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Process Biochemistry 40 (2005) 437–444

PROCESS BIOCHEMISTRY

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Bioconversion of D-galactitol to tagatose and dehydrogenase activity induction in *Gluconobacter oxydans*

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Received 16 May 2003; accepted 17 January 2004

Abstract

Tagatose, a new probiotic sweetener, is obtainable from D-galactitol by microbial oxidation employing *Gluconobacter oxydans* DSM 2343 strain. Several polyols were tested as carbon source and inducer of dehydrogenase activity and galactitol was found to be the best substitute for glycerol in the base medium (3160 mg/l tagatose) although the effects of the induction did not prove to be stable throughout cell storage. The gradual addition of galactitol in high glycerol content media resulted in relevant and stable tagatose yields (4400 mg/l) highlighting that the dehydrogenase activity induced during the adaptation procedure is correlated to the presence of glycerol. This enzyme activity can be attributable to a minor activity of a sorbitol dehydrogenase (SDH), the highest levels (200×10^{-2} IU/g) being evidenced in galactitol adapted cells with sorbitol as substrate. The adaptation procedure determined a decrease in sorbitol affinity (K_S from 37 to 57 mM) and an increase in that related to galactitol (K_S from 98 to 21 mM).

Keywords: Tagatose; D-Galactitol; *Gluconobacter*; Acetic bacteria; Biotransformation; Sweetener

1. Introduction

Approvals and newly devised applications for alternative sweeteners have extended the market of low-calorie and calorie-reduced sweet products in general, enabling the food and beverage industry to manufacture good tasting products with acceptable shelf-life at reasonable cost.

Intensive sweeteners such as aspartame, acesulfame and saccharin cannot be used as the only sweetening agents when other technological factors, like texture and mass that are correlated to sugar bulk, are important to the final product. Nowadays sugar-free bulk sweeteners are widely used, not only in the field of "tooth friendly" and calorie-reduced confectionery but also in baked and pharmaceutical products [1].

Tagatose, a ketohexose C-4 fructose epimer present in nature in low concentration, is considered a low-calorie bulk sweetener, with 92% of the sweetness of sucrose but less than half the calories [2]. It is non-cariogenic and also exhibits a probiotic effect, being a not-digestible food ingredient able to benefit humans by selectively stimulating the growth and/or activity of a limited number of bacteria, thus improving host health [3]. By increasing the growth of lactic acid bacteria, tagatose promotes the production of butyrate, that plays an important role in colon epithelium protection [4].

In April 2001 tagatose gained GRAS (Generally Recognised As Safe) status, with the subsequent possibility of commercialising it as a bulk sweetener (GAIO®, Arla Foods). Although tagatose is obtainable from natural sources, its availability appears limited and its recovery expensive, a major impediment to its use in the food industry [2].

In a previous paper, we described an alternative possibility for obtaining tagatose by microbial oxidative biotransformation of the corresponding polyol D-galactitol [5]. In fact, several metabolites, among them sorbose a precursor for Vitamin C synthesis, are obtained through such chemo- and stereo-selective oxidations, exploiting the non-induced cofactor regeneration needed for enzymic catalysis [6].

We carried out a preliminary investigation into the ability of some acetic acid bacterial strains to perform the biotransformation: 7.5 g/l of D-galactitol was added directly to *Gluconobacter oxydans* cultures, resulting in 340 mg/l tagatose at 48 h reaction. The set up of a cell adaptation procedure

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doi:10.1016/j.procbio.2004.01.028

allowed an increase of the dehydrogenase activity related to biotransformation, leading to 3160 mg/l tagatose after only 24 h reaction.

The present paper reports the effect of different dehydrogenase activity induction procedures on the biotransformation of D-galactitol to tagatose, taking into account the influence of different polyols. The role of glycerol in the induction procedure, needed to maintain the dehydrogenase enzymatic activity at high and stable levels, was also evaluated. Finally, several features related to the dehydrogenase enzymic activity responsible for the oxidation of galactitol to tagatose were clarified.

2. Materials and methods

2.1. Microorganism and culture conditions

Tagatose production was carried out employing *G. oxydans* DSM 2343 strain (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig—Germany). The culture was maintained on a GYC medium containing (g/l) glucose 50, yeast extract 10, $CaCO_3$ 30 and agar 15. The initial pH was adjusted to 5.3. The medium was sterilised at 115 °C for 30 min. The strain was incubated at 28 °C for 24–48 h.

2.2. Tagatose production

Biotransformation was carried out with growing cells employing the GLY base medium containing (g/l) glycerol 25 and yeast extract 10. The initial pH was adjusted to 5.0. The medium was sterilised at 118 °C for 20 min. Fermentation was carried out in 750 ml Erlenmeyer flasks, each containing 100 ml of the culture medium. Each flask was inoculated with a 48–72 h old culture suspended in distilled water (5 ml, 10^9 cells/ml). The flasks were incubated at 28 °C on a reciprocal shaker (60 strokes/min). After 24 h growth, galactitol was added to the cultures to final concentrations of 7.5, 12.5, 20 and 22.5 g/l. Biotransformation was then monitored for 48 h.

2.3. Induction of dehydrogenase activity

In order to increase dehydrogenase enzymic activity levels, adaptive experiments were carried out employing the *G. oxydans* strain according to two different induction procedures:

- Gradually substituting the glycerol content in the GLY base medium with different polyols (sorbitol, ribitol and erythritol comparatively) (XLY1–XLY4 series) (Table 1).
- Gradually increasing galactitol content in the GLY base medium while keeping the glycerol concentration constant (GAL1–GAL4 series) (Table 2).

Table 1
Glycerol and polyol (sorbitol, ribitol and erythritol) concentrations (g/l) in the media employed in the adaptive experiments (XLY1–XLY4 series)

Medium	Substrate (g/l)	
	Glycerol	Polyol
GLY	25.0	0
XLY1	17.5	7.5
XLY2	12.5	12.5
XLY3	7.5	20.0
XLY4	5.0	22.5

Table 2
Glycerol and galactitol concentrations (g/l) in the media employed in the adaptive experiments (GAL1–GAL4 series)

Medium	Substrate (g/l)	
	Glycerol	Galactitol
GLY	25	0
GAL1	25	7.5
GAL2	25	12.5
GAL3	25	20.0
GAL4	25	22.5

The strain, adapted to increased polyol concentration (from GLY to XLY1-GAL1, from XLY1-GAL1 to XLY2-GAL2, from XLY2-GAL2 to XLY3-GAL3 and from XLY3-GAL3 to XLY4-GAL4; see Tables 1 and 2) employing the previously described procedure [5], was then tested for oxidation activity.

2.4. Substrate affinity of the not- and adapted G. oxydans cells

For the determination of substrate affinity different polyols such as sorbitol, galactitol, mannitol and xylitol were added to the reaction mixture (7.5 g/l), and the corresponding oxidative products were determined by HPLC.

The substrate-specific constant (K_S) was determined according to the Monod model [7] on not- and galactitol (GAL3)-adapted cells, employing galactitol and sorbitol as substrates.

2.5. Crude enzyme preparation

A 250 ml culture was centrifuged and the cells washed twice with distilled water and resuspended in 15 ml of 0.1 M Tris–HCl buffer (pH 9.0). The cells were then broken with a French press at 12,000 psi. The obtained crude extract was employed in toto in the preliminary enzyme assay.

2.6. Enzyme assay

Dehydrogenase activity was determined spectrophotometrically by modifying the procedure reported for a polyol dehydrogenase assay [8]. A 250 ml culture was centrifuged and the cells, washed twice with distilled water, were