Identification of NAD-Dependent Xylitol Dehydrogenase from *Gluconobacter oxydans* WSH-003

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ABSTRACT: *Gluconobacter oxydans* plays an important role in the conversion of D-sorbitol to L-sorbose, which is an essential intermediate for the industrial-scale production of vitamin C. In the fermentation process, some D-sorbitol could be converted to D-fructose and other byproducts by uncertain dehydrogenases. Genome sequencing has revealed the presence of diverse genes encoding dehydrogenases in *G. oxydans*. However, the characteristics of most of these dehydrogenases remain unclear. Therefore, the analyses of these unknown dehydrogenases could be useful for identifying those related to the production of D-fructose and other byproducts. Accordingly, dehydrogenases in *G. oxydans* WSH-003, an industrial strain used for vitamin C production, were examined. A nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase, which was annotated as xylitol dehydrogenase 2, was identified, codon-optimized, and expressed in *Escherichia coli* BL21 (DE3) cells. The enzyme exhibited a high preference for NAD⁺ as the cofactor, while no activity with nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide, or pyrroloquinoline quinone was noted. Although this enzyme presented high similarity with NAD-dependent xylitol dehydrogenase, it showed high activity to catalyze D-sorbitol to D-fructose. Unlike the optimum temperature and pH for most of the known NAD-dependent xylitol dehydrogenases (30–40 °C and about 6–8, respectively), those for the identified enzyme were 57 °C and 12, respectively. The values of *Kₘ* and *Vₘₐₓ* of the identified dehydrogenase toward L-sorbose were 4.92 μM and 196.08 μM/min, respectively. Thus, xylitol dehydrogenase 2 can be useful for the cofactor-reduced nicotinamide adenine dinucleotide regeneration under alkaline conditions, or its knockout can improve the conversion ratio of D-sorbitol to L-sorbose.

INTRODUCTION

The genus *Gluconobacter* is a part of the group of acetic acid bacteria that are characterized by their ability to incompletely oxidize a wide range of carbohydrates and alcohols. *Gluconobacter* strains have been successfully used for the industrial production of food-related products, pharmaceuticals, and cosmetics, such as vitamin C, miglitol, dihydroxyacetone (DHA), and ketogluconates. In particular, *Gluconobacter oxydans* has applications in the production of food additives and sweeteners owing to its ability to synthesize flavoring ingredients from aromatic alcohols, aliphatic alcohols, and 5-ketofructose. Besides, *G. oxydans* enzymes, cell membranes, and whole cells are also used as sensor systems for the detection of polyols, sugars, and alcohols. In recent years, some *G. oxydans* strains have been employed for the production of enantiomeric pharmaceuticals and platform compounds; for example, *G. oxydans* DSM 2343 has been employed for the reduction of various ketones used in pharmaceutical, agrochemical, and natural products. *G. oxydans* has been utilized for L-erythrose or L-erythropentose production and D-xylulose and xylitol production. *G. oxydans* DSM 003 has been used for 3-hydroxypropionic acid production. As all of these products are related to the dehydrogenases of *G. oxydans*, identification of these enzymes in *G. oxydans* can expand the application of this bacterium.

*Gluconobacter* strains possess numerous dehydrogenases, some of which have been identified, such as alcohol dehydrogenase, which could convert ethanol to acetaldehyde, nicotinamide adenine dinucleotide phosphate (NADP)-dependent acetaldehyde dehydrogenase, which could convert acetaldehyde to acetate, pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase, which could convert D-glucose to D-gluconate, D-glucate dehydrogenase, which could convert D-glucate to 2- or 5-ketogluconate, 2-ketogluconate dehydrogenase, which could convert 2-ketogluconate to 2,5-diketogluconate, D-sorbitol dehydrogenase, which could convert D-sorbitol to L-sorbose or D-fructose, sorbose/sorbosone dehydrogenase, which...
Figure 1. Optimum pH and temperature of xylitol dehydrogenase 2. (A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the identified xylitol dehydrogenase 2 purified from E. coli BL21 (DE3) cells containing pET-28a-XDH. Lane 1: E. coli BL21 containing pET-28a after induction for 16 h at 20°C. Lane 2: Recombinant strain E. coli BL21 containing pET-28a-XDH after induction for 16 h at 20°C. Lane 3: Purified recombinant enzyme. Lane M: Molecular mass markers. (B) Effect of pH on the activity of purified xylitol dehydrogenase 2. (C) Effect of temperature on the activity of purified xylitol dehydrogenase 2.

