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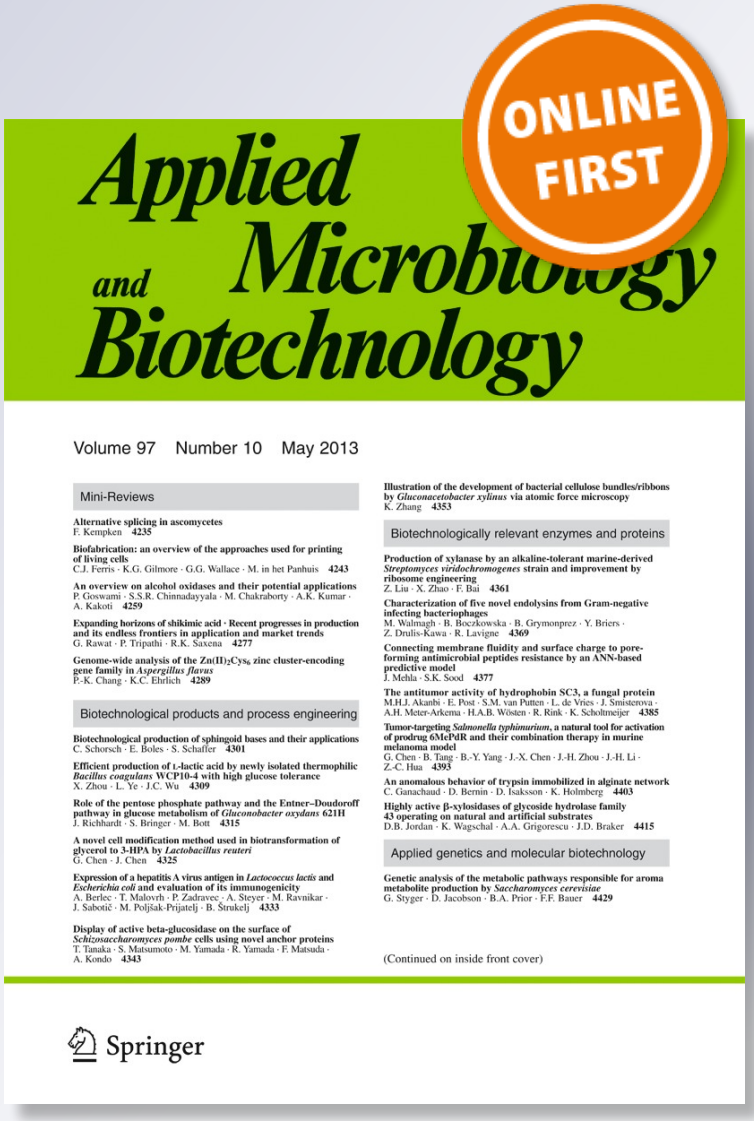

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MINI-REVIEW

Biotechnological and in situ food production of polyols by lactic acid bacteria

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Abstract Polyols such as mannitol, erythritol, sorbitol, and xylitol are naturally found in fruits and vegetables and are produced by certain bacteria, fungi, yeasts, and algae. These sugar alcohols are widely used in food and pharmaceutical industries and in medicine because of their interesting physicochemical properties. In the food industry, polyols are employed as natural sweeteners applicable in light and diabetic food products. In the last decade, biotechnological production of polyols by lactic acid bacteria (LAB) has been investigated as an alternative to their current industrial production. While heterofermentative LAB may naturally produce mannitol and erythritol under certain culture conditions, sorbitol and xylitol have been only synthesized through metabolic engineering processes. This review deals with the spontaneous formation of mannitol and erythritol in fermented foods and their biotechnological production by heterofermentative LAB and briefly presented the metabolic engineering processes applied for polyol formation.

Keywords Lactic acid bacteria · Polyols · Mannitol · Erythritol · Sorbitol · Low-calorie sugars

Introduction

Sugar alcohols, also called polyols, are noncyclic hydrogenated carbohydrates in which the carbonyl group (aldehyde or ketone) of the precursor sugar is reduced to the corresponding primary or secondary alcohol (Cummings and Stephen 2007). Mannitol, sorbitol, xylitol, and erythritol (Fig. 1) are the polyols mostly used in the food and pharmaceutical industries; moreover, mannitol is widely applied in medicine. In the food industry, they are used as sugar replacers because of their taste and sweetness. These compounds are low energy, nonmetabolizable sweeteners not affecting insulin levels making them applicable in dietetic and diabetic food products. As sugar alcohols are partially or not absorbed in the small intestine, they may reach the colon where they can be degraded by certain bacteria (Schiweck et al. 1994) leading to the production of several short chain fatty acids which have been claimed to confer health benefits to the host (van Munster and Nagengast 1993; Liong and Shah 2005). Also, because of the interesting physicochemical characteristics of polyols, they act as texturizing agents, softeners, color stabilizers, and humectants and do not take part in the Maillard reaction (Monedero et al. 2010).

Mannitol is a six-carbon polyol derived from D-fructose and produced by certain plants, algae, mushrooms, yeasts, and bacteria. It is widely used in the pharmaceutical, chemical, and food industries, while in medicine, it is used as a potent osmotic diuretic (Monedero et al. 2010; Saha and Racine 2011). In the food industry, mannitol being about half as sweet as sucrose is mainly employed as a natural sweetener, especially in food products for diabetic patients (Livesey 2003). It is also applied in "breath-freshening" products and "sugar-free" chewing gum due to its noncariogenic properties and the positive enthalpy of its solution in water imparting a refreshing taste when mannitol gets in contact with saliva. In pharmaceutical formulations, it is used as ingredient because of its low hygroscopicity and its capacity to mask undesirable flavors (Debord et al. 1987).

Sorbitol, also known as D-glucitol, is a noncyclic hexitol derived from D-glucose, which naturally occurs in several fruits such as berries, cherries, grapes, plums, pears, and

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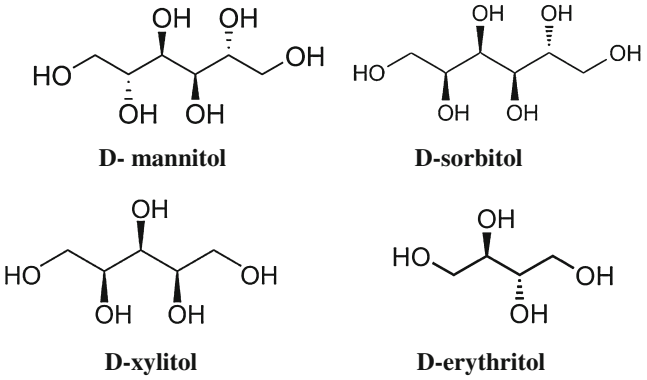

Fig. 1 Chemical structures of D-mannitol, D-sorbitol, D-xylitol, and D-erythritol

apples as well as in some vegetables (Budavari et al. 1996; Gutierrez and Gaudillere 1996). Sorbitol, which is about 60 % as sweet as sucrose, is used as sweetener, humectant, texturizer, and softener in the food industry. It is used in the manufacture of chewing gum, candies, desserts, ice creams, and diabetic foods. In addition, sorbitol is the starting material for the production of sorbose, ascorbic acid, and other pharmaceutical compounds (Ladero et al. 2007). Several yeasts and bacteria such as *Candida boidinii* and *Zymomonas mobilis* naturally synthesize sorbitol while lactic acid bacteria (LAB) do not, although they are able to metabolize it (Tani and Vongsuvanlert 1987; Silveira et al. 1999; Erzinger and Vitolo 2006).

