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Production of xylitol from D-xylose by recombinant *Lactococcus lactis*

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Abstract

The D-xylose reductase from *Pichia stipitis* CBS 5773 and the xylose transporter from *Lactobacillus brevis* ATCC 8287 were expressed in active form in *Lactococcus lactis* NZ9800. Xylitol production was investigated using non-growing recombinant cells in high cell-density under microaerobic conditions in the presence of xylose and glucose. Besides xylose, the recombinant strain with xylose reductase activity reduced L-arabinose and D-ribose in significant extent to the corresponding pentitols.

The ratio of xylitol produced per glucose consumed was almost 10-fold higher under glucose limitation than the ratio in the presence of excess initial glucose. The co-expression of the xylose transporter with the xylose reductase did not increase the efficiency of xylitol production appreciably when compared to the strain in which only the xylose reductase gene was expressed. A fed-batch experiment with high initial xylose concentration (160 g l^{-1}) under glucose limitation was carried out using the strain co-expressing xylose reductase and xylose transporter genes. The xylitol yield from xylose was 1.0 mol mol^{-1} and the ratio of xylitol produced per glucose consumed was 2.5 mol mol^{-1}. The volumetric productivity was 2.72 g l^{-1} h^{-1} at 20 h. Of the xylose initially present, 34% was consumed. Analysis of the fermentation metabolites revealed a shift from homolactic to mixed acid fermentation at early stages of the experiment.

Keywords: *Lactococcus lactis*; Xylitol; Xylose; Metabolic engineering; Xylose reductase; Xylose transport

1. Introduction

Xylitol is a sugar-alcohol used as a natural sweetener in food and confectionary industry. The metabolism of xylitol in humans is independent of insulin, which makes it a suitable sugar substitute for use by diabetics

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(Emodi, 1978). Xylitol is also well known for its anticariogenic properties (Scheinin and Mäkinen, 1975) and it is therefore added in dental products.

Xylitol is presently produced by chemical hydrogenation of the five-carbon sugar, D-xylose, with Raney-nickel as the catalyst (Albert et al., 1980; Melaja and Hämäläinen, 1977). In order to avoid poisoning of the nickel catalyst, the xylose that is obtained from hardwood hydrolysates has to be extensively purified (Ojamo, 1994). In comparison to chemical hydrogenation, an advantage of microbial xylitol production is the possibility of using industrial side-streams as raw material instead of expensive pure xylose. Thus, there has been a considerable interest in microbial conversion of xylose to xylitol with both recombinant and non-recombinant strains.

Yeasts are generally considered to be more efficient producers of xylitol than bacteria or fungi. The best productivities so far have been achieved using yeasts belonging to the genus *Candida* (Parajó et al., 1998a, 1998b; Winkelhausen and Kuzmanova, 1998). However, the use of *Candida* in food industry is problematic because of the well-known pathogenic nature of many *Candida* species (Fridkin and Jarvis, 1996). The construction of recombinant yeasts producing xylitol has been established by the introduction of xylose reduction into *Saccharomyces cerevisiae* (Meinander and Hahn-Hägerdal, 1997; Parajó et al., 1998b; Lee et al., 2000; Govinden et al., 2001), but the highest productivities obtained using natural yeast strains have usually not been met. There are also some examples on bacterial production of xylitol. The reported productivities and final xylitol concentrations have, however, been very low both with recombinant (Suzuki et al., 1999) and natural species (Yoshitake et al., 1971, 1973; Rangaswamy and Agblevor, 2002).

The energy metabolism of lactic acid bacteria (LAB) is generally not connected to their limited biosynthetic activity. Therefore, their sugar metabolism can be engineered without disturbing the biosynthesis of structural components of the cell, which has made LAB attractive targets for metabolic engineering (Hugenholtz and Kleerebezem, 1999). The most intensively investigated LAB for metabolic engineering purposes is *Lactococcus lactis*. It is widely used in dairy and food processes, and due to its GRAS (generally recognized as safe) status, it is a potential production host for food and feed applications.

It has been reported that fructose can be reduced to mannitol very effectively by non-growing LAB cells (von Weymarn et al., 2002). In the present study, we use a similar approach for the reduction of xylose to xylitol. This is carried out by expressing a yeast xylose reductase gene in *L. lactis*. We also study the effects of introducing a xylose transporter in *L. lactis* on the xylitol production. We demonstrate for the first time that xylitol productivities comparable to those of *Candida* can be achieved with a food-compatible bacterial production strain.

2. Materials and methods

2.1. Bacterial strains, cloning vectors, media and cultivation conditions

Unless otherwise indicated, *L. lactis* NZ9800 was grown at 30 °C in M17 broth (Difco) containing 5 g l^{-1} glucose. When required, the medium was supplemented with 8 μg ml^{-1} chloramphenicol. *L. lactis* NZ9800 and the vectors pNZ8032 and pNZ8037 were obtained from NIZO Laboratories (Netherlands) (de Ruyter et al., 1996).

2.2. Chemicals

Chemicals were purchased from Sigma-Aldrich unless otherwise indicated.

Nisin stock solution (25 μg ml^{-1}) was prepared by dissolving 0.1 g of 2.5% nisin into 2 ml of 1 M HCl for 2 h at room temperature. The volume was made up to 100 ml with distilled water and the solution was filtered through a 0.2 μm membrane. Bovine serum albumin was added to the solutions at 5 mg ml^{-1} in order to prevent the adsorption of nisin onto plastic surfaces.

2.3. Plasmid construction

Total DNAs from *Pichia stipitis* CBS 5773 and *Lactobacillus brevis* ATCC 8287 were isolated using Qiagen Genomic-tips according to the instructions by the manufacturer.

The DNA isolated from *P. stipitis* was used as the template for PCR amplification of the *XYL1* gene encoding the xylose reductase (Amore et al., 1991).