could convert l-sorbose to l-sorbose or 2-keto-l-gulonic acid (2-KLG),16 mannitol dehydrogenase, which could convert d-mannitol to d-fructose,17 quinate dehydrogenase, which could convert quinic acid to shikimic acid,18 glycerol dehydrogenase, which could convert glycerol to DHA,18c etc. In 2005, the complete genome of G. oxydans 621H was sequenced,19 which revealed 75 open reading frames that encode putative dehydrogenases/oxidoreductases of unknown functions. Identification of the functions of these unknown dehydrogenases/oxidoreductases is important to expand the application of G. oxydans. For instance, carbonyl reductase (GoKR) from G. oxydans DSM2343 has been employed for the reduction of various ketones,8 and membrane-bound alcohol dehydrogenase (mADH) and membrane-bound aldehyde dehydrogenase from G. oxydans DSM 2003 have been employed for 3-hydroxypropionic acid production.11

In G. oxydans, the central metabolic pathway, such as citrate cycle and Embden–Meyerhof–Parnas pathway, is incomplete because of the absence of some genes encoding succinate dehydrogenase, phosphofructokinase, phosphotransacetylase, acetate kinase, succinyl-CoA synthetase, succinate dehydrogenase, isocitratylase, and malate synthase,26 which may be the reason for the low biomass of G. oxydans when cultivated in rich medium. In a previous study, sdhCDABE genes encoding succinate dehydrogenase and flavinylation factor SdhE, ndh gene encoding a type II reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, and sucCD from Gluconacetobacter diazotrophicus encoding succinyl-CoA synthetase were expressed in G. oxydans to increase its biomass yield.20b However, G. oxydans biomass only increased by 60%, suggesting the presence of some unknown bottleneck. Except for the tricarboxylic acid cycle, all the genes were identified to encode enzymes involved in oxidative pentose phosphate and Embden–Meyerhof (ED) pathways.19 The pentose phosphate pathway is believed to be the most important route for the phosphorylative breakdown of sugars and polyols to CO₂ and provide carbon skeleton. It has been speculated that G. oxydans has the capability to uptake and channelize several polyols, sugars, and sugar derivatives into the oxidative pentose phosphate pathway; however, the gene involved in this process is still unknown. Hence, in the fermentation of D-sorbitol to l-sorbose for vitamin C production, some D-sorbitol maybe get converted to D-fructose or other byproduct to enter the pentose phosphate pathway for cell growth.19,21 Therefore, it is crucial to balance and control the conversion of D-sorbitol to l-fructose for cell growth and sorbose production.

Gene disruption and complementation experiments are often used to verify one gene function. Some G. oxydans genes have been identified by using this method, such as PQQ-dependent D-sorbitol dehydrogenase responsible for the oxidation of 1-(2-hydroxyethyl) amino-1-deoxy-D-sorbitol to 6-(2-hydroxyethyl) amino-6-deoxy-l-sorbose, which is the precursor of an antidiabetic drug miglitol,1 pyruvate decarboxylase that catalyzes the conversion of pyruvate to acetaldehyde by decarboxylation,22 mADH, membrane-bound inositol dehydrogenase, membrane-bound PQQ-dependent glucose dehydrogenase, etc.23 However, some G. oxydans genes encoding dehydrogenases are necessary for cell growth, and their knockout resulted in the absence of growth (unpublished data). Besides, G. oxydans comprises numerous dehydrogenases, some of which are isoenzymes, such as SldAB1 and SldAB2 of G. oxydans WSH-003,24 or are often associated with a broad range of substrates such as GoKR.25 Hence, the use of gene knockout strategy to identify the functions of some dehydrogenases of G. oxydans, especially the numerous unknown dehydrogenases of G. oxydans WSH-003, may not be appropriate. Therefore, in the present study, we expressed numerous unknown dehydrogenases of G. oxydans WSH-003 in Escherichia coli BL21 (DE3) cells and purified the products by one-step affinity chromatography with the Ni-NTA agarose column to identify their functions. The results revealed a new xylitol dehydrogenase (nicotinamide adenine dinucleotide
(NAD)-dependent xylitol dehydrogenase 2) that could convert D-sorbitol to D-fructose. Kinetic analysis of the novel enzyme revealed some unique traits that were quite different from the known xylitol dehydrogenases. The optimum temperature and pH of the identified xylitol dehydrogenase 2 were 57 °C and 12, respectively. This novel enzyme provides new insights into G. oxydans dehydrogenases and could have potential applications in xylitol production.

