Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Recent advances in biological production of sugar alcohols

Yong-Cheol Park[1,4], Eun Joong Oh[2,4], Jung-Hyun Jo[3], Yong-Su Jin[2] and Jin-Ho Seo[3]

Sugar alcohols, such as xylitol, mannitol, sorbitol, and erythritol are emerging food ingredients that provide similar or better sweetness/sensory properties of sucrose, but are less calorigenic. Also, sugar alcohols can be converted into commodity chemicals through chemical catalysis. Biotechnological production offers the safe and sustainable supply of sugar alcohols from renewable biomass. In contrast to early studies that aimed to produce sugar alcohols with microorganisms capable of producing sugar alcohols naturally, recent studies have focused on rational engineering of metabolic pathways to improve yield and productivity as well as to use inexpensive and abundant substrates. Metabolic engineering strategies to utilize inexpensive substrates, alleviate catabolite repression, reduce byproduct formation, and manipulate redox balances led to enhanced production of sugar alcohols.

Addresses
[1] Department of Bio and Fermentation Convergence Technology, Kookmin University, Seoul 136-702, Republic of Korea
[2] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, IL, USA
[3] Department of Agricultural Biotechnology and Center for Food and Bioconvergence, Seoul National University, Seoul 151-742, Republic of Korea

Corresponding author: Seo, Jin-Ho (jhseo94@snu.ac.kr)
[4] These authors contributed equally to this work.

Current Opinion in Biotechnology 2016, **37**:105–113

This review comes from a themed issue on **Food biotechnology**

Edited by **Yong-Su Jin** and **Jin-Ho Seo**

For a complete overview see the Issue and the Editorial

Available online 23rd December 2015

http://dx.doi.org/10.1016/j.copbio.2015.11.006

Introduction

Sugar alcohols are noncyclic hydrogenated carbohydrates which can be acquired when aldehyde or ketone in sugars is reduced to the hydroxyl group. They are widely found in fruits and vegetables, as well as in fermentation broth of microorganisms. Sugar alcohols not only offer a wide range of sweetness and cooling effect, but also non-cariogenic and less calorigenic properties. Due to their health-promoting benefits as sugar substitutes, they are used in the food and pharmaceutical industries. In addition, some sugar alcohols such as xylitol and sorbitol have potential applications as building blocks for producing various value-added derivatives [1–3].

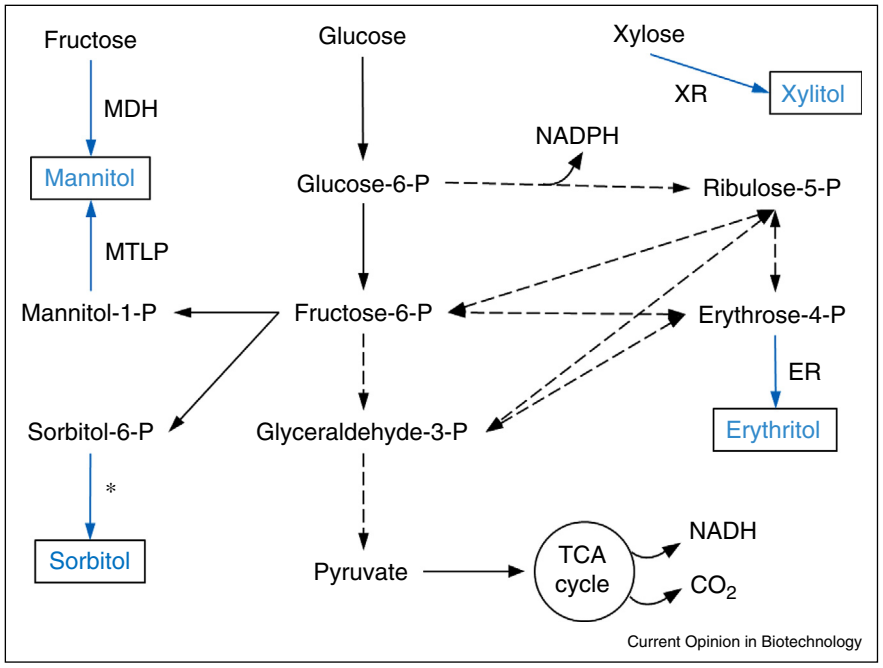
Most sugar alcohols are industrially produced by the catalytic hydrogenation of sugars under high pressure and temperature. However, current chemical processes require extreme conditions and costly chromatographic purification steps, which leads to low final product yields [1]. Because of these drawbacks, biotechnological production has been actively pursued [4,5]. Biotechnological production based on microbial fermentation offers safer and environmentally friendly processes and economic utilization of agricultural waste residues. While traditional strategies to improve sugar alcohol production through isolating better strains and optimizing culture conditions have been diminishing, metabolic engineering to improve production of sugar alcohols have been extensively pursued to establish economic microbial production platforms (Figure 1 and Table 1). In particular, metabolic engineering strategies to utilize inexpensive substrates, alleviate catabolite repression, and manipulate redox balances led to the rapid and efficient production of sugar alcohols. This review will focus on recent advances in metabolic engineering approaches to improve the biological production of sugar alcohols.

Xylitol

Xylitol is a representative five-carbon sugar alcohol that can reduce the risk of developing dental caries and is safe for diabetics. It is also a platform chemical which can be transformed to various starting materials for plastics and polymerized directly into unsaturated polyester resin [6]. The global xylitol market is more than 0.13 M tons per year, with <5.5 $/kg for bulk purchase (by pharmaceutical and chewing gum companies), and xylitol occupies 12% of the total polyol market [7]. The xylitol market continues to grow owing to an increase in chewing-gum and health markets, and new development of its application for manufacturing commodity products.

Xylitol can be produced from xylose that can be obtained from lignocellulosic biomass. Various eukaryotic and prokaryotic microorganisms have been engineered by random mutagenesis, and rational metabolic engineering. As shown in Figure 1, microorganisms naturally expressing xylose reductase (XR), such as *Candida tropicalis*, and

Figure 1



Pathway of sugar alcohol production by engineered microorganisms. MDH: mannitol dehydrogenase, MTLP: mannitol-1-phosphatase, XR: xylose reductase, ER: erythrose reductase, *: unidentified dephophorylation.

C. guillermondii, can produce xylitol during their xylose assimilation. Advances in metabolic engineering approaches enabled various microorganisms that do not contain innate XR to produce xylitol efficiently.

