American Journal of Bioscience and Bioengineering
2017; 5(5): 109-112
http://www.sciencepublishinggroup.com/j/bio
doi: 10.11648/j.bio.20170505.13
ISSN: 2328-5885 (Print); ISSN: 2328-5893 (Online)

Review Article

Biosynthesis of Xylitol from Glucose: Microorganism, Key Enzymes and Genetically Engineered Strains

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To cite this article:

Yilin Qi, Huanhuan Zhang, Junhua Yun, Miaomiao Yang, Guoyan Zhang, Yanbo Xue, Xuexia Bai, Xianghui Qi, Yanqin Liu, Longxian Ran. Biosynthesis of Xylitol from Glucose: Microorganism, Key Enzymes and Genetically Engineered Strains. *American Journal of Bioscience and Bioengineering*. Vol. 5, No. 5, 2017, pp. 109-112. doi: 10.11648/j.bio.20170505.13

Received: October 7, 2017; **Accepted**: October 19, 2017; **Published**: November 24, 2017

Abstract: Xylitol is an important prebiotics, and it is widely used in the fields of medicine and food. In view of the drawbacks of chemical method, much focus has been attracted into the study of xylitol biosynthesis. In this study, microorganism, method and key enzymes in the biosynthesis of xylitol were introduced. In addition, the production of xylitol from glucose by genetically engineered strains was summarized respectively.

Keywords: Xylitol, Biosynthesis, Glucose, Genetically Engineered Strain, Enzymes

1. Introduction

Xylitol, a kind of five carbon sugar, can be used as a substitute of sweeter, and it is a natural prebiotics with high nutritional value and low-energy. As the metabolism intermediate of carbohydrate, xylitol do not consume insulin after consumption. Considering the special anti-caries function, xylitol can be applied as diabetes, nutritional supplements, therapeutic agents and children caries food. In addition, since xylitol also has many excellent properties similar to glycerol and other polyols, it was widely used in the fields of pharmaceutics, chemistry, leather, coatings and food industries [1, 2].

At present, xylitol was mainly produced by chemical method of acidification and hydrogenation and semi-biological method of microbial fermentation of hemicellulose hydrolysate. In the semi-biological method, several natural xylose-fermenting yeasts can convert D-xylose to xylitol through reduction by a Nicotinamide Adenine Dinucleotide (Phosphate) (reduced) (NAD (P) H)-dependent xylose reductase (XR) [3]. Both of the methods rely on the hydrolysis and purification of D-xylose from hemicellulose-xylan hydrolysates. There are many serious drawbacks in this process, such as high consumption of acid-base, heavy environmental pollution and complicated technology. These drawbacks have limited the large scale production of xylitol greatly [4-6]. Therefore, the whole-biological method for producing xylitol has attracted worldwide attention. In this study, the microbes, method and key enzymes in the biosynthesis of xylitol are introduced and the production of xylitol from glucose by genetically engineered strains was also summarized.

2. Microorganisms with the Ability of Producing Xylitol

Many microbes including yeast, mold and actinomycetes, can use xylose and glucose as co-substrate to produce xylitol

(Figure 1). And a few bacteria can also generate xylitol from this co-substrate, such as *Enterobacter liquaefaciens* and *Myobactenum smegmatis*. When using mold and actinomycetes, only a small amount of xylitol can be produced. Yeast has excellent properties in the production of xylitol using xylose/glucose co-substrate. Several natural xylose-fermenting yeasts, such as *Candida* sp., *Pichia stipites*, *Aspergillus carbonarius, Debaryomyces nepalensis, Schefferomyce stipitis* and *Pachysolen tannophilus*, can use this co-substrate to generate xylitol [3, 7, 8]. Usually, *Candida* genus has strong ability of producing xylitol, such as *Candida tropicalis* and C*andida guilliermondii* being able to convert 90% xylose to xylitol in 24 h. However, these natural yeasts require a well-controlled supply of oxygen and are sensitive to ethanol levels, which limit their use [3]. Other species including *P. stipitis* and *Saccharamyces cerevisiae,* which are widely used as host strains, also have strong ability of production xylitol. So far, no bacteria in nature can bio-converse sole glucose to xylitol directly. Osmophilic yeasts were usually used first for producing D-arabitol from glucose, and then D-arabitol was metabolized to xylitol by *Gluconobacter* sp. [1, 9].