**RESULTS**

**Gene Expression and Purification of the Identified Dehydrogenase.** The selected dehydrogenase from G. oxydans WSH-003 was successfully expressed and purified. Sequence analysis revealed that the purified enzyme, annotated as xylitol dehydrogenase 2, contained a NAD(P)-binding motif and a classical active site motif belonging to the short-chain dehydrogenase family. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis showed an expected single band with a molecular weight of about 38 kDa (Figure 1A), which was consistent with the calculated molecular mass based on the deduced amino acid sequence (36.6 kDa). The optimum pH and the temperature of the purified xylitol dehydrogenase 2 were determined to be pH 12 (50 mM glycine–NaOH buffer) and 57 °C, respectively (Figure 1B,C), which are different from those for known xylitol dehydrogenases.

**Identification of Cofactor of Xylitol Dehydrogenase 2.** In general, dehydrogenases require some cofactors as electron acceptors, such as NAD(P), flavin adenine dinucleotide (FAD)/flavin mononucleotide, or PQQ. Most of the previously identified membrane dehydrogenases from G. oxydans have been reported to utilize PQQ or FAD as the cofactor. According to the prediction of transmembrane domains, xylitol dehydrogenase 2 from G. oxydans WSH-003 was noted to lack transmembrane domain. Therefore, the cofactor of the identified dehydrogenase was verified by using purified enzyme to catalyze reactions with different cofactors. The results showed that xylitol dehydrogenase 2 was highly specific for NAD+ and no detectable enzyme activity was observed with NADP+, FAD, or PQQ as the cofactor (Figure 2).

**Effect of Ethylenediaminetetraacetic Acid (EDTA) and Metal Ions on Enzyme Activity.** To determine the effects of chelator and metal ions on NAD-dependent xylitol dehydrogenase 2, ethylenediaminetetraacetic acid (EDTA) and various ions (0.5 mM Ca2+, Mg2+, Cu2+, Fe2+, Zn2+, Co2+, Ni2+, Mn2+, Cr3+, and Fe3+) were added to the reaction system. EDTA elicited no obvious effect on NAD-dependent xylitol dehydrogenase 2, indicating that the enzyme does not require a chelator for its activity. However, the enzyme could be activated by Zn2+, Co2+, and Mn2+, among which Zn2+ improved the enzyme activity by 1.8 times. In contrast, Cu2+ could almost completely inhibit the activity of NAD-dependent xylitol dehydrogenase 2 (Figure 3), while the rest of the examined metal ions had no obvious impact on the enzyme activity.

**Substrate Specificity and Kinetic Constants.** In recent years, xylitol dehydrogenase has been used for the industrial production of xylitol, and under enhanced NADH supply, NAD-dependent xylitol dehydrogenase can reduce D-xylulose to the desired xylitol. In the present study, substrate specificity analysis of the NAD-dependent xylitol dehydrogenase 2 revealed that the enzyme was highly specific toward D-sorbitol and xylitol, but showed limited activity toward D-mannitol, sorbose, and glycerol. Moreover, the enzyme showed no activity when glucose, inositol, galactose, mannose, rhamnose, xylose, fructose, glucuronic acid, glucolactone, 2-KLG, gluconic, propanol, isopropanol, methanol, and ethanol were used as substrates (Figure 4). To determine the kinetic constants, the initial velocities of the enzyme were determined in glycine–NaOH buffer (pH 12) with D-sorbitol (at increasing concentrations from 1 to 500 mM) under standard assay conditions, and Km and Vmax were noted to be 4.92 μM and 196.08 μM/min, respectively.