A baker's yeast, *Saccharomyces cerevisiae*, is a Generally Recognized as Safe (GRAS) microorganism used for producing foods, biofuels and biochemicals. In spite of its ability to use various sugars, it is unable to metabolize

Table 1

Sugar alcohol production by engineered microorganisms.

Microorganisms	Genetic perturbations	Substrate	Yield (g product/g substrate)	Productivity (g product/L·h)	References
Xylitol					
S. cerevisiae	XR, CBT, BGL	Cellobiose and xylose	0.96	1.50	[13••]
S. cerevisiae	XR, *ACS1*, *ZWF1*	Glucose and xylose	1	4.27	[21••]
C. tropicalis	XR, *Δxyl2*	Glucose and xylose	0.96	1.44	[16]
E. coli	XR, *ΔxylAB*, *ΔptsG*, *ΔptsF*	Glucose and xylose	>0.95	1.57	[25]
Mannitol					
L. reuteri	*tpfkA*, *pkaC*	Glucose and fructose	0.75	0.58	[36]
E. coli	*fdh*, *mdh*, *glf*, *xylA*	Glucose and formate	0.83	3.16	[38]
C. magnoliae	Mutagenesis	Glucose and fructose	0.81	4.00	[40]
Sorbitol					
L. plantarum	*ΔldhL*, *ΔldhD*, *srlD*	Glucose	0.66	–	[44]
L. casei	*Δldh1*, *ΔgutB*, *ΔmtlD*, *gutF*	Lactose	0.05	–	[45•]
Erythritol					
C. magnoliae	Mutagenesis	Glucose	0.11	0.12	[55]
Y. lipolytica	Mutagenesis	Glucose	0.25	0.23	[54]

XR: xylose reductase, CBT: cellobiose transporter, BGL: beta-glucosidase, *ZWF1*: glucose-6-phosphate dehydrogenase, *ACS1*: acetyl-CoA synthetase, *xyl2*: xylitol dehydrogenase, *xylA*: xylose isomerase, *xylB*: xylulose kinase, *ptsG*: phosphoenolpyruvate-dependent glucose phosphotransferase system, *ptsF*: phosphoenolpyruvate-dependent fructose phosphotransferase system, *rpfkA*: modified 6-phospho-1-fructokinase, *pkaC*: catalytic subunit of a cAMP-dependent protein kinase, *fdh*: NAD^+-dependent formate dehydrogenase, *mdh*: NAD^+-dependent mannitol 2-dehydrogenase, *glf*: glucose facilitator, *ldhL* and *ldhD*: lactate dehydrogenase, *srlD*: sorbitol-6-phosphate dehydrogenase, *ldh1*: lactate dehydrogenase, *gutB*: sorbitol phosphotransferase system, *mtlD*: mannitol-1-phosphate dehydrogenase, *gutF*: sorbitol-6-phosphate dehydrogenase.

xylose since it does not have xylose reductase (XR) and xylitol dehydrogenase (XDH). The absence of these two enzymes encouraged us to engineer xylitol-biosynthesizing microorganisms with the theoretical yield of 1 mol xylitol per mol xylose. As XR catalyzes the conversion of xylose to xylitol (Figure 1), the *XYL1* gene encoding XR from *Scheffersomyces stipitis* (formerly *Pichia stipitis*) was overexpressed in *S. cerevisiae* [8]. Because of the absence of XDH in recombinant *S. cerevisiae* for complete oxidation of xylose and xylitol to metabolic energy, additional sugars such as glucose, galactose, fructose and mannose should be supplied for cell growth and viability maintenance [9,10]. In typical batch-wise production of xylitol in a medium with glucose and xylose [9], recombinant *S. cerevisiae* expressing the *XYL1* gene consumed glucose faster than xylose because of the high affinity of sugar transporters for glucose relative to xylose. At low glucose concentrations, recombinant *S. cerevisiae* takes xylose up via high-affinity glucose transporters [8] and can metabolize xylose to xylitol simultaneously. But xylose is unable to be converted into xylitol after depletion of glucose, owing to the fact that NAD(P)H required for the XR reaction is not supplemented [11,12]. For overcoming this hurdle, two strategies have been developed: co-utilization of non-catabolite repression sugar such as cellobiose and a fed-batch scheme to feed the solution of a co-substrate until the end of the culture. An engineered *S. cerevisiae* D-10-BT expressing *S. stipitis XYL1*, and β-glucosidase (*gh1-1*), and cellodextrin transporter (*cdt-1*) from *Neurospora crassa* was able to metabolize xylose and cellodextrin simultaneously and produce xylitol with 40% higher productivity, relative to sequential utilization of glucose and xylose [13••]. Fed-batch culture strategies were devised to supply a concentrated glucose solution, maintaining low glucose concentrations in culture media. Constant feeding of xylose was also devised to provide xylose as a substrate for xylitol conversion. Control of glucose concentration less than 0.35 g/L and of xylose level up to 30 g/L in culture broth resulted in 105.2 g/L xylitol concentration, 0.95 g xylitol/g xylose yield and 1.7 g/L h productivity [9].

A natural xylitol-producing yeast, *C. tropicalis*, is able to use xylose as a sole carbon source for both xylitol production and cell growth, reducing the xylitol yield. To increase the xylitol yield from xylose, the *XYL2* gene coding for XDH in *C. tropicalis* was removed by the *URA*-blasting method [14]. By supplementation of glycerol as carbon source for energy metabolism and cofactor regeneration, the *C. tropicalis ΔXYL2* converted xylose to xylitol with 3.23 g/L h productivity and 0.98 g xylitol/g xylose yield [14]. Because XR expression in *C. tropicalis* is repressed at high glucose concentrations [15], a basal level of glucose should be maintained in culture broth. To escape from this repression, the *N. crassa* XR gene was codon-optimized and expressed in the *C. tropicalis* mutant deficient in *XYL2* [16]. This recombinant *C. tropicalis* metabolized both glucose and xylose, and showed 1.7 times higher xylitol production than without the *N. crassa* XR gene expression [16].