Xylitol is a five-carbon sugar alcohol derived from D-xylose. It is naturally found in many fruits and vegetables and can be extracted from berries, oats, and mushrooms. Yeasts are the most efficient xylitol-producing microorganisms, with strains of the genus *Candida* the best xylitol producers (Parajó et al. 1998). However, these microorganisms cannot be applied in the food industry because of well-known pathogenic status of many *Candida* species. As mannitol, xylitol is known because of its anticariogenic properties and it is therefore added in dental products and in chewing gums. Moreover, its regular consumption in adequate doses reduces the risk of tooth decay (Söderling et al. 2011). The most important beneficial effects of xylitol on oral health are as follows: inhibition of growth and metabolism of *Streptococcus mutans*—the major causative agent of caries—and *Streptococcus sobrinus*—responsible of dental plaque acid production—with the concomitant increase of pH values in the oral cavity, and its contribution to teeth remineralization (Tanzer 1995; Bahador et al. 2012). Xylitol has been also shown to reduce the incidence of ear infections (Nyyssölä et al. 2005).

Erythritol is a four-carbon polyol derived from erythrose that is naturally present in some fruits (grapes, pears, melons, and watermelons), mushrooms, and fermented foods such as soy sauce, sake, beer, and wine, where yeasts are the microorganisms associated with the fermentative process (Bernt et al. 1996; Embuscado and Patil 2001). There exists considerable evidence supporting the safe use of erythritol as a food sweetener. Metabolic studies have demonstrated that erythritol is well absorbed (60–90 %) but not systemically metabolized and is rapidly excreted intact in urine, with only small amounts available to be subject of colonic fermentation. In clinical studies, no significant gastrointestinal side effects were found at high doses (up to 1,000 mg kg body^{-1}, Bernt et al. 1996). Erythritol is industrially produced by fermentation processes with osmophilic yeasts (Embuscado and Patil 2001) since the substrate erythrose is very expensive to be used for direct catalytic hydrogenation (Monedero et al. 2010). Erythritol production has also been reported for some LAB such as *Oenococcus oeni*, *Leuconostoc mesenteroides*, and *Lactobacillus sanfranciscencis* (Veiga-da-Cunha et al. 1993; Stolz et al. 1995; Richter et al. 2001).

Current industrial production of certain sugar alcohols (i.e., mannitol and sorbitol) is performed by catalytic reduction of sugars with hydrogen gas and nickel catalyst at high temperature and pressure (Kusserow et al. 2003). However, this is an expensive method as, in addition to the extreme conditions, it requires highly pure sugar substrates and costly chromatographic purification steps. On the other hand, the enzymatic production of mannitol is highly dependent on expensive cofactors such as NAD(P)H. These limitations have led to investigate on the biotechnological synthesis of polyols through fermentation processes in the last decade (Saha 2006a, b; Saha and Racine 2010; Saha and Nakamura 2003; von Weymarn et al. 2002a, b, 2003).

LAB constitute a group of closely related microorganisms that produce lactic acid as the major or sole product from carbohydrate fermentation (Carr et al. 2002). These microorganisms lack the electron transport chain and oxidation phosphorylation and display complex nutritional requirements as they lack many biosynthetic capabilities; they have a limited capacity for biosynthesis of amino acids, vitamins, and macromolecular precursors. Thus, they are generally abundant in rich environments including milk and dairy products, vegetables, fruits and plants, cereals, meat, and meat products where the needed requirements can be provided (Mayo et al. 2010).

Many LAB species have been extensively used as starter cultures in the production of fermented foods especially dairy products such as yogurts, cheeses, buttermilk as well as pickles, olives, sauerkraut, sourdough, sausages, etc. LAB metabolism allows decreasing both carbohydrate content in the raw material they ferment and pH due to lactic acid production. This acidification process is one of the most desirable effects of their growth as it enables the growth inhibition of many microorganisms including the most common human pathogens and thus prolonging the

shelf life of fermented products. Moreover, these microorganisms may contribute to flavor, texture, and nutritional quality of fermented foods through the formation of aroma compounds, exopolysaccharides, proteins, vitamins, polyols, etc. (Hugenholtz 2008). Also, some LAB strains, namely probiotics, have been claimed to display health beneficial effects and have been included in the elaboration of functional foods with specific properties (Stanton et al. 2005).

According to the pathways of sugar fermentation, LAB are divided into homofermentative and heterofermentative microorganisms. Homofermentative species produce lactic acid as a major product in the presence of nonlimiting amounts of carbohydrates, which are fermented through glycolysis (Embden–Meyerhoff pathway) and the enzyme lactate dehydrogenase (LDH). Low glycolytic flux rates in homofermentative bacteria lead to a shift towards mixed acid fermentation yielding formate, acetate, ethanol, and lactate. This change is caused by regulation of LDH and pyruvate formate lyase activities, which are subject to control by the catabolic and anabolic flux rates and changes in the NAD(P)H/NAD(P)$^+$ ratios (Zaunmüller et al. 2006). Heterofermentative LAB such as *Leuconostoc*, *Oenococcus*, and certain *Lactobacillus* species ferment sugars generally through the phosphoketolase (PKP) or Warburg–Dickens pathway. Fermentation of pentoses leads to the formation of pyruvate and acetyl-P and consequent production of lactic and acetic acids while hexoses can be converted into lactic acid, CO_2, and ethanol. The specific enzyme of the heterofermentative pathway is D-xylulose-5 P-phosphoketolase which catalyzes the conversion of xylulose-5P to glyceraldehyse-3 P (GAP) and acetyl-P. While GAP enters glycolysis leading to the formation of lactic acid, acetyl-P is converted into ethanol. The low acetaldehyde dehydrogenase activity, and in consequence, low activity of the ethanol pathway to re-oxidase NAD(P)H, limits the heterofermentative growth on glucose. When O_2, pyruvate, citrate, or fructose is present in the medium, they may be used as alternative electron acceptors and much higher cell growth rates on hexoses are observed (Zaunmüller et al. 2006; Arsköld et al. 2008).

Nowadays, LAB are being used as "cell factories" for the production of certain metabolites either to be used as purified compounds or be produced in situ in fermented foods; examples include the production of bacteriocins, vitamins, amino acids, low-calorie sugars, etc. (Hugenholtz 2008; Patra et al. 2009). LAB are being used as cell factories because of their food grade status and beneficial effects in the gastrointestinal tract, their products being directly applicable in food products (Wisselink et al. 2002), the availability of genetic tools for strain improvement, and the possibility of using cheap substrates as crude sugar feedstocks for growth (Carvalheiro et al. 2011; Fontes et al. 2009; Monedero et al. 2010; Ortiz et al. 2012).

Certain heterofermentative LAB have been shown to be ideal microorganisms for polyol (i.e., mannitol) production as they display a fermentative metabolism associated with an important redox modulation and a limited biosynthetic capacity. Also, as LAB are used in food fermentation processes, the possibility to in situ produce polyols may be useful in the development of novel functional foods (Monedero et al. 2010).

Reviews on the biotechnological production of mannitol (Saha and Racine 2011), the polyol production by LAB by metabolic engineering processes (Monedero et al. 2010), and the technological application of polyols produced by LAB (Patra et al. 2009) have been recently published. This review will mainly deal with the state-of-the-art formation of polyols in spontaneous fermented foods and their production by wild-type and by genetically modified, albeit at lesser extent, LAB strains.

