Conversion of Disaccharides to 3-Ketodisaccharides by Nongrowing and Immobilized Cells of Agrobacterium tumefaciens

V. KLEKNER, V. LOBL, E. ŠIMOVA and M. NOVAK

Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

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ABSTRACT. High-performance liquid chromatography was used to estimate 3-ketolactose and 3-ketosucrose in cultures of agrobacteria. The activities of enzymes that convert the disaccharide substrate were evaluated during batch cultivation of Agrobacterium tumefaciens on sucrose, maltose, and lactose. The highest activity of glucoside 3-dehydrogenase and a slight activity of disaccharide-hydrolyzing enzymes were found in cells grown on lactose. Nongrowing cells converted lactose to 3-ketolactose faster than immobilized cells did. Immobilization of cells into polysaccharide gels did not stabilize the activity of glucoside 3-dehydrogenase. Glutaraldehyde cross-linking of the cell content led to an inactivation of the respiratory chain but Fe^{3+} could be used as an electron acceptor. Cells treated with glutaraldehyde converted lactose faster than nongrowing ones but the activity of glucoside 3-dehydrogenase was not stable.

The synthesis of 3-keto sugar by microorganisms was described about 30 years ago (Bernaerts and De Ley 1958). Strains of Agrobacterium converted D-glucosides and D-galactosides to their corresponding glycoside-3-uloses (Bernaerts and De Ley 1960). Glucoside 3-dehydrogenase (D-aldohexoside:(acceptor) 3-oxidoreductase EC 1.1.99.13), involved in this oxidation, was purified and characterized (Hayano and Fukui 1967). The enzyme can readily oxidize equatorial 3-hydroxyl group of the aldohexopyranosyl nonreducing part (Cl chair form) of disaccharides (Van Beeumen and De Ley 1968). Since the conversion by the enzyme is a simple way how to prepare 3-keto sugars which may be suitable compounds for the synthesis of unusual sugar derivatives (e.g., fatty esters of their endiol form were proved to prevent oxidation of oils and fats; Eltz 1968) the syntheses of 3-ketolactose, 3-ketosucrose, 3-ketotrehalose, and 3-ketomaltose were studied in detail (Fukui and Hochster 1963; Tyler and Nakamura 1971). However, the isolation of 3-keto
sugars from a culture is not a simple task. The possibility of using nongrowing cells from the exponential growth phase for the oxidation of D-glucose and D-glucosides was also described (Fukui 1965, 1970) but the activity of glucoside 3-dehydrogenase was not stable. Also Kurowski et al. (1975) described the unstable nature of this enzyme. Glucoside 3-dehydrogenase may be degraded by enzyme systems of its own bacterium or by irreversibly splitting FAD from the enzyme because FAD is tightly bound in the active enzyme (Van Beeumen and De Ley 1968).

In previous work, we tested some strains of Agrobacterium and found the A. tumefaciens strain C58 as the best producer of 3-ketolactose in a batch culture (Klekner et al. 1986). In this paper, we study the suitability of cells prepared on different carbon source for 3-ketodisaccharide synthesis and compare the conversion of lactose by nongrowing free and immobilized cells of A. tumefaciens in which the stability of the enzyme was believed to improve.

MATERIAL AND METHODS

**Microorganism and cultivation.** The strain of Agrobacterium tumefaciens C58 obtained from Prof. Nečásek (Institute of Experimental Botany, Czech. Acad. Sci., České Budějovice, Czechoslovakia) was cultivated in a medium of the following composition (in 1 L of phosphate buffer, 10 mmol/L, pH 7): sucrose (maltose, lactose) 20 g, urea 0.9 g, MgSO₄·7H₂O 0.15 g, CaCl₂ 10 mg, FeCl₃·6H₂O 10 mg, CuSO₄·5H₂O 50 mg, Na₂MoO₄·2H₂O 20 μg, H₃BO₃ 10 μg. The cultivation was carried out in a LF2 laboratory fermentor (Workshops of Czechoslovak Academy of Sciences, Prague) with a volume of 2 L of medium. The inoculum was prepared in 100 mL of the medium containing 2 % glucose (W/V) and solid CaCO₃ (0.5 %, W/V) on a reciprocal shaker (24 h). The temperature used in all cultivations was 28 °C.

Experiments with nongrowing and immobilized cells were carried out in 100-mL Erlenmeyer flasks containing 50 mL Tris-HCl buffer (0.1 mol/L, pH 7) to which the appropriate disaccharide and washed or immobilized cells were added. Flasks were shaken at 28 °C. Tris-HCl buffer was used because the conversion of the disaccharide in this buffer was about 25 % higher than in phosphate (preliminary experiments, Korandová 1987) and because the phosphate buffer can destroy calcium alginate particles. In experiments in which nongrowing and immobilized cells were compared the total biomass concentration was identical.

**Analyses.** Biomass was estimated as the constant dry matter of washed cells at 110 °C. Biomass concentration in immobilized particles was calculated from the concentration of biomass suspension taken for immobilization and from differences between buffer volumes with and without immobilized particles.

3-Ketomaltose was determined according to Fukui and Hayano (1969) by adding 3 mL of NaOH (0.1 mol/L) to 0.15 mL of a sample and measuring absorbance at 340 nm after 5 min.