In the stoichiometry of the xylose metabolism, 1 mole of NADPH or NADH is required for production of 1 mole of xylitol in the XR reaction [12]. As most XRs are more specific for NADPH than NADH, metabolic overexpression of enzymes involved in NADPH regeneration has been chosen for sufficient supplementation of NADPH in *XYL1*-expressing *S. cerevisiae* strains. Glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6-PGDH), two main metabolic enzymes for the NADPH regeneration, are located in the oxidative pentose phosphate pathway [17]. An increment of G6PDH activity by overexpression of the *ZWF1* gene coding for G6PDH in recombinant *S. cerevisiae* enhanced both cell growth and xylitol producing performance by about 24%, compared to those for the control strain. When analyzing the intracellular concentrations of cofactors, the NADPH and $NADP^+$ levels were not changed significantly, suggesting that the elevated G6PDH activity allowed rapid regeneration of NADPH and enhanced the NADPH-dependent xylitol production [17]. In *C. tropicalis*, G6PDH and 6-PGDH were also overexpressed to obtain a high level of NADPH level [18]. Similar to the case of the *ZWF1* overexpression in xylitol-producing *S. cerevisiae* [17], a 21% enhancement in xylitol productivity was obtained, compared to the control *C. tropicalis* [14,18]. To direct the carbon flux from glycolysis to the oxidative pentose phosphate pathway, the expression level of phosphoglucose isomerase (PGI) was reduced by changing its own promoter to the *ADH1* promoter with low transcriptional activity [19]. Reduced PGI activity did not affect xylitol production, but application of both the *ZWF1* overexpression and reduced PGI activity resulted in a 1.9 times enhancement in specific xylitol productivity in a glucose-limited fed-batch cultivation [19]. A bacterial enzyme of transhydrogenase controls the redox balance inside the cells by transferring a proton from NADH to $NADP^+$, or NADPH to NAD^+. The *Azotobacter vinelandii* transhydrogenase was expressed actively in *XYL1*-expressing *S. cerevisiae*, but xylitol productivity was reduced contrary to the expectation [11]. The bacterial transhydrogenase in *S. cerevisiae* might be unfavorable to deliver a proton in NADH to $NADP^+$ [11]. Acetyl-CoA synthetase (ACS1p) and acetaldehyde dehydrogenase 6 (ALD6p) were related directly or indirectly to the regeneration of NAD(P)(H) and also overexpressed to elevate the cofactor pools [20]. *S. cerevisiae* was further engineered by introducing NADPH-dependent XR and NADH-preferring mutant XR along with coexpression of *ZWF1* for NADPH regeneration and of *ACS1* for NADH regeneration. The resulting *S. cerevisiae* strain was cultivated in an optimized fed-batch fermentation to enhance both xylitol productivity and xylitol concentration. Integration of metabolic engineering approach with optimization of a

fermentation strategy allowed production of xylitol with 4.27 g/L h productivity and 196.2 g/L concentration [21••].

Compared to xylitol production by yeast fermentation, bacterial fermentation provides potential advantages for the industrial production of xylitol, such as rapid cell growth, easy genetic manipulation, and usage of inexpensive growth medium [22]. *Escherichia coli*, which has been served as a metabolic engineering host strain to produce numerous value-added chemicals, has been metabolically engineered to produce xylitol using the following strategies. First, heterologous xylose reductases from various xylose-fermenting strains have been introduced because *E. coli* does not have a xylose reduction pathway capable of converting xylose to xylitol [23,24]. Second, catabolic repression of glucose on xylose transport has been overcome for producing xylitol from a mixture of glucose and xylose. Expression of a relaxed catabolic repression mutant of cAMP receptor protein (*crp**) [23], deletion of *araC* followed by adaptive evolution [22], and deletion of the glucose-specific PTS permease gene *ptsG* [25] allowed simultaneous utilization of glucose and xylose for enhanced xylitol production. Third, NADPH production was enhanced to increase in vivo XR activity. Deletion of *pfkA* coding for phosphofructokinase and *sthA* coding for soluble transhydrogenase increased the carbon flux toward the pentose phosphate pathway, which is the main source of NADPH, resulting in an improved xylitol yield [26]. Fourth, as xylose is the only known substrate for xylitol production, the native xylose catabolic pathway (*xylA* and *xylB*) was blocked to avoid xylose consumption [25,27]. Notably, the optimization of xylose reductase expression in *E. coli* resulted in a drastic improvement of xylitol production in *E. coli* [25]. Synonymous mutations were introduced in the translation initiation region of xylose reductase, which affected the mRNA secondary structure for expressing xylose reductase. The resulting engineered *E. coli* was able to produce 150 g/L of xylitol from hemicellulosic hydrolyzates with high productivity (1.4 g/L h) and yield (>0.95 g xylitol/g xylose).

