Advances in Biological Sciences Research, volume 3
International Conference on Biological Engineering and Pharmacy (BEP 2016)

Microbial Production of Xylitol from D-arabitol by *Gluconobacter Oxydans*

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***Abstract*—Xylitol, a five carbon sugar alcohol, has numerous applications in the fields of food and pharmaceuticals. *Gluconobacter oxydans* contains two enzymes which are membrane-bound D-arabitol dehydrogenase and soluble xylitol dehydrogenase enabling the production of xylitol from D-arabitol. This review provides comprehensive insights regarding properties of key enzymes and advances of xylitol production of *G. oxydans* by microbial methods.**

Keywords-Gluconobacter oxydans; xylitol; D-arabitol dehydrogenase; xylitol dehydrogenase

I. INTRODUCTION

Xylitol, a five-carbon sugar-alcohol, widely exists in fruits and vegetables and some other plants. Since its sweetness is similar to sucrose, it is used as an alternative to natural sweeteners [1.2]. Xylitol is also used as a sugar substitute by for diabetic patients. In addition, xylitol is reported to play a role in preventing dental caries. Because of its negative heat of dissolution, it offers a fresh and cooling in the oral cavity [3.4]. Therefore, the method of producing xylitol has caused extensive attention.

Though xylitol extensively exists in fruit and vegetables, it is difficult and costly to extract from natural objects directly for its content is relatively low. At present, large-scale production is typically obtained by a chemical process of D-xylose hydrogenation [5]. However, due to too many steps involved in this process, it costs a lot and had a negative impact on the environment. So the biotransformation method has aroused researcher's attention.

Traditional biological method for production of xylitol cannot transform glucose to xylitol directly. And until now, there have no any microbe can transform glucose to xylitol directly in the nature. *G.oxydans* can produce xylitol from D-arabitol based on two simple steps including two key enzymes (ArDH and XDH) in the metabolic pathway. This review introduces the research of xylitol production from D-arabitol by *G. oxydan* based on the special mechanisms, and the biotransformation process of D-arablitol to xylitol, and some achievements of microbial production of xylitol from D-arabitol by *G.oxydans.*

II. BIOSYNTHETIC PATHWAY AND MICROORGANISM INVOLVING XYLITOL PRODUCTION

A. Three-step Fermentation Process.

In 1969, Onishi and Suzuki [6] put forward a three-step process for bioconversion of glucose to xylitol firstly. (Fig.1). The first step, completed the conversion of D-glucose to D-arabitol with *Debaryomyces hansenii*; for the second step, *Acetobacter suboxydans* was took charge of biotransformation from D-arabitol to D-xylulose; for the third step, *Candida guilliermondii* answered for the biotransformation from D-xylulose to xylitol. The three procedures, last 211 h and the final yield obtained from D-glucose was 11%. Due to the low yield productivity, people found the procedure to be less effective and gave up it.

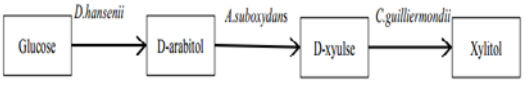

Figure 1. Three-step fermentation process from D-glucose to xylitol

B. Two-step Fermentation Process

Suzuki [7] screened a *G. oxydans* strain which can shorten the three-step process. For first procedure, *Debaryomyces hansenii* was responsible for biotransformation from D-glucose to D-arabitol, for second procedure, *G. oxydans* consumed D-xylulose to xylitol (Fig. 2). The *G. oxydans* possess two enzymes enabling the production of xylitol from D-arabitol. The two enzymes are the membrane-bound D-arabitol dehydrogenase (ArDH) and the soluble NADP-dependent xylitol dehydrogenase (XDH).

Figure 2. Two-step fermentation process

III. D-ARABITOL DEHYDROGENASE AND XYLITOL DEHYDROGENASE

The transformation from D-arabitol to xylitol is realized by *G.oxydans* because of its membrane-bound ArDH and soluble NAD-dependent XDH. This process involves oxidation of D-arabitol to D-xylulose by D-arabitol dehydrogenase (AraDH), followed by reduction of the D-xylulose to xylitol by xylitol dehydrogenase (XDH) (Fig. 3) [8].

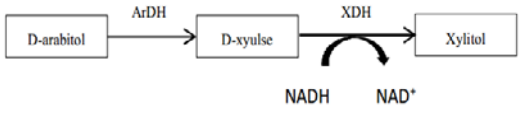

Figure 3. Schematic representation of xylitol production from D-arabitol

A. Properties of ArDH

ArDH is a kind of polyhydric alcohol dehydrogenase, plays a great role in the pentose metabolism process. ArDH is part of the short-chain dehydrogenase family and its subunit is composed of 250~300 amino acids residues, in which only 20 amino acid residues were highly conserved [9]. Hairong Cheng [9] cloned the D-arabitol dehydrogenase gene from *G. oxydans* and demonstrated the optimum pH of polyols oxidation with ArDH was around 8.5 in Tris–Cl buffer, while the optimum pH of reductive sugar reduction was 6.5. Adachi O[10] investigated the membrane-bound quinoprotein D-Arabitol dehydrogenase of *G. suboxydans* IFO 3257, in which the optimal pH of D-arabitol oxidation with ArDH was at 5.0. According to relevant researches, the optimum pH of oxidation D-arabitol into D-xylulose is pH5.0 ~ 6.0 [11].

