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Sorbitol synthesis by an engineered *Lactobacillus casei* strain expressing a sorbitol-6-phosphate dehydrogenase gene within the lactose operon

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Abstract

Sorbitol is claimed to have important health-promoting effects and *Lactobacillus casei* is a lactic acid bacterium relevant as probiotic and used as a cheese starter culture. A sorbitol-producing *L. casei* strain might therefore be of considerable interest in the food industry. A recombinant strain of *L. casei* was constructed by the integration of a D-sorbitol-6-phosphate dehydrogenase-encoding gene (*gutF*) in the chromosomal lactose operon (strain BL232). *gutF* expression in this strain followed the same regulation as that of the *lac* genes, that is, it was repressed by glucose and induced by lactose. ^{13}C-nuclear magnetic resonance analysis of supernatants of BL232 resting cells demonstrated that, when pre-grown on lactose, cells were able to synthesize sorbitol from glucose. Inactivation of the L-lactate dehydrogenase gene in BL232 led to an increase in sorbitol production, suggesting that the engineered route provides an alternative pathway for NAD^+ regeneration.

Keywords: *Lactobacillus casei*; Sorbitol; Metabolic engineering

1. Introduction

Lactic acid bacteria (LAB) are fermentative microorganisms that use two main sugar catabolic pathways. The Embden–Meyerhof–Parnas pathway results in almost exclusively lactic acid as end-product (homolactic fermentation). The NAD^+ consumed during glycolysis is regenerated during pyruvate reduction by the L-lactate dehydrogenase (Ldh), thus maintaining the cellular redox balance. The 6-phosphogluconate/phosphoketolase pathway results in significant amounts of other end-products such as ethanol, acetate, and carbon dioxide in addition to lactic acid (heterolactic fermentation). Under certain conditions, LAB can also use alternative electron acceptors to regenerate NAD^+ through suitable dehydrogenases. For instance, some heterofermentative LAB can reduce directly fructose to mannitol, by means of a mannitol dehydrogenase [1]. In homofermentative LAB, similar reactions can take place and fructose-6-phosphate can be reduced to mannitol-1-phosphate and sorbitol-6-phosphate (Fig. 1), which are catalized by mannitol-1-phosphate dehydrogenase and sorbitol-6-phosphate dehydrogenase (S6PDH), respectively. However, their genes are clustered with those of the corresponding hexitol transporter of the phosphoenol-pyruvate-dependent phosphotransferase system (PTS) family, indicating that their regular physiological role

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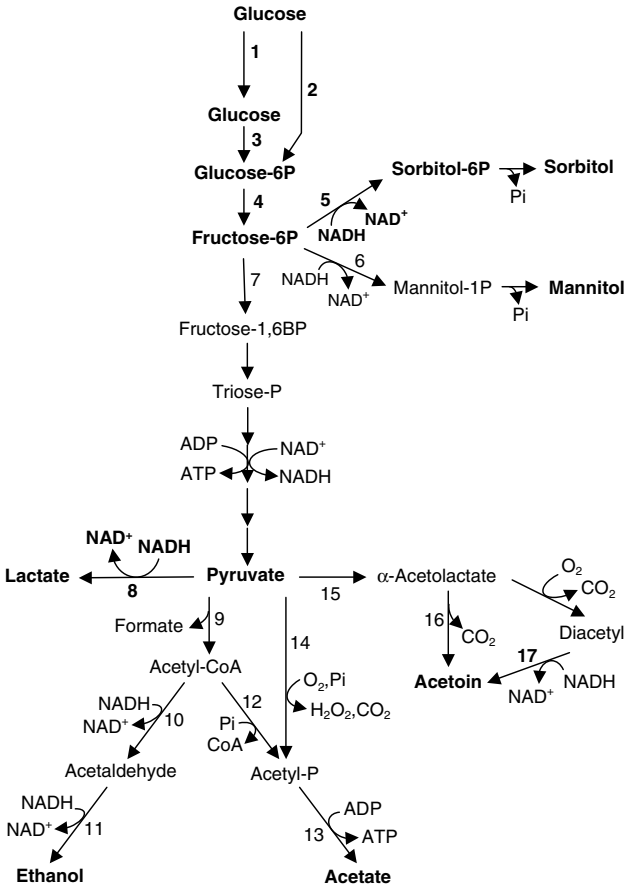