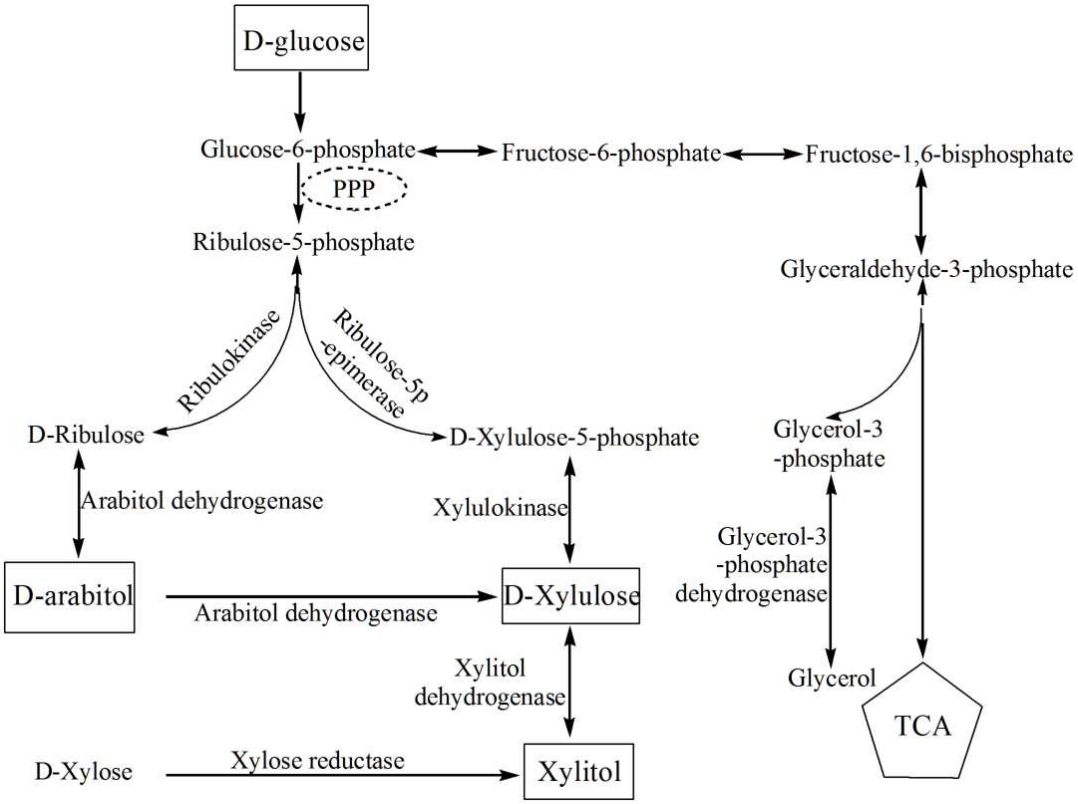

Figure 1. *The possible biosynthetic pathway for conversion of glucose to D-arabitol in yeast.*

3. Whole-Biological Method

As early as the last century, Onishi and Suzuki had already disclosed the preparation of xylitol through multi-step process using multi-bacteria [10]. The process included three steps as shown in Figure 2: First, D-arabitol was produced efficiently from glucose by fermentation with osmophilic yeasts; Second, the D-arabitol was oxidized to D-xylulose with *Acetobacter suboxydans*; and Third, the D-xylulose was reduced to xylitol using yeast of *Candida guilliermondii* [11]. However, only about 9.0 g/L xylitol with a yield of 11% was produced from 77.5 g/L glucose after 211 h of fermentation using the above process [12]. Because of the long time process and low yield, this method has not been applied in practice. In 2002, Suzuki, et al. isolated a strain of *Gluconobacter oxydan*, which can convert D-arabitol to xylitol by one step [13]. Therefore, the multi-step biosynthesis process of xylitol could be simplified into two steps. In the first step, the bio-conversion of D-arabitol from glucose was also executed by osmophilic yeasts. And in the second step, *G. oxydans* was used merely for converting D-arabitol to xylose, which involved the oxidation of D-arabitol to D-xylulose by D-arabitol dehydrogenase (ArDH), and the followed reduction of D-xylulose to xylitol by xylitol dehydrogenase (XDH) [9] (Figure 3).

