of 2 ml of 2 mol.l^{-1} Na_2CO_3. A release of nitrophenol was determined spectrophotometrically at 420 nm. Cells were separated from the reaction mixture, dried and the enzyme activity was calculated for 1 g of dry weight [3]. The enzyme activity is expressed in katals. Protein content was determined by the method of Bradford [20] using bovine serum albumin as a standard.

Cell viability

The cell viability was determined with 2,3,5-triphenyltetrazolium chloride (TTC), with fluoresceindiacetate and with an oxygen electrode, respectively [21].

Results and discussion

Cells immmobilized by entrapment were cultivated in a similar way as cell suspension cultures [1,8,22]. However, the microscopic investigation of cells immobilized by glutaraldehyde showed evident morphological changes, compared with intact cell suspensions. The most striking was the thinnig of cell walls after permeabilization by Tween 80 and hexadecyltrimethylammonium bromide respectively. The appearance of the cell plasmolysis and the low degree of the aggregation of cells occuring by the immobilization was also important. The viability of the immobilized cells was determined by respiratory activity, measured with an oxygen electrode, and by vital staining (fluorescein, or 2,3,5-triphenyltetrazolium chloride). It has been found that cells immobilized by glutaraldehyde were not viable.

The permeabilization of cells led to substantial loss of proteins, while the enzyme activity showed a moderate decrease. Sawicka and Kacperska [15] described the presence of soluble and insoluble (cell wall-associated) β–galactosidase, which could be solubilized. Using Tween 80 and hexadecyltrimethylammonium bromide, respectively, the cell wall might be permeabilized. After glutaraldehyde crosslinking, a moderate decrease in the enzyme activity was found.

Table 1. The β–galactosidase activity in the cell suspension and in immobilized cells of tomato.
Tabuľka 1. Aktivita β–galaktozidázy v suspenznej kultúre a v imobilizovaných bunkách rajčiaka.

Cells[1]	Protein[2] mg / g dry weight[3]	Activity[4] nkat / g dry weight	Specific activity[5] pkat / mg protein[6]
Suspension[7]	16.9	4.4	0.26
Permeabilized[8]:			
- Tween 80	8.7	3.3	0.38
- HTAB	8.6	4.6	0.53
Immobilized after permeabilization[9]:			
- Tween 80	8.6	3.2	0.37
- HTAB	8.5	4.5	0.53

HTAB - hexadecyltrimethylammonium bromide.
HTAB - hexadecyltrimetylamonium bromid, 1 - bunky, 2 - bielkoviny, 3 - g sušiny, 4 - aktivita, 5 - merná aktivita, 6 - mg bielkovín, 7 - suspenzia, 8 - permeabilizované, 9 - imobilizované po permeabilizácii.

After permeabilization of the cell wall by hexadecyltrimethylammonium bromide, a significant increase of the intracellular activity of the enzyme has been observed. A moderate decrease in the enzyme activity was found after glutaraldehyde crosslinking (Tab. 1.).

Sucrose is the most widely used carbon source of plant tissue cultures. Glucose and fructose are present in the media in roughly equal amounts after the first few days of inoculation, but the cells do not consume fructose while glucose is present [23]. The cells immobilized in alginate gels utilize glucose [23] while the glutaraldehyde crosslinked cells do not (Fig.1.).

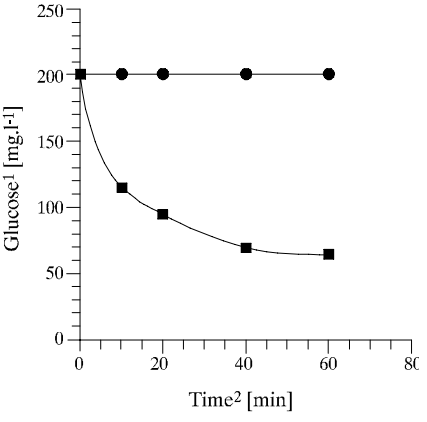

Fig. 1. Time course of glucose utilization by cells immobilized by glutaraldehyde (●) and by cells in suspension (■).

Obr. 1. Časový priebeh utilizácie glukózy glutaraldehydom imobilizovanými bunkami (●) a bunkami suspenznej kultúry (■).

1 - glukóza, 2 - čas.

