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Identification and determination of invertase secreted by tomato cells

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A simple and rapid procedure for the identification and determination of extracellular invertase from a culture medium of tomato cell suspension cultures is described. Sucrose was used as substrate for the determination of the extracellular and intracellular activities of the enzyme. The culture medium (without cells) was used for identification and determination of extracellular enzyme activity. Intracellular activity was estimated from the cell suspension.

Glycosidases are involved in several important biological processes such as digestion, biosynthesis of glycoproteins, and catabolism of glycoconjugates.

Invertase (β-D-fructofuranosidase EC 3.2.11.26, saccharase called also sucrase) catalyses the hydrolysis of sucrose to glucose and fructose under concentrations lower than 10% (wt./vol.) and has transfructosylating activity under sucrose concentrations higher than 10% (wt./vol.) (Rubio et al. 2002). The enzyme is an important industrial product with applications in the production of non-crystalizable invert sugars and soft-centered chocolates (Wiseman 1979). Invertase has been widely studied especially in yeast and fungi (Costalioli et al. 1997; Romero-Gómez et al. 2000).

The development of a new method for identification and determination of biocatalysts is highly connected with progress of biotechnological processes. Although invertase is generally present in plants, this source has not used previously.

It is the aim of this paper to report on our investigation concerning the hydrolysis of sucrose by intra- and extracelullar invertase from tomato cells. Sucrose was used for the study of intracelullar and extracelullar invertase activity.

Homogenized cell suspension cultures and culture medium alone after 7 days cultivation were used for assaying the activity of intracellular and extracellular invertase. The distribution of intracellular and extracellular enzyme activity is shown in the Table.

The data indicate an 90% intracellular and 10% extracellular distribution of the enzyme activity tested. The intracellular specific enzyme activity is 2.5 times higher.

The production of extracellular galactosidases as well as proteolytic enzymes (Mulinami and Devendra 1999; Stano

Table: Invertase activity in tomato cell culture and culture medium after 7 days cultivation

Fraction	Volume (ml)	Protein (mg/g fresh weight)	Activity (nkat/g fresh weight)	Specific activity nkat/mg protein
Intracellular activity (Homogenate of isolated cells)	2.0	1.8	345	191.7
Extracellular activity (Culture medium without cells)*	5.1	0.5	39	78.0

*Corresponding to the amount of isolated cells

et al. 2002), which are released from plant cells might be of some importance for biotechnological application in food and pharmaceutical research and industry (Asano et al. 2000; Mučaji et al. 2001). These enzymes are generally present in plants. Until now they have not been used in biotechnological processes (Mulinami and Devendra 1999; Paek et al. 1998).

Experimental

1. Plant material

Long term callus and suspension cultures were derived from tomato seedlings and were cultivated as described previously (Poór et al. 1998). The cell suspension culture analysed contained 11.8 g (f. w.) cells in 60 ml medium.

2. Identification and determination of intra- and extracellular invertase

Using sucrose as substrate we identified and determined the intra- and extracellular activity of invertase. Cells suspension cultures were used to determine the intracellular enzyme activity. The cells (10 g) were filtered and washed twice with 1500 ml of distilled water. Soluble proteins extracted by grinding the cells in a precooled mortar using a ratio 1 : 2 [g/ml] of cells and McIlvaine buffer pH 4.6 at 4 °C. The homogenate was filtered through two layers of nylon cloth and centrifuged at 15000 × g at 4 °C.
For determination of extracellular enzyme activity the cultivation medium (without cells) was used after centrifugation (15 000 × g 10 min at 4 °C).

3. Enzyme assay

Enzyme activity was determined by a modified method of Rubio et al. (2002) using sucrose as the substrate. The reaction mixture contained a suitable amount of enzyme (0.2–0.4 ml) and 0.4 mM sucrose in McIlvaine buffer pH 4.6, in a final volume of 2 ml. Enzyme activity was determined at 30 °C for 30–60 min. The control contained temperature inactivated enzyme (100 °C/10 min). The enzyme activity was expressed in katals. Proteins were determined by the method of Bradford (1976) using bovine serum albumin as the standard protein. The glucose content released by the enzyme was determined by the method of Trinder (1969).

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