Spontaneous polyol production in fermented foods

To date, reviews on polyol production by LAB have dealt with the optimization of their synthesis by different processes or their applications (Patra et al. 2009; Monedero et al. 2010; Saha and Racine 2011), while the in situ production of polyols in fermented foods and the benefits that these compounds confer to the producing microorganisms as well as the final product have been ignored so far. Interestingly, functional lactic starter cultures with the ability to produce polyols may lead to the production of novel fermented foods naturally sweetened with these low-calorie sugars. Fermented foods displaying in situ polyol formation by BAL are shown in Table 1.

Fermented vegetables

Vegetable fermentation has been performed since ancient times to prolong the shelf-life of fresh vegetables and to improve their organoleptic properties. Addition of salt to fresh vegetables allows the naturally present microbiota to initiate spontaneous fermentation process making the environment more appropriate for the development of LAB, which have to overgrow other microorganisms such as Gram-negative bacteria, yeasts, and fungi. Certain LAB can use vegetables as carbohydrate source as they provide sucrose, glucose, and fructose that can be fermented producing lactic acid, acetic acid, ethanol, and/or mannitol. Recently, Wouters et al. (2012) studied the dynamics of microbial communities, LAB diversity, and metabolite kinetics of Romanian spontaneously fermented vegetables. Several vegetable (green tomato, carrot, and cauliflower mixture as well as cauliflower alone) fermentations were performed at 25 °C for 3 days and then kept at 16 °C for

Table 1 Spontaneous polyol production by LAB in fermented foods

Food matrix or fermented food	LAB species	Polyol/s produced	Fermentation conditions	Reference
Romanian mixed vegetables (green tomatoes, carrots and cauliflower)	*L. plantarum* *L. brevis* *Leuconostoc mesenteroides* *Leuconostoc citreum*	Mannitol	25 °C for 3 days, then 16 °C until the end of fermentations (varying from 2 weeks to 2 months)	Wouters et al. (2012)
Kimchi (Chinese cabbage)	*Leuconostoc mesenteroides* *Lactobacillus* spp.	Mannitol	Packaged into airtight bags and stored at 4 °C for 29 days	Jung et al. (2011, 2012)
Doenjang (soybean fermented food)	*Leuconostoc mesenteroides* *Tetragenococcus halophilus* *E. faecium*	Mannitol, xylitol	Prepared from *meju*, 2-month fermentation	Namgung et al. (2010), Kim et al. (2009)
Cocoa pulp and cocoa beans	*Leuconostoc pseudomesenteroides* *Fructobacillus tropaeoli*-like *L. fermentum*	Mannitol	4–6 days, room temperature	Papalexandratou et al. (2011a, b)
Wheat and spelt sourdoughs	*L. plantarum* *L. fermentum* *L. rossiae* *L. brevis* *L. paraplantarum*	Mannitol, erythritol	10 days with daily backslopping	Van der Meulen et al. (2007)
Gluten-free sourdough	*L. curvatus* *L. reuteri* *L. animalis*	Mannitol	48 h at 30 or 37 °C	Rühmkorf et al. (2012)
Sorghum and wheat sourdoughs	*L. sanfranciscensis*	Mannitol	24 h at 30 °C	Galle et al. (2010)

2 weeks to 2 months. At the end of fermentation, glucose, fructose, and sucrose were totally consumed in nearly all samples, while lactic acid (11.3 g l^{-1}), ethanol (11.2 g l^{-1}), acetic acid (1.7 g l^{-1}), and in some cases, mannitol (5.4 g l^{-1}) were produced. *Lactobacillus plantarum* and *Lactobacillus brevis* were the most frequently isolated LAB species and probably responsible for mannitol production.

Kimchi, a traditional Korean fermented vegetable food, is made with Chinese cabbage and radish. Metagenomics and metabolomics studies to elucidate the connection between *kimchi* microbiota and the metabolites present in this food were conducted by Jung et al. (2011). Metagenomics studies revealed that dominant genera were *Leuconostoc* (the most abundant), *Lactobacillus*, and *Weissella*, with *Leuconostoc mesenteroides* subsp. *mesenteroides* and *L. sakei* subsp. *sakei* the most represented species. Metabolomics studies showed a reduction of free sugars (glucose and fructose) with concomitant increase of lactate, acetate, ethanol, and mannitol. The presence of mannitol resulted in a refreshing taste. Production of lactate, acetate, and mannitol were barely associated with growth of the dominant genera. Also, Jung et al. (2012) studied the production of *kimchi* using *Leuconostoc mesenteroides* as a starter culture; the authors observed that the same species (*Leuconostoc*, *Lactobacillus*, and *Weissella*) were predominant despite of the type of *kimchi* produced. The use of *Leuconostoc mesenteroides* as starter culture caused earlier consumption of free sugars and higher organic acid and mannitol production.

Doenjang is a Korean soybean fermented food traditionally prepared by spontaneous fermentation of *meju*. In this fermentation process, *Bacillus* and LAB species such as *Leuconostoc mesenteroides*, *Tetragenococcus halophilus*, and *Enterococcus faecium* were found (Kim et al. 2009). The metabolomic study carried out by Namgung et al. (2010) showed that polyols such as xylitol, mannitol, and sorbitol were produced in significant amounts during *doenjang* fermentation. While sorbitol showed a steady increase throughout the fermentation process, the amount of xylitol was doubled after 140 days of fermentation compared to the value found at day 0.

Cocoa bean fermentation

For chocolate production, the cocoa pulp-bean mass undergoes a spontaneous fermentation process. Key microbial species involved in a successful fermentation are (1) yeasts, which act in pectin degradation and ethanol production; (2)

LAB, which are involved in glucose and/or fructose and citric acid co-fermentation, lactic acid production, and mannitol formation; and (3) acetic acid bacteria (AAB), which produce acetic acid and overoxidate acetic and lactic acids.

Different cocoa bean fermentation practices, which vary considerably according to the region, are carried out. For instance, the Brazilian practice involves placing beans in large wooden boxes and a spontaneous and vigorous fermentation takes place for 7 days. During this time, beans are turned every day to intensify aeration (Schwan 1998). On the other hand, the Ecuadorian technique is characterized by short-time (4 days) fermentations and by spreading out of cocoa pulp-bean mass during the day and piled into heaps during the night to aid drying (Camu et al. 2008; Lehrian and Patterson 1983). These practices cause changes in the microenvironment within the fermenting mass displaying negatives effects such as volatile compound evaporation, heat dispersion, carbohydrate-rich pulp losing, a strong presence of yeasts, a broad LAB species diversity, and/or absence or too early AAB species.