**DISCUSSION**

In this study, a xylitol dehydrogenase from 44 uncharacterized dehydrogenases of G. oxydans WSH-003 was identified and characterized. This novel NAD-dependent xylitol dehydrogenase 2 could convert D-sorbitol to D-fructose, indicating certain correlation of this enzyme with pentose phosphate pathway. The optimum temperature and pH for the identified xylitol dehydrogenase 2 revealed its unique characteristics, when compared with some of the previously identified xylitol dehydrogenases.
dehydrogenases. It has been reported that D-fructose is the major byproduct formed during the conversion of D-sorbitol to L-sorbose by \textit{G. oxydans} in industrial-scale vitamin C production,\textsuperscript{21} and that knockout of genes involved in D-fructose production can further improve the conversion rate of D-sorbitol to L-sorbose. Owing to its unique characteristics, the NAD-dependent xylitol dehydrogenase 2 identified in the present study can be applied for the production of D-xylitol.\textsuperscript{10a}

To characterize all the dehydrogenases of \textit{G. oxydans} WSH-003, the enzymes were predicted and heterologously overexpressed in \textit{E. coli} BL21 (DE3) cells. Then, the expressed dehydrogenases were purified by one-step affinity chromatography with Ni-NTA agarose column. While most of the dehydrogenases with obvious expression levels in \textit{E. coli} showed no activities, NAD-dependent xylitol dehydrogenase 2 could efficiently convert D-sorbitol to D-fructose.

Previous studies have indicated that majority of the numerous dehydrogenases in \textit{G. oxydans} are membrane-bound, PQQ- or FAD-dependent enzymes with more than one subunit; for example, alcohol dehydrogenases have three subunits,\textsuperscript{25} aldehyde dehydrogenases have two subunits,\textsuperscript{26} NADP-dependent D-sorbitol dehydrogenases have one or three subunits,\textsuperscript{15a,d,26} and polyol dehydrogenase have two subunits.\textsuperscript{28} Most of the cytoplasmic-soluble polyol dehydrogenases are NADP-dependent with more than one subunit; for instance, NADP-dependent D-sorbitol dehydrogenase have four subunits, NADP-dependent D-sorbitol dehydrogenase have two subunits,\textsuperscript{29} and NAD-dependent ribitol dehydrogenase have four subunits.\textsuperscript{30} However, the xylitol dehydrogenase 2 identified in the present study was noted to be NAD-dependent with only one subunit. The amino acid sequence of the NAD-dependent xylitol dehydrogenase 2 showed similarity to those of the enzymes in the MDR superfamily. However, the optimum pH and temperature for the oxidation activity of the NAD-dependent xylitol dehydrogenase 2 were observed to be slightly higher than those reported in earlier studies for the same reaction of xylitol dehydrogenases isolated from different strains of \textit{G. oxydans}.\textsuperscript{31} The reason for this variation in the optimum pH and temperature for xylitol dehydrogenase activity could be due to different source strains from which the enzymes were isolated.

With regard to the substrate specificity of xylitol dehydrogenases, xylitol dehydrogenase from \textit{G. oxydans} ATCC 621 has been noted to present a higher catalytic activity toward sorbitol and xylitol,\textsuperscript{32} whereas xylitol dehydrogenase from \textit{Gluconobacter thailandicus} CGMCC1.3748 has been demonstrated to exhibit catalytic activity toward xylitol, D-sorbitol, D-mannitol, and D-fructose.\textsuperscript{31} Besides, while most of the known xylitol dehydrogenases have been reported to be dependent on cofactor NAD\textsuperscript{+}, an NADP\textsuperscript{+}-dependent xylitol dehydrogenase has been found to increase ethanol production from xylene in recombinant \textit{Saccharomyces cerevisiae} though protein engineering.\textsuperscript{33}