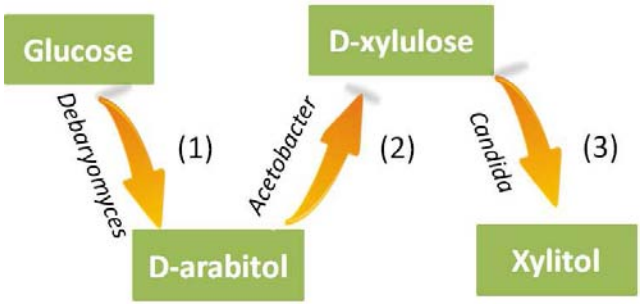

Figure 2. *Schematic plot of xylitol production by multi-bacteria.*

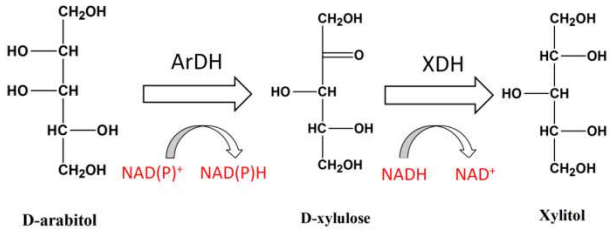

Figure 3. *The biotransformation of D-arabitol to xylitol in G. oxydans.*

However, the yield of xylitol by whole-biological method was relatively lower to be industrialized compared with chemical method. With the development of gene engineering technology in microbial biotransformation, it will be facilitated that xylitol is produced from glucose by genetically engineered strain efficiently.

4. Key Enzymes in the Pathway of Xylitol Biosynthesis

4.1. Xylitol Dehydrogenase

Xylitol dehydrogenase (XDH, EC1.1.1.9) belongs to a family of short-chain dehydrogenase with a molecular weight of about 28 kDa. It is one of the key enzymes in the xylose metabolism of yeast and D-xylulose metabolism of *Gluconobacter* sp. In this process, xylose is first converted to xylitol by XR and then xylitol is catalyzed to D-xylulose by XDH. XDH is a homodimer in 3 D structure, and its active site is highly conserved. XDH is a two-way enzyme, and requires cofactors NADH or NAD^+ in the forward and reverse reactions. Since the redox reaction is carried out in an unbalanced manner between XR and XDH due to the different coenzyme, xylitol is accumulated in this process. The biosynthesis of xylitol can be enhanced by providing sufficient Nicotinamide Adenine Dinucleotide (reduced) (NADH). Compared with the addition of expensive NADH, the method of coenzyme regeneration according to the mechanism of NADH/ Nicotinamide Adenine Dinucleotide (NAD^+) is more economical. It was reported that xylitol production was improved by increasing the coenzyme regeneration efficiency of the pentose phosphate pathway via overexpression of Glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in *G. oxydans* [14].

4.2. D-Arabitol Dehydrogenase

D-arabitol dehydrogenase (ArDH, EC1.1.1.11) is an important dehydrogenase in the process of pentose metabolism. It can be divided into two types according to its product in the dehydrogenation: one is ribulose, and the other is D-xylulose. ArDH is a one-way enzyme, and usually it is located in the cell membrane or cytoplasm. Most of ArDHs use Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$) as a cofactor, and only a few of them are both NADP- or NAD-dependent. Until now, at least two NADP-dependent ArDH genes had been cloned from *G. oxydans* ATCC 621 [13] and *G. oxydans* CGMCC 1.110 [15]. It is noteworthy that two novel NAD-dependent ArDHs from *A. suboxydans* and *Gluconobacter* sp. were cloned, expressed and characterized [9, 16]. In view of the cofactors NADH/NAD^+ imbalance during the conversion of D-arabitol to xylitol, this kind of NAD-dependent ArDH may be preferable to the biosynthesis of xylitol.

5. Production of Xylitol by Genetically Engineered Stains

Xylitol can be produced from glucose using yeast plus *Gluconobacter* sp. Since glucose can be derived from the starch of resource-rich cassava, corn and other crops, the production of xylitol using glucose as substrate has attracted the attention of many researchers and motivated the sustainable development of xylitol industry [17, 18]. During this process, D-arabitol is generated through conversion of glucose by osmophilic yeast first. Then, D-arabitol is converted finally to xylitol by ArDH and XDH. It has showed that glucose can be converted to D-arabitol by strains of osmotolerant yeast efficiently [13], and that the maximum D-arabitol production of 93.48±2.79 g/L and volumetric productivity of 1.380 g/L h could be achieved from 200 g glucose by *Zygosaccharomyces rouxii* JM-C46 and *Kodamaea ohmeri* [19, 20]. Besides, the conversion of D-arabitol to xylitol by ArDH and XDH is the rate-limiting step [14, 21, 22], therefore, much focus has been attracted into the study in this area. So far, the yield of D-arabitol to xylitol had been achieved more than 83% by genetic engineering strains of BL21-*xhd* and *Gluconobacter oxydans* [1], and 87% by genetic engineering strains of BL21-*xhd* and BL21-*xdh* through the co-biotransformation of whole cells. The xylitol production was increased by more than two times as compared with that of *Gluconobacter* sp. alone, and was improved 10.1% than that of *Gluconobacter* sp. mixed with BL21-*xdh* [9].