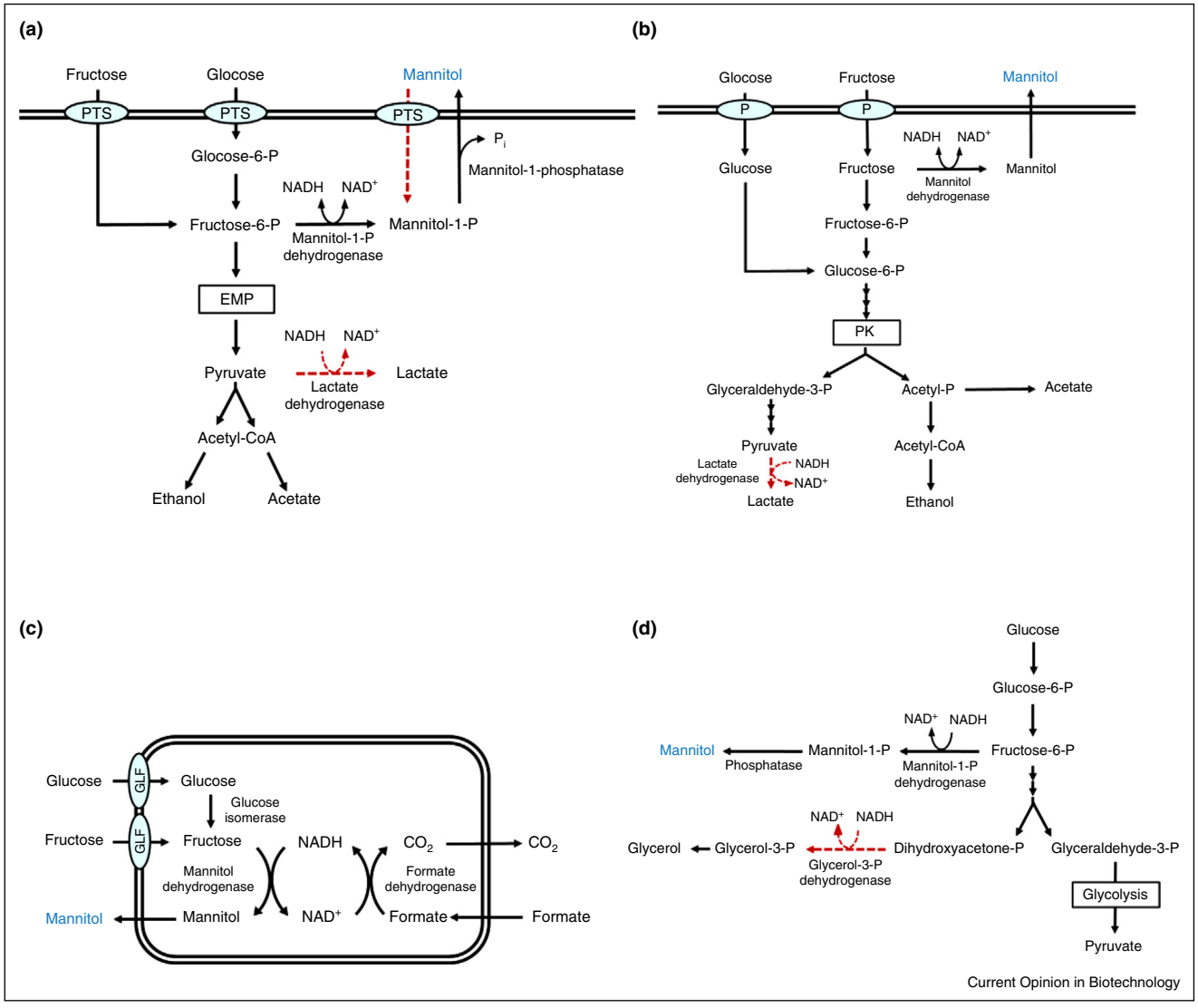
Mannitol

Mannitol is a six-carbon sugar alcohol naturally found in fruits and vegetables. It is widely used in food and pharmaceutical products because of its low caloric and cariogenic properties. Mannitol is also useful in medicine and the chemical industry [4]. Many microorganisms including bacteria and yeasts are known to produce mannitol by the fermentation of glucose or fructose [4,28]. Metabolic engineering approaches have been undertaken to improve mannitol production from natural mannitol-producing strains, and to introduce heterologous genes into other hosts which cannot produce mannitol naturally.

Lactic acid bacteria (LAB) have been extensively studied for the biotechnological production of mannitol [28]. As shown in Figure 2a and b, homofermentative and heterofermentative LAB have different hexose metabolic pathways. Although mannitol production by homofermentative LAB is lower as compared to heterofermentative LAB, homofermentative LAB have widely been used in the dairy industry. Therefore, engineering of homofermentative LAB to produce mannitol might offer the production of extra-value dairy products [4]. Metabolic engineering strategies to overproduce mannitol in LAB focused on reducing by-products formation, such as lactic acid and acetic acid, and facilitating efficient regeneration of NADH which is necessary for conversion of fructose-6-phosphate (F6P) into mannitol. Indeed, a lactate dehydrogenase (LDH)-deficient *Lactococcus lactis* exhibited mannitol-accumulating phenotypes, indicating that surplus NADH in the LAB can enhance the mannitol-1-phosphate dehydrogenase reaction [29]. Additional overexpression of two genes (*Lactobacillus plantarum mtlD* gene and *Eimeria tenella* M1pase gene) encoding mannitol-1-phosphate dehydrogenase and mannitol-1-phosphatase in the LDH-deficient strain achieved a much higher mannitol yield (0.27 g mannitol/g glucose) than the wild-type strain (<0.003 g mannitol/g glucose) [30]. Because the produced mannitol can be utilized as a carbon source after depletion of glucose, prevention of re-assimilation of mannitol was an effective strategy to increase mannitol production. Gaspar *et al.* deleted the mannitol transport system (*mtlF*) in the LDH-deficient strain to minimize the re-assimilation of extracellular mannitol [31•]. The resulting double mutant of *L. lactis* (*Δldh ΔmtlF*) produced mannitol with a high yield (0.33 g mannitol/g glucose). Additional deletion of the lactate dehydrogenase gene (*ldhB*) to generate surplus NADH further improved the mannitol yield up to 0.42 (g mannitol/g glucose) [32•].

Heterofermentative LAB were also engineered to produce mannitol. Unlike homofermentative LAB using mannitol-1-phosphate dehydrogenase and mannitol-1-phosphatase, heterofermentative LAB produce mannitol from fructose by a single enzymatic reaction using mannitol dehydrogenase (MDH). The strategy to delete lactic acid dehydrogenase genes (*ldhD* and *ldhL*) to limit by-products including lactic acid and supply more NADH was also effective in a heterofermentative LAB *L. fermentum*. While the yield of mannitol from fructose increased through this strategy, volumetric productivity of mannitol decreased [33]. In heterofermentative LAB, fructose is not only a precursor of mannitol production, but also a gateway substrate of the phophoketolase pathway. Therefore, reducing leakage of fructose into the phosphoketolase pathway can increase the production of fructose into mannitol. Helanto and coworkers obtained a mutant of *Leuconostoc pseudomesenteroides* showing only 10% of the fructokinase activity of the parental strain by random mutagenesis. The fructokinase mutant produced mannitol with a higher yield and volumetric productivity

Figure 2



Metabolic engineering approaches to produce mannitol in engineered microorganisms. Disruption of lactate dehydrogenase (LDH) in LAB enhanced mannitol production because of surplus NADH. **(a)** Prevention of re-assimilation of mannitol by deleting mannitol transport system was an effective strategy in homofermentative LAB. **(b)** Reducing the flux of fructose to the phosphoketolase pathway increased the mannitol production in heterofermentative LAB. **(c)** Heterologous enzyme expression in *E. coli* resulted in efficient conversion of fructose to mannitol. **(d)** Deletion of *GPD1* and *GPD2* provided surplus NADH for mannitol production in *S. cerevisiae*. EMP: Embden–Meyerhof–Parnas pathway, PTS: phosphotransferase system, PK: phosphoketolase pathway, P: permease, GLF: glucose facilitator. Red dashed arrows indicate inactivation of the pathways.

than the parental strain [34]. Co-utilization of fructose and glucose can improve mannitol production by increasing NADH availability [35]. As *L. reuteri* simultaneously uses the phophoketolase pathway (PKP) and the Embden–Meyerhof–Parnas (EMP) pathway, a truncated gene encoding modified 6-phospho-1-fructokinase (PFK) from *Aspergillus niger* along with its activator *pkaC* were introduced into *L. reuteri* to increase the PFK activity. The resulting strain showed substantially higher mannitol productivity (0.58 g/L h) than the parental strain (0.10 g/L h) in a mixture of glucose and fructose due to the enhanced flux through the EMP pathway [36].