In most of the identified \textit{G. oxydans} strains, glycolysis, and citric acid cycle are incomplete owing to the lack of phosphofructokinase and succinate dehydrogenase,\textsuperscript{34} which is the main reason for the low biomass yield of \textit{G. oxydans}, when compared with other common bacteria, and a major limitation to the use of \textit{G. oxydans} whole-cell biotransformation. It has been reported that pentose phosphate pathway and ED pathway are the main catabolic routes for biomass and energy supply in \textit{Gluconobacter} strains.\textsuperscript{34} Despite its industrial application for several decades, the metabolic pathways and regulatory mechanisms of \textit{Gluconobacter} spp. are not yet fully elucidated.\textsuperscript{35} To improve the biomass of \textit{G. oxydans}, Krajewski et al. knocked out the membrane-bound glucose dehydrogenase and soluble glucose dehydrogenase and improved the biomass by 271%.\textsuperscript{36} An understanding of the mechanisms of catabolism of polyols, sugars, and sugar derivatives into the pentose phosphate pathway is essential for increasing the biomass and catalysis efficiency of \textit{G. oxydans} strains. As D-sorbitol and L-sorbose cannot directly enter into the pentose phosphate pathway, they must be catabolized via some intermediates. The NAD-dependent xylitol dehydrogenase 2 identified in the present study can catalyze D-sorbitol to D-fructose, which can directly enter the pentose phosphate pathway through phosphorylation, suggesting that the over-expression of this enzyme may increase the biomass of \textit{G. oxydans} by utilizing more D-sorbitol.

In conclusion, a novel NAD-dependent xylitol dehydrogenase 2 from \textit{G. oxydans} WSH-003 was identified in this study. Owing to its unique characteristics, such as optimum pH and temperature, the identified dehydrogenase could be used in the production of xylitol or fructose, or in regeneration of cofactor under specific conditions. Although \textit{G. oxydans} WSH-003 has been mutated from wild-type strain at least 90 times by different methods with reliable records to improve the L-sorbose production and tolerance to saccharides and alditols such as L-sorbose and D-sorbitol, generation of D-fructose as the byproduct of the strain could not be resolved. However, knockout of this xylitol dehydrogenase and similar dehydrogenases could facilitate further increase in the yield of D-sorbitol to L-sorbose, which could be important for the current industrial-scale production of vitamin C.

\section{MATERIALS AND METHODS}

\textbf{Genes, Plasmids, and Strains.} The vector pMD19-T Simple and pET-28a(+) were used for vector construction and protein expression, respectively. \textit{E. coli} JM109 cells were employed for plasmid construction, and \textit{E. coli} BL21 (DE3) cells were used for protein expression. The dehydrogenase gene (GenBank accession no. 29878874) was PCR-amplified from the genomic DNA of \textit{G. oxydans} WSH-003 using the primer pair CCG GAA TTT CAG GCT -
Gene Expression and Purification of Dehydrogenase.

The recombinant strain was cultured in 250 mL shake flasks containing 25 mL of terrific broth medium. After growth to log phase (OD600 = 0.6), the cells were precoupled to 20 °C. Then, 0.5 mM isopropyl-β-D-thiogalactopyranoside was added to induce protein expression, and the cells were incubated at 20 °C for another 16 h for protein expression.

Subsequently, the cells were collected by centrifugation at 5000 rpm for 5 min, washed twice with binding buffer (50 mM phosphate bu-

Effect of Metal Ions and EDTA.

In order to determine the effect of the metal ions and the EDTA on the enzyme, various metal ions (0.5 mM) and EDTA (5 mM) were added individually to the reaction mixture. Relative activity was used to investigate, while the reaction mixture without any additional treatment served as a control (100%).

Substrate Specificity and Determination of Kinetic Constants.