In a multiphasic analyses of Ecuadorian cocoa fermentation, microbiological and metabolite target analyses of fermented cocoa samples as well as the sensory analyses of chocolates produced from fermented beans were performed to evaluate the influence of these practices on the microbial species diversity and dynamics during fermentation of cocoa bean (Papalexandratou et al. 2011a). Box and platform fermentations were carried out and metabolites present in the pulp, beans, and drainage samples were determined. Glucose and fructose were the main carbohydrates in the fresh pulp, being consumed simultaneously within 36–50 h of both platform and box fermentations. Large amounts of mannitol (29–35 g kg^{-1}) were produced in the pulp, while no sucrose was found. Inside fresh beans, mannitol amounts were low (~1.5 g kg^{-1}), while in drainage samples, mannitol was hardly found. *Leuconostoc pseudomesenteroides*, *Fructobacillus tropaeoli*-like, and *Lactobacillus fermentum* were the main LAB species found. This succession of cocoa-specific microbial species during fermentations has been reflected in low quantities of acetic acid and large amounts of mannitol and gluconic acid. The high amounts of mannitol produced by *Leuconostoc* spp. (strictly heterofermentative LAB) and *F. tropaeoli*-like resulted in a lower or absent additional supply of pyruvate and, hence, a lower conversion to volatile flavor compounds such as diacetyl, acetoin, and 2,3-butanediol. The traditional Ecuadorian cocoa bean fermentation method caused an incomplete fermentation impacting negatively on the succession of microbial activities and on the quality of the fermented dry cocoa beans.

Spontaneous cocoa bean box fermentations using the Brazilian methodology were also studied by Papalexandratou et al. (2011b). Physical parameters, microbial growth, bacterial species diversity (LAB and AAB), and metabolite kinetics were determined. The composition of fresh pulp of cocoa pods varies according to source but typically contains glucose, fructose, and citric acid. The main end-products of metabolism of these pulp substrates by associated microbiota were ethanol, lactic acid, mannitol, and/or acetic acid. Glucose and fructose were almost depleted after 30 h of fermentation while citric acid was consumed after 2 days. Mannitol production was found during the first day and reached a peak after 84 h of fermentation (24.7± 0.3 g kg^{-1}). In fresh cocoa beans, sucrose was the main carbohydrate (28.0 g kg^{-1}) which was converted into glucose and fructose during fermentation with lactic acid remaining at low amounts in the beans. *Fructobacillus pseudoficulneus* and *L. plantarum* were the predominant LAB species during the initial fermentation phase while *L. fermentum* and *Acetobacter pasteurianus* were the prevalent species during the fermentation.

Sourdough

Sourdough is a bread product made by a mixture of ground cereals—such as wheat or rye—and water that is spontaneously fermented by naturally occurring LAB and yeasts. The typical mildly sour taste of sourdough is due to the lactic acid produced by LAB strains. Sourdough fermentations have several beneficial effects on breads as they improve dough leavening due to gas formation, enhance texture and flavor, stimulate aroma formation, and promote dough acidification delaying spoilage (Hammes and Gänzle 1998; Arendt et al. 2007).

Sourdough fermentation processes and their ecosystems have been intensively studied during the last decade (De Vuyst and Vancanneyt 2007; Scheirlinck et al. 2007; Font de Valdez et al. 2010). Depending on the origin and type of sourdough fermentation, different LAB species and yeasts are involved. In this regard, Van der Meulen et al. (2007) studied population dynamics and metabolic profiling of laboratory wheat and spelt sourdough during 10-day backslopping. *L. plantarum*, *L. fermentum*, *Lactobacillus rossiae*, *L. brevis*, and *Lactobacillus paraplantarum* were the dominant LAB strains of sourdough ecosystems, while *L. fermentum* dominated one wheat sourdough. Metabolite concentrations strongly varied during the last fermentation days. Lactic acid, ethanol, and mannitol were the main products found. Other metabolites present were succinic acid, erythritol, phenyllactic acid, hydroxyphenyllactic acid, and indolelactic acid, which contributed to cell homeostasis to restore redox balance equilibration. Mannitol was mainly produced during days 4 and 5 of fermentation. The only exception was a wheat sourdough dominated by *L. fermentum* where mannitol was found after 10 days, suggesting that this species was responsible for mannitol

production. Mannitol concentrations were never higher than 1.0 g kg^{-1}; besides mannitol, erythritol (0.14 g kg^{-1}) was found in all sourdoughs.

More recently, Rühmkorf et al. (2012) reported on the ability of the indigenous sourdough strain *Lactobacillus reuteri* TMW 1.106 to in situ produce mannitol (between 40.3 and 49.2 g kg^{-1}) during fermentation of different gluten-free sourdoughs. This strain showed a strong mannitol dehydrogenase activity in all assayed flours since no fructose but high amounts of mannitol and acetate were found at the end of fermentation. When increasing sucrose concentration in the dough, less lactate and ethanol and more acetate and mannitol (up to 75.78 g kg^{-1}) were produced. The mannitol-producing *L. reuteri* strain grew faster than other assayed lactobacilli in some of the flours used, suggesting that the use of fructose as external electron acceptor, present in the sourdough ecosystem, enhances microbial competitiveness (Gänzle et al. 2007).

Although polyols have been determined as fermentation metabolites in several fermented foods as mentioned above, the involvement of these compounds has only been associated to an improved bacterial growth of the producer microorganism. The implication of polyols on the organoleptic properties of fermented foods has only been referred to cocoa bean fermentation where the synthesis of pyruvate and the concomitantly formation of aroma compounds were deviated towards the production of acetate and mannitol, negatively affecting the quality of fermented beans. More detailed studies concerning the effect of polyols on the organoleptic properties of fermented foods remain to be investigated.

Production of polyols by wild-type LAB

Mannitol

Current industrial production of mannitol is made by chemical synthesis through catalytic hydrogenation of a glucose/fructose (1:1) mixture at high temperatures and pressures (Makkee et al. 1985). This process, associated with low purity and yield due to poor catalyst selectivity, converts fructose into mannitol as well as its isomer sorbitol, while glucose is completely converted into sorbitol. This low efficient process yields about 25 % mannitol and 75 % sorbitol in the final mixture and the need of purification steps is required.

Biotechnological production of mannitol is an interesting alternative to the current industrial chemical production, and it has been actively investigated in the last 10 years. Certain heterofermentative LAB belonging to the genera *Leuconostoc*, *Oenococcus*, and *Lactobacillus* are efficient mannitol producers when D-fructose is available in the medium. Mannitol production by a fermentative process has several advantages as compared with chemical synthesis such as a complete conversion of D-fructose into D-mannitol without co-formation of sorbitol, mild production conditions, and no requirement of highly purified substrates (Soetaert et al. 1995; von Weymarn et al. 2002a).

Production of mannitol by heterofermentative LAB is based on their ability to use D-fructose as an alternative electron acceptor and convert it exclusively into D-mannitol in a one-step enzymatic reaction catalyzed by the enzyme mannitol 2-dehydrogenase (MDH). Besides lactate and ethanol production, reducing equivalents of [NAD(P)H] coming from sugar metabolism can be regenerated during this conversion (Monedero et al. 2010). Mannitol formation provides an advantage for microbial growth thanks to the MDH reaction enabling the regeneration of the $NAD(P)^+$ pool. Part of acetyl-P formed during heterofermentation can be channeled towards acetate production instead of ethanol with the extra gain of ATP via acetate kinase resulting in improved cell growth. Homofermentative LAB, which lack the enzyme MDH and use the glycolytic pathway for sugar fermentation, are unable to naturally produce mannitol (Song and Vieille 2009). Mannitol production by these LAB species has been achieved through different metabolic engineering strategies that will be later discussed.

Optimization of the culture conditions for mannitol production by heterofermentative LAB

In 1995, Stolz et al. reported that growth and metabolism of the sourdough strain *Lactobacillus sanfranciscensis* were strongly influenced by agitation and the presence of maltose and electron acceptors such as oxygen and fructose. Under agitation culture conditions or the addition of fructose to cultures growing on maltose, acetate was formed instead of ethanol and fructose was reduced to mannitol, features that contributed to the competitiveness of this *Lactobacillus* species in the sourdough ecosystem.