Fig. 1. Pathways proposed for glucose metabolism in *Lactobacillus casei* BL23 (wild-type) and in the engineered strains obtained in this work. 1, glucose permease; 2, glucose-specific phosphoenolpyruvate-dependent phosphotransferase system (PTS^{Man}); 3, glucose kinase; 4, phophoglucose isomerase; 5, sorbitol-6-phosphate dehydrogenase; 6, mannitol-1-phosphate dehydrogenase; 7, 6-phosphofructokinase; 8, lactate dehydrogenase; 9, pyruvate formate lyase; 10, acetaldehyde dehydrogenase; 11, alcohol dehydrogenase; 12, phosphotransacetylase; 13, acetate kinase; 14, pyruvate oxidase; 15, α-acetolactate synthase; 16, α-acetolactate decarboxylase; 17, 2,3-butanediol dehydrogenase.

should be mediating hexitol assimilation [2,3]. This could be confirmed by the fact that hexitol accumulation has only been demonstrated in mutants lacking Ldh, where NAD^+ regeneration was seriously compromised [4–7].

Lactobacillus casei is a facultative heterofermentative LAB associated with dairy products and some strains have health-promoting properties [8–12]. In *L. casei*, two S6PDH-encoding genes have been found [3,13]. However, sorbitol production is not likely in this bacterium during its growth on glucose or lactose, since both S6PDH genes are subject to catabolite repression and substrate induction [3,13]. The aim of this work has been the construction of sorbitol-producing *L. casei* strains. The *gutF* gene, encoding a S6PDH, was introduced in the lactose operon of *L. casei* and expression of the S6PDH was subject to the same regulation as lactose genes [14–16]. Production of sorbitol and other metabolites in this recombinant strain and in an L-Ldh deficient variant were determined by ^{13}C-nuclear magnetic resonance (NMR) analysis.

2. Materials and methods

2.1. Bacterial strains, plasmids, primers and growth conditions

The strains, plasmids and primers used in this work are listed in Table 1. *L. casei* strains were grown in MRS medium (Oxoid LTD., Hampshire, England) or MRS fermentation medium (Scharlau Chemie S.A., Barcelona, Spain) supplemented with 0.5% (w/v) of different carbohydrates at 37 °C under static conditions. *Escherichia coli* DH5α was used in all cloning experiments and was grown in Luria–Bertani (LB) medium at 37 °C under agitation. When required, erythromycin

Table 1
Strains, plasmids and oligonucleotides used in this study

Strain, plasmid or oligonucleotide	Description	Source or reference
Lactobacillus casei		
BL23	CECT [a] 5275 (wild type)	B. Chassy (University of Illinois, Urbana)
BL155	BL23 with a frameshift in *lacF*	[16]
BL232	BL155 with *gutF* integrated in *lac* operon	This work
BL233	BL232 with *ldhL*::pRV300	This work
BL234	BL232 with *gutF*::pRV300 in gut operon	This work
Plasmids		
pRV300	Suicide vector carrying Er^r from pAMβ1	[29]
PVBldh	pRV300 containing an internal *ldhL* fragment	[24]
pRVgut3	pRV300 containing an internal *gutF* fragment	[3]
pIlac	*lacG* 3′ end and *lacF* gene in pRV300	[21]
PIlgutF	pIlac containing *gutF*	This work
Oligonucleotides		
gutNdeI	5′ AGAGATTGCATATGTCTGATTGG	Isogen Bioscience
gutEcoRI	5′ CCAAGCTGAATTCTTAACCTCTGGAC	Isogen Bioscience

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