In addition to LAB, *E. coli* was engineered to produce mannitol from fructose. Because *E. coli* cannot produce mannitol naturally, it is necessary to introduce a fructose transporter, MDH, NADH supplying reactions for producing mannitol from fructose. Kaup *et al.* expressed NAD^+-dependent MDH from *L. pseudomesenteroides* to reduce fructose to mannitol, NAD^+-dependent formate dehydrogenase from *Mycobacterium vaccae* to supply NADH, and a glucose facilitator from *Zymomonas mobilis* to take up fructose in *E. coli* (Figure 2c). This engineered *E. coli* produced 66 g/L of mannitol from 90 g/L of fructose and 500 mM of sodium formate within 8 hours with

high specific mannitol productivity (>4 g/g dry cell weight h) [37]. Furthermore, additional co-expression of glucose isomerase (*xylA*) to convert glucose to fructose enabled the engineered *E. coli* to produce mannitol from glucose [38].

Several studies have reported mannitol production in yeast. *C. magnoliae* HH-01, isolated from fermentation sludge, produced 209 g/L of mannitol from a mixture of glucose and fructose with a yield of 0.83 (g mannitol/g fructose) in fed-batch fermentation [39]. In another approach, UV mutagenesis of a mannitol-producing *C. magnoliae* resulted in a high titer (240 g/L) of mannitol without any by-products [40]. *S. cerevisiae* was also engineered to produce mannitol. Because *S. cerevisiae* does not produce mannitol naturally, the heterologous metabolic pathway to produce mannitol from glucose has been introduced as follows. First, the *E. coli* gene *mtlD* coding for mannitol-1-phosphate dehydrogenase was expressed to convert fructose-6-phosphate to mannitol-1-phosphate. Second, *GPD1* and *GPD2* coding for NADH-dependent glycerol-3-phosphate dehydrogenase were disrupted to provide surplus NADH for reducing fructose-6-phosphate (Figure 2d). The resulting *S. cerevisiae* was able to produce mannitol from glucose anaerobically with a yield of 0.12 (g mannitol/g glucose) [41]. Although *S. cerevisiae* and LAB have different sugar metabolisms, common engineering approaches to both improving the redox balance and substrate availability were similarly effective for enhancing mannitol production.

Sorbitol

Sorbitol is a six-carbon sugar alcohol, and it is widely used in food products and the chemical industry due to its relatively low sweetness and high solubility. In addition, it can be used as a building block for many value-added derivatives. On the basis of the broad range of its applications, estimated annual production of sorbitol is over 500,000 tons, and it is about 50% of the polyol market [42]. To produce sorbitol biotechnologically, previous studies extensively employed *Z. mobilis*, which can convert both fructose and glucose into sorbitol via glucose–fructose oxidoreductase [42,43].

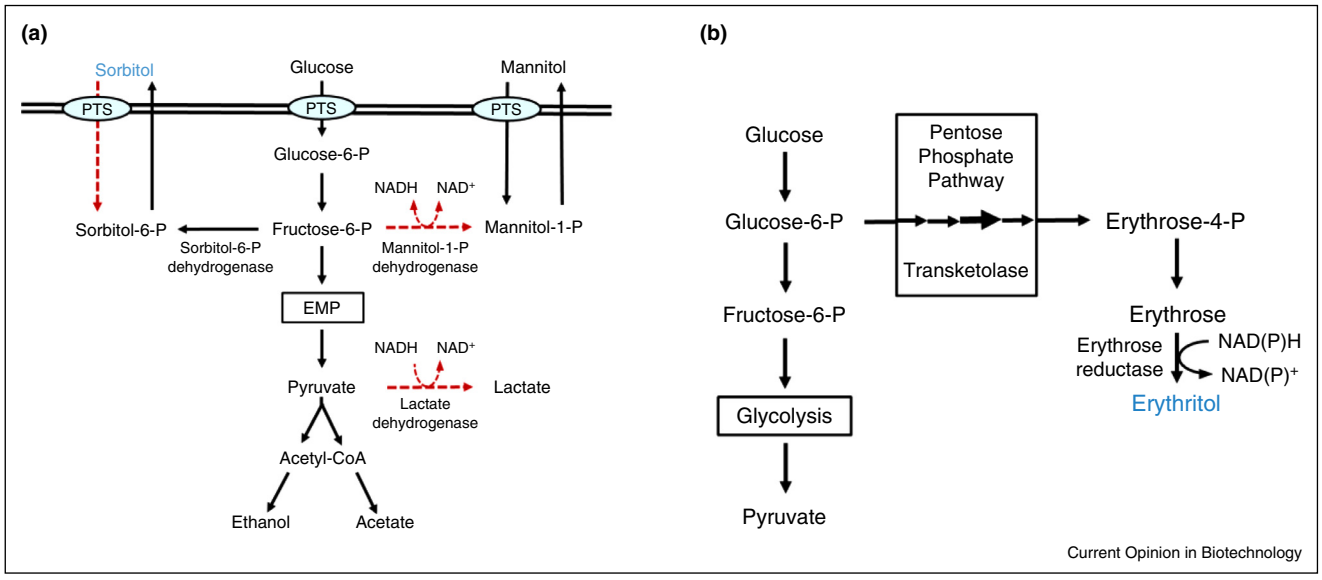
As LAB have the abilities to produce food-grade and value-added metabolites in an industrial scale, *L. plantarum* and *L. casei* have been engineered to produce sorbitol. Engineering strategies were similar to those for mannitol production in LAB. Overexpression of the key enzymes for converting a substrate to sorbitol, blocking re-utilization of the produced sorbitol, reducing other by-products, and improving redox balance have been attempted. As a host strain, LDH-deficient mutants were mainly used to minimize carbon loss for the production of lactic acid. As shown in Figure 3a, overexpression of sorbitol-6-phosphate dehydrogenase in an LDH-deficient *L. plantarum* led to the production of sorbitol from glucose (0.66 g sorbitol/g glucose) although mannitol was co-produced with sorbitol [44]. De Boeck *et al.* engineered *L. casei* using the combination of rational approaches to eliminate the mannitol production. The *gutF* gene encoding for the sorbitol-6-phosphate dehydrogenase was expressed in LDH-deficient *L. casei*, which is similar to the engineering approaches in *L. plantarum*. After depletion of glucose, however, the produced sorbitol was re-utilized. The problem with the re-utilization of sorbitol was solved by disrupting the *gutB* gene related to the sorbitol phosphotransferase system. In addition, inactivation of the *mtlD* gene coding for mannitol-1-phosphate dehydrogenase was performed to avoid mannitol production. The engineered *L. casei* was able to produce sorbitol from glucose with no mannitol production. In fed-batch fermentation, the engineered *L. casei* was able to produce sorbitol from lactose with a yield of 0.05 (g sorbitol/g lactose) [45•].

