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Improved xylitol production by expressing a novel D-arabitol dehydrogenase from isolated *Gluconobacter* sp. JX-05 and co-biotransformation of whole cells

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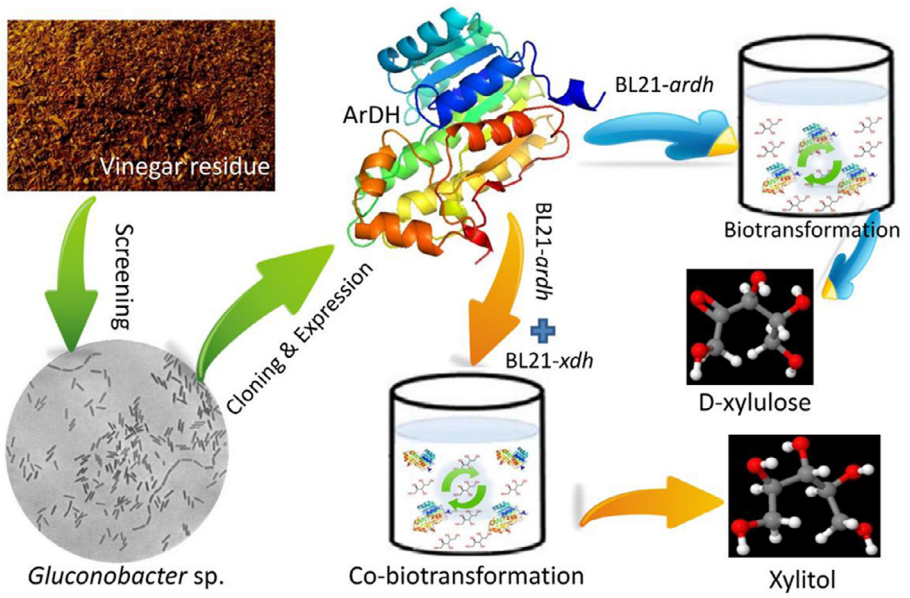
HIGHLIGHTS

- An *ardh* gene from a new isolated *Gluconobacter* sp. JX-05 was expressed and analyzed.
- A novel NAD-dependent D-arabitol dehydrogenase was characterized and modeled.
- The NAD-dependent ArDH was firstly used in the co-biotransformation of D-arabitol.
- The xylitol production was improved by the whole cell microbial co-biotransformation.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, a novel *ardh* gene encoding D-arabitol dehydrogenase (ArDH) was cloned and expressed in *Escherichia coli* from a new isolated strain of *Gluconobacter* sp. JX-05. Sequence analysis revealed that ArDH containing a NAD(P)-binding motif and a classical active site motif belongs to the short-chain dehydrogenase family. Subsequently, the optimal pH and temperature, specific activities and kinetic parameter of ArDH were determined. In the co-biotransformation by the whole cells of BL21-*ardh* and BL21-*xdh*, 26.1 g/L xylitol was produced from 30 g/L D-arabitol in 22 h with a yield of

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0.87 g/g. 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*.

1. Introduction

Xylitol is an important functional sugar alcohol. Because its hygroscopicity, sweetness and appearance are similar to sucrose, xylitol is used as an alternative natural sweetener. Moreover, due to its insulin–independent metabolism, xylitol is conducive to the prevention of dental caries and suitable for diabetic patients (Makinen, 2000; Parajó et al., 1998; Zhang et al., 2015). Therefore, xylitol is widely used in food, medicine and chemical industry and has an important application value and market potential (Parajó et al., 1998; Ur-Rehman et al., 2015).