The β–galactosidase activity of immobilized cells had pH optimum at 4.6 as the viable cells. Enzyme hydrolysis of β–PNG was linear within 3.5 h and reached 65 % of conversion, than almost stoped. The temperature optimum of β–galactosidase activity of immobilized cells and cells in suspensions was at 50 °C. Similar properties were reported for β–galactosidase isolated from winter rape [15], gherkin, ginseng and poppy [2-3]. The inhibitory effect of 1.10^{-4} - 5.10^{-4} mol.l^{-1} *p*-chloromercuribenzoic acid can be eliminated with 5.10^{-3} - 10.10^{-3} mol.l^{-1} cysteine, dithiothreitol or 2-mercaptoethanol, respectively [4,5,24]. These results indicate that the –SH groups are essential for the enzyme activities of α– and β–galactosidase [2,3,24].

The partially purified β–galactosidase from gherkin and poppy seedings was inhibited by galactose and glucose in a moderate way [2-5,15,24]. A similar inhibitory effect was observed in immobilized cells, too. The activity

TABLE 2. Stability of β–galactosidase in immobilized tomato cells during storage.
TABUĽKA 2. Stabilita β–galaktozidázy v imobilizovaných bunkách rajčiaka v priebehu ich uchovávania.

Preservative[1]	% of original activity after[2]				
	0 month[3]	1 month	2 months	3 months	6 months
None	64	-	-	-	-
CLCTC (50 mg.l^{-1})	63	65	69	79	90
ATDNO (100 mg.l^{-1})	63	67	70	80	91
chloramphenicol[4] (50 mg.l^{-1})	63	67	70	81	93
sodium azide[5] (200 mg.l^{-1})	62	67	70	81	95
freezing in 0.15 mol.l^{-1} NaCl[6]	62	69	70	83	98

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

Pôvodná aktivita = aktivita enzýmu (100 %) v suspenznej kultúre pred imobilizáciou.
CLCTC - chlórtetracyklín hydrochlorid, ATDNO - (1-metyldodecyl)-dimetylamín-4-oxid, 1 - konzervans, 2 - % pôvodnej aktivity po, 3 - mesiac, 4 - chloramfenikol, 5 - azid sodný, 6 - mrazenie v 0,15 mol.l^{-1} NaCl.

of enzyme in tomato cells immobilized by glutaraldehyde (in 0.15 mol.l^{-1} NaCl with all preservatives tested) was relatively high even after 6 months as illustrated in Table 2.

Dissociation of galactose, glucose and other potential inhibitors during storage might be explained by the phenomenon which has as yet remained unexplained by experiments. The results achieved showed that the best preservation conditions were by using sodium azide, chloramphenicol, ATDNO, CLCTC, and 0.15 mol.l^{-1} NaCl solutions at -20 °C, respectively.

It is known that immobilization of plant cells by entrapment in beads brings some important advantages compared with a free cell suspension [1].

The immobilization of gherkin, poppy and ginseng cells by glutaraldehyde and their storage in 0.15 mol.l^{-1} NaCl with 0.02 % sodium azide solution seems to be a very convenient method for long-term preservation of different catalysts [4,5,25]. However, the conservation of multifunctional enzyme systems of the cells needs further study. The results obtained inthis study suggest the possibility of using immobilized plant cells for α– and β–galactosidase enzyme activity in the biotechnology of precious saccharides.

It is known that the immobilization of cells by entrapment in beads has some important advantages over cell suspension. It supports the release of the product, promotes cell aggregation, protects cells from shear stress,

gives good cell-to-cell contact and preserves the activity of multifunctional enzyme systems [1,8,22].

Our results suggest, that treating the cell wall leads to a change of its permeability and to a significant increase in the enzyme activity. However, the effect of the cell wall permeability on the enzyme activity requires further study.

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Aktivita β–galaktozidázy v imobilizovaných bunkách rajčiaka

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SÚHRN. Suspenzné kultúry rajčiaka sa permeabilizovali Tweenom 80 alebo hexadecyltrimetylamoniumbromidom a imobilizovali glutaraldehydom. pH-Optimum β–galaktozidázy v imobilizovaných bunkách je 4,6 a tepelné optimum je pri 50 °C. Enzýmová hydrolýza má lineárny priebeh počas 3,5 h a konverzia dosahuje 65 %. Dobrá stabilita imobilizovaných buniek sa dosiahla ich uchovávaním v roztoku 0,15 mol.l^{-1} NaCl s prídavkom: chloramfenikolu, (1-metyldodecyl)-dimetylamín-4-oxidu (ATDNO) alebo chlortetracyklínu (CLCTC). Imobilizované bunky majú vysokú enzýmovú aktivitu, dlhodobú stabilitu a vhodné mechanické vlastnosti.

KĽÚČOVÉ SLOVÁ: imobilizácia, permeabilizácia, β–galaktozidáza, rajčiak