In the last decade, several studies have focused on the optimization of the culture conditions for mannitol production by LAB strains (Table 2). von Weymarn et al. (2002a) showed that *L. fermentum* NRRL B-1932 displayed efficient mannitol production capabilities in growing state; maximum mannitol yield of 94 mol% and volumetric productivities of 16 g l^{-1} h^{-1} using a simplified MRS-based medium at the highest temperature (35 °C) assayed were obtained. To reduce biomass production costs, mannitol production by different LAB strains under resting state was evaluated (von Weymarn et al. 2002b). *Leuconostoc mesenteroides* ATCC-9135 was the most efficient mannitol producer either in resting or slowly growing state. Optimization studies using response surface methodology showed that mannitol yield and specific productivity were strongly influenced by pH,

Table 2 Main examples of culture conditions used for the optimization of mannitol production by different LAB strains

LAB strain	Culture medium	Fermentation conditions	Mannitol production	Reference
L. fermentum NRRLB-1932	Simplified production (SP) medium containing(g l^{-1}): tryptone, 10; yeast extract, 5; K_2HPO_4, 2; fructose, 20; glucose, 10; $MgSO_4$, 0.4; $MnSO_4$, 0.02	Free pH batch cultures 25, 30, and 35 °C Agitation—200 and 400 rpm	83 g l^{-1} Yield—93.6 mol% Volumetric productivity—16 g l^{-1} h^{-1}	von Weymarn et al. (2002a)
Leuconostoc mesenteroides ATCC-9135	Bioconversion media:	Controlled pH (4.8–6.0) semicontinuous process (membrane cell recycle bioreactor)	Yield—96.6 mol%	von Weymarn et al. (2002b)
	• BC1 (for resting cell cultures with low sugar concentration) (g l^{-1}) yeast extract, 0.25; glucose, 10; fructose, 20; K_2HPO_4, 2; $MgSO_4$, 0.2; $MnSO_4$, 0.01; tryptone, 0.5	28–38 °C	Volumetric productivity—26.2 g l^{-1} h^{-1}	
	• BC2 (for resting cell cultures with high sugar concentration) (g l^{-1}) yeast extract, 0.5; glucose, 50; fructose, 100; K_2HPO_4, 1; $MgSO_4$, 0.2; MnSO4, 0.02; tryptone, 1.0	Agitation—200 and 400 rpm		
L. intermedius NRRL B-3693	Simplified MRS medium (without beef extract and Tween 80) with fructose (30 %, *w*/*v*)	Controlled pH (5.0), fed-batch fermentation 37 °C Agitation—130 rpm	202 g l^{-1} Yield—67 mol%	Saha and Nakamura (2003)
	Simplified medium (g l^{-1}) fructose, 300; soy peptone, 5.0; CSL, 50; ammonium citrate, 2.0; $MgSO_4$, 0.1; $MnSO_4$, 0.05; Na_2HPO_4, 2.0 (final pH 5.0)	Controlled pH (5.0) fed-batch fermentation 37 °C Agitation—130 rpm	201 g l^{-1}	Saha (2006a)
	Medium containing sugarcane molasses and fructose syrup in a 1:1 ratio (total sugars, 150 g l^{-1}; fructose/glucose 4:1), soy peptone D, 5 g l^{-1}; CSL, 50 g l^{-1}	Controlled pH (5.0) batch culture 37 °C Agitation—130 rpm	104 g l^{-1}	Saha (2006b)
	Inulin-based medium containing (g l^{-1}) soy peptone, 5.0; CSL, 50; ammonium citrate, 2.0; $MgSO_4$, 0.1; $MnSO_4$, 0.05; Na_2HPO_4, 2 (final pH 5.0) plus acid hydrolyzed inulin, inulin, or fructose and inulin mixture (3:5, total 400 g l^{-1})	Controlled pH (5.0) batch culture 37 °C Agitation—130 rpm	228 g l^{-1}	Saha (2006c)
Leuconostoc fructosum	Hydrolyzed carob syrup-based medium supplemented with MRS nutrients except for ammonium citrate, which was replaced by sodium citrate buffer	Free pH cultures 30 °C Static culture	44 g l^{-1} Volumetric productivity—2.36 g l^{-1} h^{-1}	Carvalheiro et al. (2011)
Leuconostoc mesenteroides	Cashew apple juice-based medium with yeast extract and K_2HPO_4	Controlled pH (6.5) culture 30 °C Agitation—150 rpm	18 g l^{-1} Yield—67 mol% Volumetric productivity—1.8 g l^{-1} h^{-1}	Fontes et al. (2009)
L. fermentum CRL 573	Modified MRS medium: MRS with glucose (1 %, *w*/*v*) and fructose (6.5 %, *w*/*v*)	Free and controlled pH cultures—4.0, 5.0, and 6.0 37 °C Agitation—100 rpm	56 g l^{-1} Yield—93.5 mol%	Rodríguez et al. (2012)
L. reuteri CRL 1101	Modified MRS containing sugarcane molasses instead of glucose at final sugar concentration of 3.0, 5.0, 7.5, or 10.0 % (*w*/*v*)	Free pH cultures 30 and 37 °C With (100 rpm) and without agitation	38 g l^{-1} Yield—86.9 mol%	Ortiz et al. (2012)

CSL corn steep liquor (» 50 % solids, *w*/*v*)

being the best values (97 mol% and 26.2 g l^{-1} h^{-1}, respectively) obtained at pH 4.5. Increasing the initial fructose concentration from 100 to 120 and 140 g l^{-1} had a negative effect on productivities, due to substrate and end-product inhibition of the enzyme MDH while an increase in biomass concentration in the resting cell bioconversion had no effect on the specific mannitol productivities. In a semicontinuous process using a membrane cell recycle bioreactor with the same initial cell density, conversion of fructose to mannitol (~0.9 mol mol^{-1}) and volumetric mannitol productivity (~20 g l^{-1} h^{-1}) remained stable for 14 successive batch cultures (von Weymarn et al. 2003). The scaling-up of this semicontinuous process from 2-l laboratory to a 100-l pilot allowed achieving higher mannitol yields (93–97 %) and volumetric mannitol productivities (>20 g l^{-1} h^{-1}). The mannitol-enriched permeate solution contained 112 g l^{-1} mannitol. The downstream processing protocol comprised simple steps such as evaporation, crystallization (easy to carry out due to the low mannitol solubility in water), crystal separation, and drying. This bioprocess offered several advantages such as lower raw material costs, improved mannitol yield, no need for addition of inert gases into the reactor, and a simplified purification protocol (von Weymarn et al. 2003).

Saha and Nakamura (2003) found that the strain *Lactobacillus intermedius* NRRL B-3693 was able to synthesize high amounts of mannitol (up to 200 g l^{-1}) in a fructose-rich medium at controlled pH (5.0) in batch fermentation with agitation (130 rpm) (Table 2). This strain used fructose both as substrate fermentation and as alternative electron acceptor at the same time depending on glucose availability in the medium. When the strain was grown in fructose as sole carbon source, lactic acid and ethanol together with mannitol (yield 0.70 g g^{-1} of fructose) were produced. When using a fed-batch fermentation approach with fructose (300 g l^{-1}) as unique substrate, the time needed for maximum mannitol production (202.5±4.3 g l^{-1}) decreased from 136 to 92 h. In the presence of a glucose/fructose mixture in a 1:2 ratio, glucose was used as carbon source forming lactic acid and acetic acid instead of ethanol, and fructose was almost exclusively used as electron acceptor forming a large amount of mannitol with conversion efficiencies yielding 89–96 %. The authors suggested that one third of fructose could be replaced by glucose or carbohydrates such as maltose, galactose, mannose, raffinose, or starch plus glucoamylase, and two thirds of fructose could be replaced by sucrose. When mannitol concentration in the fermentation broth is around 200 g l^{-1}, it can be crystallized by simply removing the cells from the fermented medium and cooling it slowly to 4 °C since the solubility limit of mannitol is 180 g l^{-1} at 25 °C.