B. Properties of XDH

XDH is an oxidordeuctase, which catalytic activity is reversible. Xylulose is reduced to xylitol using NADH as coenzyme, while xylitol is oxidized to xylulose using NAD^+ as coenzyme [12]. In this paper, we describe the characterization of XDH from *G. oxydans*. The *xdh* from *G.oxydans* was investigated by Masakazu [12]. The optimal pH of XDH oxidation for xylitol in the presence of NAD^+ was around pH 11.0, while that of reduction for D-xylulose was around pH 5.0. Shen's [13] research got the same information. It implies that the optimal pH value of XDH oxidation is usually within the range of alkalinity, while that of reduction reaction usually in the range of acidity.

IV. BIOTRANSFORMATION OF XYLITOL FROM D-ARABITOL

The enzyme activity and coenzyme regeneration efficiency were increased in the recombinant *G. oxydans* strains compared to wild type of *G.oxydans*, so as to achieve efficient conversion of D-arabitol and obtained the high yield of xylitol. Sha Li et al [14] made Glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) overexpressed in *G. oxydans*. The xylitol productivity (0.62 $g{\cdot}L^{-1}/h$) of the recombinant *G. oxydans* strain was 3.26-fold of the wild type strain (0.19 $g{\cdot}L^{-1}/h$). The *G. oxydans* PZ strain produced 29.3 $g{\cdot}L^{-1}$ xylitol from 40 $g{\cdot}L^{-1}$ D-arabitol. Zhang et al [15] constructed a recombinant strain of *G. oxydans* PXPG to coexpress the XDH gene and a cofactor regeneration enzyme (glucose dehydrogenase, GDH) gene from *Bacillus subtilis*. The results showed that *G. oxydans* PXPG can produce 12.23 g·L-1 xylitol from 30 $g{\cdot}L^{-1}$ D-arabitol with a yield of 40.8 %. On the contrary, the wild-type strain of *G. oxydans* only can produce 7.56 $g{\cdot}L^{-1}$ xylitol with a yield of 25.2%. So the yield was much improved through engineering strain expressed with XDH and GDH genes.

Qi et al [16] cloned the xylitol dehydrogenase gene from *G. oxydans* CGMCC 1.49 and overexpressed it in *E.coli* BL21, Approximately 25.10 $g{\cdot}L^{-1}$ xylitol was obtained at 22 h with a yield of 0.837 g/g from 30 $g{\cdot}L^{-1}$ D-arabitol in the batch biotransformation with the recombinant strain BL21-*xdh*, whereas only 8.10 $g{\cdot}L^{-1}$ xylitol was produced in 30 h by the wild strain with a yield of 0.270 g/g. Zhou et al[17] cloned a xylitol dehydrogenase gene from *G. oxydans* and a cofactor regeneration enzyme gene which was a glucose dehydrogenase gene from *Bacillus subtilis* and co-expressed in *E. coli*. Approximately 26.91 $g{\cdot}L^{-1}$ xylitol was obtained from around 30 $g{\cdot}L^{-1}$ D-xylulose with a 92% conversion yield. The xylitol yield of the recombinant strain was more than 3 fold higher compared to that of the *G. oxydans* NH-10 (7.32 $g{\cdot}L^{-1}$). Genetic engineering bacteria constructed by molecular cloning and functional expression of ArDH and XDH genes of *G. oxydans* certainly can provide the production of xylitol by biological method with high performance.

V. CONCLUSION

G. oxydans possess two enzymes enabling the production of xylitol from D-arabitol. The two enzymes were XDH and ArDH. Since the complexity of two independent catalytic reactions by XDH and ArDH, there are much more researches should be done to discover novel genes and enzymes which can be used in the process of xylitol production. We found that efficient conversion of D-arabiatol and high yield of xylitol can be achieved by engineering *G. oxydans* and *E.coli* respectively. So if we

carry out more detailed research work on genetic engineering strain, the yield of xylitol will be further improved through biological methods. Meanwhile, in view of the high price of coenzymes ($NADH/NAD^+$), the probably method to lighten production cost is to construct engineering strain which can let coenzyme regenerate efficiently.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (Nos. 31571806 and 21006041) and Project of Jiangsu University (No. 08JDG009) and PAPD.

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