At present, xylitol is mainly produce via chemical hydrogenation and microbial transformation of D-xylose. However, both these methods rely on the hydrolysis and purification of D-xylose from hemicellulose-xylan dydrolysates (Sugiyama et al., 2003; Suzuki et al., 2002).These not only cause severe contaminations, but also require complicated technologies and high consumption of acid-base (Perez-Bibbins et al., 2015; Povelainen and Miasnikov, 2006). In recent years, xylitol production through microbial conversion of D-glucose, which is cheap and can be derived from the starch of resource-rich cassava, corn and other crops, has attracted the attention of many researchers and motivated the sustainable development of xylitol industry (Granstrom et al., 2007; Povelainen and Miasnikov, 2006). During the biotransformation for xylitol production, D-arabitol is generated through fermentation of osmophilic yeast from glucose first. Then, D-arabitol is oxidized to D-xylulose by NAD(P)-dependent D-arabitol dehytrogenase (ArDH), and xylitol is produced finally through the reduction of D-xylulose by NADH-dependent xylitol dehydrogenase (XDH) (Suzuki et al., 2002). Previous studies showed that D-glucose can be converted to D-arabitol efficiently by strains of osmotolerant yeast (Suzuki et al., 2002), 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 generated from 200 g D-glucose as the substrate by *Zygosaccharomyces rouxii* JM-C46 and *Kodamaea ohmeri* (Qi et al., 2015; Zhu et al., 2010). Since ArDH and XDH are the key enzymes that play crucial roles in the biosynthesis pathway of xylitol from glucose and the conversion of D-arabitol to xylitol by these two enzymes is the rate-limiting step (Li et al., 2016; Zhang et al., 2013; Zhou et al., 2012), much focus has been attracted into the study in this area. So far, we have improved the biological production of xylitol from D-arabitol through the genetic engineering strains BL21-*xhd* mixed *Gluconobacter oxydans* with exogenous NADH. Using this method, the yield of xylitol converted from D-arabitol was more than 83% (Qi et al., 2016). Until now, at least two NADP-dependent D-arabitol dehydrogenase genes had been cloned from *G. oxydans* ATCC 621 (Suzuki et al., 2002) and *G. oxydans* CGMCC 1.110 (Cheng et al., 2005). It is noteworthy that a novel NAD-dependent D-arabitol dehydrogenase (aArDH) from *Acetobacter suboxydans* was cloned and characterized before (Cheng et al., 2009). 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 NADP-dependent ArDH for this purpose.

In this study, a *Gluconobacter* sp. strain was screened and identified from the sample of vinegar residue. Based on the sequence of the putative polyol dehydrogenase of *G. oxydans* ATCC621H, a novel NAD-dependent D-arabitol dehydrogenase encoded by the *ardh* gene was cloned, expressed and characterized. Furthermore, an efficient method for xylitol production using the recombinant *E. coli* strain with the heterologous *ardh* gene and our previously constructed strain of BL21-*xdh* was established.

2. Materials and methods

2.1. Strain, plasmid, culture medium and conditions

E. coli BL21 (DE3) (Stratagene, USA) was used as the host for all following steps. pMD18-T purchased from TakaRa was used for cloning. Plasmid pET28a (+) (Novagen, USA) carring an N-terminal His·Tag/thrombin/T7·Tag configuration plus an optional C-terminal His·Tag sequence was used as the overexpression vector. The seed culture medium (SCM, g/L) for *Gluconobacter* sp. JX-05 was composed of as the following: yeast extract 5.0, peptone 3.0, glucose 10.0, D-arabitol 10.0, $CaCO_3$ 15.0. The enrichment medium (EM), containing yeast extract 10.0, peptone 5.0, glucose 20.0, D-arabitol 30.0, $CaCO_3$ 15.0, was used for high-density cultivation of *Gluconobacter* sp. JX-05. *Gluconobacter* sp. JX-05 was initially grown in SCM at 30 °C with 200 rpm for 20 h, and then inoculated with 2% (v/v) quantity into 200 mL of EM in a 1 L flask. Thereafter *Gluconobacter* sp. JX-05 was cultured at 30 °C with 200 rpm for 48 h. LB medium was used for *E. coli* strain BL21 and LB medium with 100 μg/mL ampicillin (LBA) was used to select recombinant strain.

2.2. Screening and isolation

Samples of vinegar residue for screening *Gluconobacter* sp. strain were collected from Hengshun of Zhenjiang, China. 10 g sample was added into 250 mL flask contained 50 mL of SCM, and then incubated at 30 °C and 200 rpm for 24 h. The sample of culture broth was serially diluted and then transferred to the plates of EM containing 20 g/L agar for the preliminary screening (Yamada et al., 1976). Through this method, acetic acid bacteria including *Gluconobacter* sp. with the capacity of dissolving calcium carbonate were picked up and inoculated in EM at 30 °C for 24 h.

2.3. Identification of screening

The method of preliminary identification was developed based on the characterization of *Gluconobacter* sp. taxonomically (Asai et al., 1964). And the identification was carried out via the observation of morphology of colonies and cells, and the determination of their physiological and biochemical properties (Asai et al., 1964; Tanasupawat et al., 2009). Next, the strains that passed the initial check and could produce xylitol were chosen for the molecular examination. The 16s rDNA sequence was amplified by PCR with universal primers of bacteria. The sense primer is 5′-AGAGTTT GATCCTGGCTCAG-3′, and the anti-sense primer is 5′-TACGGC TACCTTGTTACGACTT-3′. PCR procedure was performed with denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 2 min, 55 °C for 30 s, and extension at 72 °C for 1.5 min, with