The effect of several nutrient salts (ammonium citrate, sodium phosphate, magnesium sulfate, and manganese sulfate) and corn steep liquor (CSL) on mannitol production by *L. intermedius* NRRL B-3693 was studied by Saha (2006a). CSL, an inexpensive source of nitrogen, essential vitamins, amino acids, and minerals, and the major by-product of the corn starch processing may effectively replace yeast extract and peptone in the growth media (Amartey and Leung 2000). Different concentrations of nutrient salts were added to a simplified medium containing very high fructose amounts (300 g) and soy peptone (5 g l^{-1}). Mannitol production increased when magnesium sulfate and manganese sulfate were added to the medium separately; manganese sulfate (0.033 g l^{-1}) was essential for mannitol production and caused a more pronounced positive effect on mannitol yield than magnesium sulfate. Using this simplified medium with high fructose concentration (300 g l^{-1}), *L. intermedius* NRRL B-3693 produced 200.6±0.2 g l^{-1} of mannitol at pH 5.0 and 37 °C (Table 2). In another approach, Saha (2006b) evaluated CSL supplemented with different inexpensive carbon sources (sugarcane molasses and fructose syrup) and inexpensive organic and inorganic nitrogen sources to replace the traditional but more expensive carbon (glucose and fructose) and nitrogen (bacteriological peptone and yeast extract) sources usually used in culture media. Molasses, a by-product of sugar manufacturing processing, generally contains sucrose (40–42 %) and glucose and fructose (1:1). *L. intermedius* NRRL B-3693 was grown in stirred cultures at pH 5.0 in a medium containing sugarcane molasses and fructose syrup (1:1 ratio) (total sugars, 150 g l^{-1}; fructose/glucose ratio 4:1), soy peptone D (5 g l^{-1}), and CSL (50 g l^{-1}). Under these conditions, the strain produced high concentrations of mannitol (105 g l^{-1}) after 22 h of fermentation. The production of mannitol was strongly influenced by the variability of CSL (Saha and Racine 2010).

Carvalheiro et al. (2011) performed a detailed evaluation of eight mannitol-producing LAB strains in a hydrolyzed carob syrup-supplemented medium (Table 2). Carbohydrates typically present in carob syrup are sucrose (50 % of total sugars), fructose, glucose, and pinitol in a total concentration of 200 g l^{-1} (Moniz et al. 2009). All LAB strains tested were able to grow in this medium and produced mannitol at high yields (0.7 g mannitol g^{-1} fructose) and volumetric productivities (1.3 g l^{-1} h^{-1}). The strains consumed fructose and glucose simultaneously with fructose assimilation rates being always higher than glucose rates. *L. fructosum* NRRL B-2041 exhibited the best mannitol production value (43.7 g l^{-1}), volumetric productivity (2.36 g l^{-1} h^{-1}), and conversion efficiency (fructose to mannitol 100 %).

To evaluate mannitol production by LAB, Fontes et al. (2009) used cashew apple juice as carbon source. Cashew apple is the cashew fruit peduncle rich in fructose, glucose, vitamins, minerals, and some amino acids (Oliveira et al. 2002; Campos et al. 2004). Cashew trees are cultivated for cashew nut production, and the pulp representing 90 % of

the fruit weight is discarded. *Leuconostoc mesenteroides* B-512 produced 18 g l^{-1} of mannitol with 1.8 g l^{-1} h^{-1} productivity and a 67 % yield in a cashew apple juice-supplemented medium containing only a final concentration of 50 g l^{-1} of reducing sugar (28 g l^{-1} of fructose).

More recently, Rodríguez et al. (2012) reported on the ability of *L. reuteri* CRL 1101 and *L. fermentum* CRL 573 to produce mannitol in a rich medium (modified MRS) containing a fructose/glucose (6.5:1.0) mixture as carbon source. Batch fermentations with variable and constant pH (6.0 and 5.0) were undertaken. Fructose was reduced into mannitol while glucose supported cell growth. Interestingly, these strains were not able to grow on fructose as the sole energy source at concentrations between 30 and 75 g l^{-1}. Mannitol production and yields by *L. reuteri* CRL 1101 (22.22 g l^{-1} and 75.7 mol%, respectively) and *L. fermentum* CRL 573 (56.84 g l^{-1} and 93.5 mol%, respectively) were maximal in fermentations at constant pH (5.0) compared to variable pH cultures (*L. reuteri* CRL 1101, 16.58 g l^{-1} and 96.7 mol%; *L. fermentum* CRL 573, 12.02 g l^{-1} and 55.4 mol%, respectively). *L. fermentum* CRL 573 produced almost 5-fold higher amount of mannitol at controlled pH 5.0 than at free pH; moreover, the maximum mannitol production was reached after 12 h of incubation as compared to variable pH fermentations (24 h). Depending on the pH values, *L. reuteri* CRL 1101 used fructose only as an alternative external electron acceptor (variable pH or constant pH 6.0) since fructose was completely converted into mannitol, or both as electron acceptor and energy source (pH 5.0). Interestingly, *L. fermentum* CRL 573 reduced fructose into mannitol by using it as an alternative electron acceptor and simultaneously employing it as energy source, independent of the pH conditions. These features demonstrate that the optimum culture conditions for mannitol production are strain dependent.

Sugarcane molasses as low-cost carbon source for mannitol production by *L. reuteri* CRL 1101 was evaluated in free pH batch fermentations. Different culture conditions comprising molasses concentrations between 3 and 10 % (*w*/*v*), incubation temperatures of 37 and 30 °C, and static and agitated conditions were studied (Ortiz et al. 2012). *L. reuteri* CRL 1101 grew well in all assayed media; glucose was fermented into lactic and acetic acids and ethanol, while fructose was reduced to mannitol. Maximal mannitol concentrations (32.8 g l^{-1}) were achieved using 7.5 % (*w*/*v*) of molasses at 37 °C after 24 h of incubation. No significant increase in mannitol production was observed when increasing molasses concentration up to 10 % (*w*/*v*) or prolonging fermentation time to 48 h. Agitation conditions did not significantly improve mannitol production (38.4 g l^{-1}); however, a 25-fold increase in mannitol synthesis was obtained after 8 h of incubation as compared with static cultures at the same time point.