Furthermore, construction of coenzyme regeneration system, such as G6PDH/6PGDH, glucose dehydrogenase/alcohol dehydrogenase, can reduce the amount of exogenous NADH, and is economically feasible for xylitol production in industrial bioconversion. In a previously report, G6PDH/6PGDH was used for the system of coenzyme regeneration in *G. oxydans*. Approximately 29.3 g/L xylitol was obtained, with a yield of 73.2%, from 40 g/L D-arabitol in the batch biotransformation, and the xylitol productivity (0.62 g/L/h) was 3.26-fold of the wild type strain (0.19 g/L/h) in repetitive batch biotransformation [14, 21, 22]. Hence, NADH supply can be enhanced by regeneration system in the conversion and is beneficial to the practical synthesis of xylitol.

6. Conclusion

Xylitol production based on D-arabitol which can be biosynthesized by osmophilic yeast from the cheap substrate of glucose is an economical, effective and less pollution method. There are several studies on genetically engineered strains to produce xylitol, and various progresses had been achieved recently. Although the coenzyme regeneration can reduce the cost of xylitol production, this approach has still a

certain distance from industrial production because of the low efficiency of regeneration. Construction of high-efficiency genetically engineered strains which can convert glucose to xylitol in one-step may be a potential method for high production of xylitol. Therefore, in order to compete with the chemical method, more strategies including novel coenzyme regeneration system, enzymes and strains modification should be investigated to achieve industrial large-scale production.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 31571806) and High-level talents project of Six Talent Peaks in Jiangsu Province (Grant No. SWYY-018).

References

[1] Qi, X. H., Zhu, J. F., Yun, J. H., Lin, J., Qi, Y. L., Guo, Q., Xu, H., 2016. Enhanced xylitol production: Expression of xylitol dehydrogenase from *Gluconobacter oxydans* and mixed culture of resting cell. *Journal of Bioscience and Bioengineering*. 122, 257-262.

[2] Qi, X. H., Wang, X., Lin, J., Zhu, J. F., Luo, Y., Deng, W. Y., Wang, F., Sun, W. J., Meng, J. Z., 2014. Microbial and enzymatic process for xylitol production from D-glucose. *Current Organic Chemistry*, 18, 3131-3135.

[3] Zhang, C., Zong, H., Zhuge, B., Lu, X., Fang, H., Zhuge, J., 2015. Production of Xylitol from D-Xylose by Overexpression of Xylose Reductase in Osmotolerant Yeast *Candida glycerinogenes* WL2002-5. *Applied Biochemistry and Biotechnology,* 176, 1511-1527.

[4] Makinen, K. K., 2000. The rocky road of xylitol to its clinical application. *Journal of Dental Research*, 79, 1352-1355.

[5] Mayer, G., Kulbe, K. D., Nidetzky, B., 2002. Utilization of xylitol dehydrogenase in a combined microbial/enzymatic process for production of xylitol from D-glucose. *Applied Biochemistry and Biotechnology*, 98-100, 577-589.

[6] Matsushika, A., Watanabe, S., Kodaki, T., Makino, K., Sawayama, S., 2008. Bioethanol production from xylose by recombinant *Saccharomyces cerevisiae* expressing xylose reductase, NADP (+)-dependent xylitol dehydrogenase, and xylulokinase. *Journal of Bioscience and Bioengineering*, 105, 296-299.

[7] Tamburini, E., Costa, S., Marchetti, M. G., Pedrini, P., 2015. Optimized Production of Xylitol from Xylose Using a Hyper-Acidophilic *Candida tropicalis*. *Biomolecules*. 5, 1979-1989.