Erythritol

Erythritol is a four-carbon sugar alcohol. Due to its less calorigenic and non-insulin stimulant properties, erythritol has been used in food and pharmaceutical industries. Erythritol is widely distributed in nature such as in seaweeds and fruits [46]. Osmophilic yeasts including *Torula sp.*, *C. magnoliae*, and *Yarrowia lipolytica* and LAB such as *L. oenos* can produce erythritol naturally [47–51]. Among erythritol-producing strains, *Trichosporonoides megachiliensis* and *Pseudozyma tsukubaensis* have been used to produce erythritol commercially because of their high yield and productivity [52,53].

Biotechnological production of erythritol has relied on isolation of microorganisms capable of producing erythritol at a high yield with reduced glycerol formation [5]. To further improve erythritol production by the isolated producers, two strategies have been implemented. First, optimization of culture conditions for microbial fermentation improved erythritol production. Although erythritol-producing strains were able to convert glucose or fructose to erythritol with a decent rate, even greater volumetric productivities and yields were achieved by controlling initial glucose concentration, using a fed-batch fermentation with optimized media, and by supplementation of minerals [5]. Second, random mutagenesis by UV and chemical mutagen was performed to enhance erythritol production. The selected mutants produced more erythritol and less by-products than the parental strains because of improved activities and expression levels of key enzymes involved in the pentose phosphate pathway (PPP) [52,54,55]. As shown in Figure 3b, the substrate for erythritol production, erythrose-4-phosphate is synthesized via the PPP, and erythrose reductase (ER) catalyzes the final reaction for converting it to erythritol [5]. As such, ER is hypothesized as a rate-limiting enzyme in erythritol production. Indeed, *C. magnoliae* and *Y. lipolytica* mutants exhibiting

Figure 3



Metabolic engineering approaches to produce sorbitol **(a)** and erythritol **(b)** in engineered microorganisms. (a) Genetic perturbations to prevent re-assimilation of the produced sorbitol, to eliminate of mannitol production, and to increase reducing power were combined to improve sorbitol production in LAB. (b) Enhanced carbon flux into the pentose phosphate pathway including the reaction of erythrose reductase and transketolase improved erythritol production in yeast. EMP: Embden–Meyerhof–Parnas pathway, PTS: phosphotransferase system. Red dashed arrows indicate inactivation of the pathways.

enhanced erythritol production showed higher ER activities [54,55]. In addition, Sawada *et al.* reported that mutant *T. megachiliensis*, which has been employed for commercial erythritol production, showed higher enzyme activities of ER and transketolase (TKL) [52]. These results suggested that the increased carbon flux into the pentose phosphate pathway including the reaction of ER and TKL might be crucial for efficient erythritol production. Recently, the Cas9/CRISPR system has enabled editing of genomes of microorganisms rapidly and efficiently [56]. Furthermore, the Cas9/CRISPR system does not leave any antibiotic markers in the host strains, which is beneficial for industrial application [57]. Using the Cas9/CRISPR system, overexpression of key enzymes such as ER and NADPH-regenerating enzymes in erythritol-producing strains might be conducted to improve erythritol production.

Conclusions

Numerous studies to improve biotechnological production of sugar alcohols have been reported. Specifically, recent metabolic engineering approaches have focused on utilizing inexpensive and abundant substrates, overcoming catabolic repression, and maintaining redox balance in the microbial platforms. However, not all the fermentative processes are applicable for industrial-scale manufacturing yet because of several issues, such as high production and purification costs, and low productivity. To make the biotechnological processes for sugar alcohol more competitive and economic, diversification of value-added chemicals derived from sugar alcohols, improvement of applicability of inexpensive substrates, such as cellulosic hydrolyzates, and development of efficient bioconversion processes to produce higher-value derivatives of sugar alcohols will be necessary. Metabolic engineering of non-conventional host strains through emerging genome editing tools based on the Cas9/CRISPR system will enable economic and sustainable production of sugar alcohols from renewable biomass.

Conflict of interest statement

Nothing declared.

Acknowledgements

This research was supported by The Advanced Biomass R&D Center (ABC) of Korea Grant (2011-0031359) and The National Research Foundation of Korea Grant (2013R1A2A2A01004806) funded by the Ministry of Science, ICT & Future Planning and Technology. The authors would like to express our great appreciation to Timothy L. Turner for his proofreading and suggestions on the writings.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Schiweck H, Bär A, Vogel R, Schwarz E, Kunz M: *Sugar Alcohols. Ullmann's Encyclopedia of Industrial Chemistry*. Wiley–VCH; 2000.

2. Bieleski R: **Sugar alcohols**. *Plant Carbohydrates I*. Springer; 1982:: 158-192.

3. Granström TB, Izumori K, Leisola M: **A rare sugar xylitol. Part I: the biochemistry and biosynthesis of xylitol**. *Appl Microbiol Biotechnol* 2007, **74**:277-281.

4. Song SH, Vieille C: **Recent advances in the biological production of mannitol**. *Appl Microbiol Biotechnol* 2009, **84**:55-62.

5. Moon HJ, Jeya M, Kim IW, Lee JK: **Biotechnological production of erythritol and its applications**. *Appl Microbiol Biotechnol* 2010, **86**:1017-1025.

6. Werpy T, Petersen G: *Top Value Added Chemicals from Biomass: Volume 1—Results of Screening for Potential Candidates from Sugars and Synthesis Gas*. U.S. Department of Energy; 2004.

7. da Silva SS, Chandel AK: *D-Xylitol: Fermentative Production, Application and Commercialization*. Springer; 2012.

8. Lee WJ, Kim MD, Ryu YW, Bisson L, Seo JH: **Kinetic studies on glucose and xylose transport in *Saccharomyces cerevisiae***. *Appl Microbiol Biotechnol* 2002, **60**:186-191.

9. Lee WJ, Ryu YW, Seo JH: **Characterization of two-substrate fermentation processes for xylitol production using recombinant *Saccharomyces cerevisiae* containing xylose reductase gene**. *Process Biochem* 2000, **35**:1199-1203.

10. Li Z, Qu H, Li C, Zhou X: **Direct and efficient xylitol production from xylan by *Saccharomyces cerevisiae* through transcriptional level and fermentation processing optimizations**. *Bioresour Technol* 2013, **149**:413-419.