Mannitol 2-dehydrogenase activity

To our knowledge, the enzyme MDH was for the first time described in *L. brevis* by Martinez et al. (1963) who showed that the enzyme was present in extracts of fructose-grown cells in quantities equivalent to about 3 % of soluble proteins. More recently, MDH enzymes and their respective genes (*mdh*) have been studied from some LAB species including *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *L. reuteri*, *L. intermedius*, and *L. sanfranciscensis* (Aarnikunnas et al. 2002; Hahn et al. 2003; Korakli and Vogel 2003; Saha 2004; Sasaki et al. 2005). Differences in MDH structure, molecular weight, and cofactor dependency are shown in Table 3. Although several MDH from LAB have been characterized, only Ortiz et al. (2012) have investigated on the correlation of MDH activity and mannitol production during growth of *L. reuteri* CRL 1101. Enzymatic activity was determined at different incubation times (4–48 h) under the assayed conditions [variable pH batch cultures, (3–10 %, *w*/*v*) sugar concentrations, 30 and 37 °C, static and agitated cultures]. In general, the highest MDH activity values (3.646–2.064 U/mg cell protein) were obtained at log growth phase (4 h) in all fermentations assayed, which agrees with the cell-associated mannitol synthesis during log and early stationary phases. Interestingly, highest MDH activity (3.646±0.052 U/mg cell protein) was detected in the presence of 3 % (*w*/*v*) carbon source at 37 °C after 24 h, which was not correlated with maximum mannitol production values scored at higher sugar concentrations (7.5 or 10 %, *w*/*v*).

Erythritol

The wine heterofermentative LAB *O. oeni* produces erythritol as an alternative pathway for NAD(P)H reoxidation during anaerobic glucose metabolism. The rate of erythritol production represents 37 % of the glucose consumption rate (Veiga-Da-Cunha et al. 1993). The pathway of erythritol formation from glucose involves four enzymes: (1) a phosphoglucose isomerase that converts glucose 6-P into fructose 6-P, (2) a phosphoketolase that cleaves fructose 6-P into erythrose 4-P and acetyl-P, (3) the erythritol 4-P dehydrogenase that reduces erythrose 4-P into erythritol 4-P, and (4) a phosphatase that converts erythritol 4-P into erythritol. Acetate or ethanol is produced from acetyl-P (Veiga-da-Cunha et al. 1993).

O. oeni can also produce erythritol when growing in a pantothenate-deficient medium. *O. oeni*, like other LAB strains, is auxotrophic for pantothenic acid, which is an intermediate of coenzyme A (CoA) synthesis (Garvie 1967). CoA is required for fatty acid activation and for reactions involving fatty acid CoA-esters. During heterolactic

Table 3 Characteristics of the enzyme mannitol 2-dehydrogenase (MDH) from different LAB species

		LAB species				
		L. reuteri	*L. intermedius*	*L. sanfranciscensis*	*Leuconostoc pseudomesenteroides*	*Leuconostoc mesenteroides*
Structural arrangement		Homodimer	Heterotetramer	Monomer	Homotetramer	ND
Molecular weight	Native	75 kDa	171 kDa	53 kDa	155 kDa	ND
	Subunits	40 kDa	43 and 34.5 kDa	44 kDa	43 kDa	36 kDa
Optimum pH		5.4	5.5	5.8	5.4	ND
Km $_{Fru \rightarrow Man}$		34 mM	20 mM	24 mM	44 mM	71 mM
Vmax $_{Fru \rightarrow Man}$		72 U/mg prot	396 U/mg prot	29 U/mg prot	ND	ND
Cofactor dependency		NADPH	NADPH	NADPH (100 %) NADH (40 %)	NADPH (100 %) NADH (32 %)	NADH
Substrate specificity		D-Fructose	D-Fructose	D-Fructose	D-Fructose	D-Fructose Fru-1-P (11 %)
Gene and protein (accession number[a])		1,008 pb (AY485531.1) 336 aa (AAS55855.1)	ND	ND	1,017 pb (AJ486977.1) 338 aa (CAD31644.1)	1,014 pb (AY090766.1) 338 aa (AAM09029.1)
Specific activity (U/mg prot)	Crude extract	ND	0.99	1.0	15.0	0.68
	MDH purified	124.6	331.2	15.5	450.0	70.0
Notes		► No affinity: Fru-6-P, mannose, arabinose, sorbitol ► Activated by Zn^{2+} ► Inhibited by EDTA	► No affinity: glucose, xylose, arabinose ► Activated: Zn^{2+}, Mn^{2+}, and Co^{2+} ► Inhibited by *p*-chloromercuribenzoate	► No affinity: glucose, xylose, arabinose ► Activated by NH_4^+, Ca^{2+}, Li^{2+}, Mg^{2+}, K^+, and EDTA ► Inhibited by Zn^{2+}, Mn^{2+}, and Fe^{2+}	► Activated by Zn^{2+} ► Inhibited by EDTA, citrate, imidazole	► Inhibited by *p*-chloromercuribenzoate
Reference		Sasaki et al. (2005)	Saha (2004)	Korakli and Vogel (2003)	Hahn et al. (2003)	Aarnikunnas et al. (2002)

ND not described

[a] Accession number of nucleotide sequences available in GenBank

glucose fermentation, CoA and acetyl-CoA are substrates of two different enzymes. In this sense, CoA plays an important role in the ethanol pathway, but not in pathways that lead to formation of erythritol, glycerol, and lactate. Richter et al. (2001) demonstrated that pantothenate deficiency, and a consequent CoA deficiency, is the major reason for the shift from heterolactic to erythritol/acetate fermentation as a cell strategy to NAD(P)$^+$ regeneration in *O. oeni*.

Using several *L. sanfranciscensis* strains mostly isolated from sourdough, Stolz et al. (1995) have shown that erythritol is synthesized under stress conditions as an additional metabolic product. Starved resting cells of *L. sanfranciscencis* LTH 1729 and LTH 2581 formed up to 20 % erythritol from maltose in the presence of citrate under anaerobic conditions.

Polyol production by genetically modified LAB

Sorbitol and xylitol are not naturally produced by LAB. However, efficient genetic tools such as gene inactivation and expression of homologous or heterologous genes have been successfully developed in LAB and applied to reroute the carbon flux to the formation of these sugar alcohols. The metabolic engineering processes leading to the synthesis of these polyols and the strategies used to produce mannitol by homofermentative LAB are briefly presented here (for a detailed revision, readers may check reviews of Monedero et al. 2010 and Akinterinwa et al. 2008).

In LAB, cofactor NAD^+ is consumed during glycolysis and regenerated by the reduction of pyruvate to lactate by the LDH enzyme maintaining the cellular redox balance. In heterofermentative LAB, fermentation of pentoses leads to the formation of pyruvate and acetyl-P and their consequent conversion to lactate and acetate while hexoses can be converted to lactate, CO_2, and ethanol. The low activity of the ethanol pathway in the reoxidation of NAD(P)H due to a low acetaldehyde dehydrogenase activity limits heterofermentative growth on glucose (Maicas et al. 2002). Heterofermentative LAB bypass the limiting ethanol pathway by using alternative pathways for NAD(P)H reoxidation. Thus, when external electron acceptors such as O_2, pyruvate, citrate, or fructose are present, the microorganism can use them as such to regenerate NAD^+ using

other dehydrogenase enzymes. For polyol synthesis, adequate metabolic conditions should be achieved to provide abundant NADH for efficient reduction of sugar precursors. An important strategy frequently employed during metabolic engineering consists in blocking the formation of natural proton sinks such as lactate or ethanol in the final steps of glycolysis (Neves et al. 2002; Viana et al. 2005).