[8] Pratter, S. M., Eixelsberger, T., Nidetzky, B., 2015. Systematic strain construction and process development: Xylitol production by *Saccharomyces cerevisiae* expressing *Candida tenuis* xylose reductase in wild-type or mutant form. *Bioresoure Technology*, 198, 732-738.

[9] Qi, X., Zhang, H., Magocha, T. A., An, Y., Yun, J., Yang, M., Xue, Y., Liang, S., Sun, W., Cao, Z., 2017. Improved xylitol production by expressing a novel D-arabitol dehydrogenase from isolated *Gluconobacter* sp. JX-05 and co-biotransformation of whole cells. *Bioresoure Technology*, 235, 50-58.

[10] Onishi, H., Suzuki, T., 1969. Microbial production of xylitol from glucose. *Applied Microbiology*, 18, 1031-1035.

[11] Sampaio, F. C., Mantovani, H. C., Passos, F. J. V., Moraes, C. A. d., Converti, A., Passos, F. M. L., 2005. Bioconversion of d-xylose to xylitol by *Debaryomyces hansenii* UFV-170: Product formation versus growth. *Process Biochemistry,* 40, 3600-3606.

[12] Suzuki, T., Yokoyama, S., Kinoshita, Y., Yamada, H., Hatsu, M., Takamizawa, K., Kawai, K., 1999. Expression of xyrA gene encoding for D-Xylose reductase of *Candida tropicalis* and production of xylitol in *Escherichia coli*. *Journal of Bioscience and Bioengineering*, 87, 280-284.

[13] Suzuki, S., Sugiyama, M., Mihara, Y., Hashiguchi, K., Yokozeki, K. 2002. Novel enzymatic method for the production of xylitol from D-arabitol by *Gluconobacter oxydans*. *Bioscience Biotechnology and Biochemistry*, Vol. 66, pp. 2614-2620.

[14] Li, S., Zhang, J. L., Xu, H., Feng, X. H., 2016. Improved xylitol production from D-arabitol by enhancing the coenzyme regeneration efficiency of the pentose phosphate pathway in *Gluconobacter oxydans*. *Journal of Agricultural and Food Chemistry,* 64, 1144-1150.

[15] Cheng, H. R., Jiang, N., Shen, A., Feng, Y. J., 2005. Molecular cloning and functional expression of D-arabitol dehydrogenase gene from *Gluconobacter oxydans* in *Escherichia coli*. *FEMS Microbiology Letters*, 252, 35-42.

[16] Cheng, H. R., Li, Z. L., Jiang, N., Deng, Z. X., 2009. Cloning, Purification and characterization of an NAD-dependent D-arabitol dehydrogenase from Acetic Acid Bacterium, *Acetobacter suboxydans*. *Protein Journal*, 28, 263-272.

[17] Granstrom, T. B., Izumori, K., Leisola, M., 2007. A rare sugar xylitol. Part II: biotechnological production and future applications of xylitol. *Applied Microbiology and Biotechnology*, 74, 273-276.

[18] Povelainen, M., Miasnikov, A. N., 2006. Production of D-arabitol by a metabolic engineered strain of *Bacillus subtilis*. *Biotechnology Journal*, 1, 214-219.

[19] Qi, X., Luo, Y., Wang, X., Zhu, J., Lin, J., Zhang, H., Chen, F., Sun, W., 2015. Enhanced D-arabitol production by *Zygosaccharomyces rouxii* JM-C46: isolation of strains and process of repeated-batch fermentation. *Journal of Industrial Microbiology & Biotechnology,* 42, 807-812.

[20] Zhu, H. Y., Xu, H., Dai, X. Y., Zhang, Y., Ying, H.J., Ouyang, P. K., 2010. Production of D-arabitol by a newly isolated *Kodamaea ohmeri*. *Bioprocess and Biosystems Engineering*, 33, 565-571.

[21] Zhang, J., Li, S., Xu, H., Zhou, P., Zhang, L., Ouyang, P., 2013. Purification of xylitol dehydrogenase and improved production of xylitol by increasing XDH activity and NADH supply in *Gluconobacter oxydans*. *Journal of Agricultural and Food Chemistry,* 61, 2861-2867.

[22] Zhou, P., Li, S., Xu, H., Feng, X., Ouyang, P., 2012. Construction and co-expression of plasmid encoding xylitol dehydrogenase and a cofactor regeneration enzyme for the production of xylitol from D-arabitol. *Enzyme and Microbial Technology*, 51, 119-124.