11. Jeun YS, Kim MD, Park YC, Lee TH, Yoo MS, Ryu YW, Seo JH: **Expression of *Azotobacter vinelandii* soluble transhydrogenase perturbs xylose reductase-mediated conversion of xylose to xylitol by recombinant *Saccharomyces cerevisiae***. *J Mol Catal B Enzym* 2003, **26**:251-256.

12. Rizzi M, Erlemann P, Bui-Thanh NA, Dellweg H: **Xylose fermentation by yeasts**. *Appl Microbiol Biotechnol* 1988, **29**:148-154.

13. •• Oh EJ, Ha S-J, Rin Kim S, Lee W-H, Galazka JM, Cate JHD, Jin Y-S: **Enhanced xylitol production through simultaneous co-utilization of cellobiose and xylose by engineered *Saccharomyces cerevisiae***. *Metab Eng* 2013, **15**:226-234.
Introduction of cellodextrin tranporter in a xylitol producing *S. cerevisiae* enabled efficient and rapid production of xylitol from a mixture of cellobiose and xylose, suggesting the feasiblity of using inexpesnsive and abundant cellulosic hydrolysate for xylitol production.

14. Ko BS, Kim J, Kim JH: **Production of xylitol from D-xylose by a xylitol dehydrogenase gene-disrupted mutant of *Candida tropicalis***. *Appl Environ Microbiol* 2006, **72**:4207-4213.

15. Ikeuchi T, Kiritani R, Azuma M, Ooshima H: **Effect of D-glucose on induction of xylose reductase and xylitol dehydrogenase in *Candida tropicalis* in the presence of NaCl**. *J Basic Microbiol* 2000, **40**:167-175.

16. Jeon W, Yoon B, Ko B, Shim W, Kim J: **Xylitol production is increased by expression of codon-optimized *Neurospora crassa* xylose reductase gene in *Candida tropicalis***. *Bioprocess Biosys Eng* 2012, **35**:191-198.

17. Kwon DH, Kim MD, Lee TH, Oh YJ, Ryu YW, Seo JH: **Elevation of glucose 6-phosphate dehydrogenase activity increases xylitol production in recombinant *Saccharomyces cerevisiae***. *J Mol Catal B Enzym* 2006, **43**:86-89.

18. Ahmad I, Shim W, Jeon W, Yoon B, Kim JH: **Enhancement of xylitol production in *Candida tropicalis* by co-expression of two genes involved in pentose phosphate pathway**. *Bioprocess Biosys Eng* 2012, **35**:199-204.

19. Oh YJ, Lee TH, Lee SH, Oh EJ, Ryu YW, Kim MD, Seo JH: **Dual modulation of glucose 6-phosphate metabolism to increase NADPH-dependent xylitol production in recombinant *Saccharomyces cerevisiae***. *J Mol Catal B Enzym* 2007, **47**:37-42.

20. Oh EJ, Bae YH, Kim KH, Park YC, Seo JH: **Effects of overexpression of acetaldehyde dehydrogenase 6 and acetyl-CoA synthetase 1 on xylitol production in recombinant *Saccharomyces cerevisiae***. *Biocatal Agric Biotechnol* 2012, **1**:15-19.

21. •• Jo JH, Oh SY, Lee HS, Park YC, Seo JH: **Dual utilization of NADPH and NADH cofactors enhances xylitol production in engineered *Saccharomyces cerevisiae***. *Biotechnol J* 2015 http://dx.doi.org/10.1002/biot.201500068. [Epub ahead of print].
Combination of metabolic engineering approches with optimization of a fermentation strategy resulted in an impressive yield and productivity of xylitol production by engineered *S. cerevisiae*.

22. Kim SM, Choi BY, Ryu YS, Jung SH, Park JM, Kim G-H, Lee SK: **Simultaneous utilization of glucose and xylose via novel mechanisms in engineered *Escherichia coli***. *Metab Eng* 2015, **30**:141-148.

23. Cirino PC, Chin JW, Ingram LO: **Engineering *Escherichia coli* for xylitol production from glucose–xylose mixtures**. *Biotechnol Bioeng* 2006, **95**:1167-1176.

24. Woodyer R, Simurdiak M, van der Donk WA, Zhao H: **Heterologous expression, purification, and characterization of a highly active xylose reductase from *Neurospora crassa***. *Appl Environ Microbiol* 2005, **71**:1642-1647.

25. Su B, Wu M, Zhang Z, Lin J, Yang L: **Efficient production of xylitol from hemicellulosic hydrolysate using engineered *Escherichia coli***. *Metab Eng* 2015, **31**:112-122.

26. Chin JW, Cirino PC: **Improved NADPH supply for xylitol production by engineered *Escherichia coli* with glycolytic mutations**. *Biotechnol Prog* 2011, **27**:333-341.

27. Nair NU, Zhao H: **Selective reduction of xylose to xylitol from a mixture of hemicellulosic sugars**. *Metab Eng* 2010, **12**:462-468.

28. Saha BC, Racine FM: **Biotechnological production of mannitol and its applications**. *Appl Microbiol Biotechnol* 2011, **89**:879-891.

29. Neves AR, Ramos A, Shearman C, Gasson MJ, Almeida JS, Santos H: **Metabolic characterization of *Lactococcus lactis* deficient in lactate dehydrogenase using in vivo ^{13}C-NMR**. *Eur J Biochem* 2000, **267**:3859-3868.

30. Wisselink HW, Moers AP, Mars AE, Hoefnagel MH, De Vos WM, Hugenholtz J: **Overproduction of heterologous mannitol 1-phosphatase: a key factor for engineering mannitol production by *Lactococcus lactis***. *Appl Environ Microbiol* 2005, **71**:1507-1514.

31. • Gaspar P, Neves AR, Ramos A, Gasson MJ, Shearman CA, Santos H: **Engineering *Lactococcus lactis* for production of mannitol: high yields from food-grade strains deficient in lactate dehydrogenase and the mannitol transport system**. *Appl Environ Microbiol* 2004, **70**:1466-1474.
Mannitol production by food-grade strains may offer direct production of mannitol in food during the food fermentation.

32. • Gaspar P, Neves AR, Gasson MJ, Shearman CA, Santos H: **High yields of 2,3-butanediol and mannitol in *Lactococcus lactis* through engineering of NAD+ cofactor recycling**. *Appl Environ Microbiol* 2011, **77**:6826-6835.
To increase reducing power and decrease byproduct formation in LAB, the authors deleted genes (*ldhB* and *ldhX*) coding for lactate dehydrogenase. When combined with the overexpression of *mtlD* coding for mannitol-1-phosphate dehydrogeanse, the resulting strain produced mannitol from glucose.