Sorbitol

Very few reports have been published concerning sorbitol production by LAB. Nissen et al. (2005) and Ladero et al. (2007) studied sorbitol production by strains of *L. casei* and *L. plantarum* reverting the sorbitol catabolic pathway taking advantage of the reversibility of the enzyme sorbitol 6-P dehydrogenase (S6PDH). This enzyme is responsible for the interconversion of sorbitol 6-P to fructose 6-P. Nissen et al. (2005) generated the strain *L. casei* BL232 with S6PDH activity inducible by lactose by integration of the gene *gutF* (encoding S6PDH) in the chromosomal lactose operon. The recombinant strain pregrown on lactose was able to produce sorbitol from glucose with conversion rates of 2.4 % (moles of sorbitol per mole of glucose). Sorbitol synthesis was increased 2.5 times (conversion rate of 4.3 %) when the gene encoding for the LDH enzyme (*ldhL*) of *L. casei* BL232 was inactivated. A further inactivation of the *mtl*D gene, encoding a mannitol 1-P dehydrogenase, showed an increase in sorbitol production without mannitol synthesis, avoiding thus a polyol mixture (De Boeck et al. 2010).

Probiotic strain *L. plantarum* WCFS1 has two possible operons related to sorbitol catabolism. Ladero et al. (2007) generated plasmids harboring the *L. plantarum* WCFS1 enzymes S6PDH (*srl*D1 and *srl*D2) translationally fused with the expression signals of the *L. plantarum ldhL* gene. The recombinant plasmids were introduced into *L. plantarum* NCIBM8826 (wild type) and its D- and L-LDH-deficient derivatives. Production of sorbitol (5.5 mM) was detected only in LDH-deficient cells containing the overexpressing *srl*D plasmid when using cell suspensions without pH control. In fermentations with resting cells at controlled pH (6.5), greater amount of sorbitol (13.1 mM) and increased glucose to sorbitol efficiency conversion (65 %) were found. In both cases, production of mannitol (2.0 and 2.7 mM and 5 and 13 % glucose conversion, respectively) was also detected (indicating competition for fructose 6-P rerouting by natively expressed mannitol 1-P dehydrogenase). Growing cells obtained lower production levels of sorbitol than resting cells, a difference that may be caused by a higher ATP demand for biomass production.

Xylitol

While metabolic engineering strategies using homologous genes have been applied for sorbitol synthesis by LAB, xylitol production was obtained by heterologous expression of D-xylose reductase and xylose transporter genes from the yeast *Pichia stipitis* CBS 5773 and the strain *L. brevis* ATCC 8287, respectively. In this work, Nyyssölä et al. (2005) cloned both genes in *Lactococcus lactis* NZ9800 in a plasmid under control of the *nisA* promoter. The recombinant *Lactococcus* strain, which was not capable of metabolizing D-xylose and D-xylitol, produced D-xylitol during co-metabolism of glucose and D-xylose. D-Xylose was quantitatively converted to D-xylitol reaching an amount of 54.4 g l^{-1} and a volumetric productivity of 2.72 g l^{-1} h^{-1} after 20 h, these values being similar to those obtained with yeasts. The final xylitol concentration obtained was 74.1 g l^{-1} after a fermentation of 40.5 h.

Mannitol

Mannitol can be scarcely produced (~16 g l^{-1}) by LDH-deficient homofermentative LAB as reported by Neves et al. (2000) with *Lactococcus lactis* strains. In this case, fructose 6-P is converted into mannitol 1-P (Mtl1P) in an NADH-dependent reaction catalyzed by mannitol 1-P dehydrogenase (Mtl1PDH), which is subsequently dephosphorylated to mannitol by mannitol phosphatase. In contrast, utilization of mannitol by homofermentative LAB is more common than production (in the absence of glucose) (Wisselink et al. 2002). To prevent mannitol consumption, Gaspar et al. (2004) depleted the mannitol transport system (*mtlF* or *mtlA* genes) by gene replacement in the *ldh*-deficient *Lactococcus lactis* FI9630 strain. The double mutant strains of *Lactococcus lactis* (Δ*ldh*Δ*mtlA* and Δ*ldh*Δ*mtlF*) were able to produce mannitol (2.4 g l^{-1}) from glucose. More recently, Gaspar et al. (2011) blocked different *ldh* genes (*ldh*X and *ldh*B) creating, respectively, single (Δ*ldh*B) and double mutant strains (Δ*ldh*B Δ*ldh*X). When the *lactococcal mtl*D gene (mannitol 1-P dehydrogenase) and the heterologous gen coding for mannitol 1-P phosphatase (MTLP) from *Eimeria tenella* were cloned under a nisin-inducible promoter, mannitol yield increased 3–4-fold in the mutant strains with regard to the wild-type strain.

In another approach, Wisselink et al. (2004) overexpressed the mannitol 1-P dehydrogenase gene (*mtlD*) from *L. plantarum* in three strains of *Lactococcus lactis*: parental strain NZ9000 (wild type), LDH-deficient strain NZ9010, and the phosphofructokinase (PFK)-reduced HWA217 strain. While the wild-type strain (expressing *mtlD*) was unable to produce mannitol, mannitol formation (0.008 mmol $mmol^{-1}$ glucose consumed) was observed in the culture supernatant of the strain displaying reduced PFK expression and an even higher mannitol conversion (0.011 mmol $mmol^{-1}$ glucose consumed) in the supernatant of the LDH-deficient and *mtl*D-transformed strain.

Mannitol production values by homofermentative LAB through metabolic engineering approaches are still lower than those achieved using wild-type heterofermentative

LAB. Genetic modification of homofermentative LAB strains is only justified when they are required for a specific purpose or process such as the use of *L. casei* or *Lactococcus lactis* strains in the manufacture of dairy products and not for mannitol production itself.

A few studies have been conducted to improve mannitol synthesis by heterofermentative LAB through metabolic engineering processes. Again, Aarnikunnas et al. (2003) observed that disrupting both genes encoding D- and L-specific LDH (*ldh*) in *L. fermentum* led to the production of mannitol and pyruvic acid instead of lactic acid. In some heterofermentative LAB, total conversion of fructose into mannitol cannot be achieved during fermentation as part of fructose is converted into fructose 6-P by fructokinase enzyme in the phosphoketolase pathway. Helanto et al. (2005) obtained a random mutant of *Leuconostoc pseudomesenteroides* (altered in the expression of the *fruK* gene) unable to grow on fructose as sole carbon source. This strain showed reduced fructokinase activity, normal fructose uptake, and improved conversion (74 to 86 mol%) of fructose into mannitol.

Despite the importance of alternative sources for mannitol production, where its synthesis by heterofermentative LAB has been a promising alternative to its current industrial production, further metabolic engineering studies to overexpress MDH activity in these bacteria are needed.

Conclusions

LAB are food grade microorganisms extensively used as starter cultures in the elaboration of fermented foods and beverages and in the production of nutraceutics or as probiotics. Heterofermentative LAB naturally produce polyols such as mannitol and erythritol; formation of these compounds as food or pharmaceutical ingredients or in situ in fermented foods has been studied. Optimization of culture conditions including fermentation parameters and formulation of inexpensive culture media for an efficient polyol synthesis by LAB has been reported for different species. In particular, mannitol formation by LAB is still pursued as an interesting alternative of the current chemical industrial process. Moreover, genetic engineering processes for the synthesis of mannitol by homofermentative LAB or production of xylitol and sorbitol by LAB have been investigated. Less is known yet on the influence of these polyols on organoleptic properties of fermented foods where these compounds are naturally formed as in fermented vegetables, cocoa bean fermentation, and sourdough. The future challenge, however, is the formulation of novel fermented foods naturally sweetened with mannitol or erythritol; further studies in this direction remain to be done.

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