33. Aarnikunnas J, von Weymarn N, Rönnholm K, Leisola M, Palva A: **Metabolic engineering of *Lactobacillus fermentum* for production of mannitol and pure L-lactic acid or pyruvate**. *Biotechnol Bioeng* 2003, **82**:653-663.

34. Helanto M, Aarnikunnas J, von Weymarn N, Airaksinen U, Palva A, Leisola M: **Improved mannitol production by a random mutant of *Leuconostoc pseudomesenteroides***. *J Biotechnol* 2005, **116**:283-294.

35. Wisselink H, Weusthuis R, Eggink G, Hugenholtz J, Grobben G: **Mannitol production by lactic acid bacteria: a review**. *Int Dairy J* 2002, **12**:151-161.

36. Papagianni M, Legiša M: **Increased mannitol production in *Lactobacillus reuteri* ATCC 55730 production strain with a modified 6-phosphofructo-1-kinase**. *J Biotechnol* 2014, **181**:20-26.

37. Kaup B, Bringer-Meyer S, Sahm H: **Metabolic engineering of *Escherichia coli*: construction of an efficient biocatalyst for D-mannitol formation in a whole-cell biotransformation**. *Appl Microbiol Biotechnol* 2004, **64**:333-339.

38. Kaup B, Bringer-Meyer S, Sahm H: **D-Mannitol formation from D-glucose in a whole-cell biotransformation with recombinant *Escherichia coli***. *Appl Microbiol Biotechnol* 2005, **69**:397-403.

39. Song KH, Lee JK, Song JY, Hong SG, Baek H, Kim SY, Hyun HH: **Production of mannitol by a novel strain of *Candida magnoliae***. *Biotechnol lett* 2002, **24**:9-12.

40. Savergave LS, Gadre RV, Vaidya BK, Jogdand VV: **Two-stage fermentation process for enhanced mannitol production using *Candida magnoliae* mutant R9**. *Bioprocess Biosyst Eng* 2013, **36**:193-203.

41. Costenoble R, Adler L, Niklasson C, Lidén G: **Engineering of the metabolism of *Saccharomyces cerevisiae* for anaerobic production of mannitol**. *FEMS Yeast Res* 2003, **3**:17-25.

42. Silveira M, Jonas R: **The biotechnological production of sorbitol**. *Appl Microbiol Biotechnol* 2002, **59**:400-408.

43. Barrow KD, Collins JG, Leight DA, Rogers PL, Warr RG: **Sorbitol production by *Zymomonas mobilis***. *Appl Microbiol Biotechnol* 1984, **20**:225-232.

44. Ladero V, Ramos A, Wiersma A, Goffin P, Schanck A, Kleerebezem M, Hugenholtz J, Smid EJ, Hols P: **High-level production of the low-calorie sugar sorbitol by *Lactobacillus plantarum* through metabolic engineering**. *Appl Environ Microbiol* 2007, **73**:1864-1872.

45. • De Boeck R, Sarmiento-Rubiano LA, Nadal I, Monedero V, Pérez-Martínez G, Yebra MJ: **Sorbitol production from lactose by engineered *Lactobacillus casei* deficient in sorbitol transport system and mannitol-1-phosphate dehydrogenase**. *Appl Microbiol Biotechnol* 2010, **85**:1915-1922.
Multiple metabolic engineering strategies to prevent re-assimilation of the produced sorbitol, to eliminate mannitol production, and to increase reducing power were combined to produce sorbitol from glucose without mannitol production.

46. Röper H, Goossens J: **Erythritol, a new raw material for food and non-food applications**. *Starke* 1993, **45**:400-405.

47. Oh D, Cho C, Lee J, Kim S: **Increased erythritol production in fed-batch cultures of *Torula sp.* by controlling glucose concentration**. *J Ind Microbiol Biotechnol* 2001, **26**:248-252.

48. Koh ES, Lee TH, Lee DY, Kim HJ, Ryu YW, Seo J-H: **Scale-up of erythritol production by an osmophilic mutant of *Candida magnoliae***. *Biotechnol Lett* 2003, **25**:2103-2105.

49. Yang SW, Park JB, Han NS, Ryu YW, Seo J-H: **Production of erythritol from glucose by an osmophilic mutant of *Candida magnoliae***. *Biotechnol Lett* 1999, **21**:887-890.

50. Rymowicz W, Rywińska A, Marcinkiewicz M: **High-yield production of erythritol from raw glycerol in fed-batch cultures of *Yarrowia lipolytica***. *Biotechnol lett* 2009, **31**:377-380.

51. Veiga-da-Cunha M, Santos H, Van Schaftingen E: **Pathway and regulation of erythritol formation in *Leuconostoc oenos***. *J Bacteriol* 1993, **175**:3941-3948.

52. Sawada K, Taki A, Yamakawa T, Seki M: **Key role for transketolase activity in erythritol production by *Trichosporonoides megachiliensis* SN-G42**. *J Biosci Bioeng* 2009, **108**:385-390.

53. Jeya M, Lee KM, Tiwari MK, Kim JS, Gunasekaran P, Kim SY, Kim IW, Lee JK: **Isolation of a novel high erythritol-producing *Pseudozyma tsukubaensis* and scale-up of erythritol fermentation to industrial level**. *Appl Microbiol Biotechnol* 2009, **83**:225-231.

54. Ghezelbash GR, Nahvi I, Emamzadeh R: **Improvement of erythrose reductase activity, deletion of by-products and statistical media optimization for enhanced erythritol production from *Yarrowia lipolytica* Mutant 49**. *Curr Microbiol* 2014, **69**:149-157.

55. Ghezelbash G, Nahvi I, Malekpour A: **Erythritol production with minimum by-product using *Candida magnoliae* mutant**. *Appl Biochem Microbiol* 2014, **50**:292-296.

56. Sander JD, Joung JK: **CRISPR-Cas systems for editing, regulating and targeting genomes**. *Nat Biotechnol* 2014, **32**:347-355.

57. Zhang GC, Kong II, Kim H, Liu JJ, Cate JH, Jin YS: **Construction of a quadruple auxotrophic mutant of an industrial polyploid *Saccharomyces cerevisiae* strain by using RNA-guided Cas9 nuclease**. *Appl Environ Microbiol* 2014, **80**:7694-7701.