Substrate specificity of the identified dehydrogenase was tested using 20 mM xyitol, glucose, d-mannitol, inositol, sorbose, galactose, sorbitol, mannose, rhamnose, xylose, fructose, glucuronic acid, glucolactone, 2-KLG, gluconic acid, propanol, glycerol, inopropanol, methanol, and ethanol in the above-mentioned buffers. For kinetics experiments, the substrate concentrations were varied between 1 and 500 mM and the cofactor concentration was 2 mM.

Determination of Optimum Temperature and pH for the Identified Dehydrogenase.

To determine the optimum pH, the enzyme activity was assessed in a pH range of 3–13 in the following buffers (50 mM): NaAc–HAc (pH 3.0–5.0), phosphate-buffered saline (pH 5.0–9.0), Tris–HCl (pH 9.0–10.0), and glycine–NaOH (pH 9.0–13.0). Similarly, the optimal temperature for the identified dehydrogenase was determined by analyzing the enzyme activity to be from 20 to 70 °C.

REFERENCES

(1) Deppenmeier, U.; Hoffmeister, M.; Prust, C. Biochemistry and biotechnological applications of Gluconobacter strains. Appl. Microbiol. Biotechnol. 2002, 60, 233–242.

(2) Giridhar, R.; Srivastava, A. K. Model based constant feed batch 1-sorbose production process for improvement in 1-sorbose productivity. Chem. Biochem. Eng. Q. 2000, 14, 133–140.

(3) Yang, X.-P.; Wei, L.-J.; Lin, J.-P.; Yin, B.; Wei, D.-Z. A membrane-bound PQQ-dependent dehydrogenase in Gluconobacter oxydans M5, responsible for production of 6-(2-hydroxyethyl) amino-6-deoxy-sorbose. Appl. Microbiol. Biotechnol. 2008, 79, 5250–5253.

(4) Polujengred, L.; Boonyaratankalin, S. Dihydroxyacetone production by Gluconobacter frateruli in a minimum medium using fed-batch fermentation. J. Chem. Technol. Biotechnol. 2017, 92, 2635–2641.

(5) (a) Li, K.; Mao, X.; Liu, L.; Lin, J.; Sun, M.; Wei, D.; Yang, S. Overexpression of membrane-bound glutonate-2-dehydrogenase to enhance the production of 2-keto-3-gluconic acid by Gluconobacter oxydans. Microb. Cell Fact. 2016, 15, No. 121. (b) Shi, Y.-y.; Li, K.-f.; Lin, J.-p.; Yang, S.-l.; Wei, D.-z. Engineered Expression Vectors Significantly Enhanced the Production of 2-Keto-3-gluconic Acid by Gluconobacter oxydans. J. Agric. Food Chem. 2015, 63, 5492–5498.

(6) (a) Siemen, A.; Kosciow, K.; Schweiger, P.; Deppenmeier, U. Production of 5-keto-fructose from fructose or sucrose using genetically modified Gluconobacter oxydans strains. Appl. Microbiol. Biotechnol. 2018, 102, 1699–1710. (b) Rabenhorst, J.; Gatfield, I.; Hilner, J.-M. Natural, Aliphatic and Thiocarboxylic Acids Obtainable by Fermentation and a Microorganism Therefore, EP10789902001.

(7) (a) Bertoková, A.; Bertok, T.; Filip, J.; Tkac, J. Gluconobacter sp cells for manufacturing of effective electrochemical biosensors and biofuel cells. Chem. Pap. 2015, 69, 27–41. (b) Macauley, S.; McNeil, B.; Harvey, L. M. The genus Gluconobacter and its applications in biotechnology. Crit. Rev. Biotechnol. 2001, 21, 1–25. (c) Schenkmanová, A.; Bertóková, A.; Šefcovcová, J.; Štefuca, V.; Bučko, M.; Vikartovská, A.; Gemeiner, P.; Tkáč, J.; Katrilik, J. Whole-cell Gluconobacter oxydans biosensor for 2-phenylethanol biooxidation monitoring. Anal. Chim. Acta 2015, 854, 140–144.

(8) Chen, R.; Liu, X.; Wang, J.; Lin, J.; Wei, D. Cloning, expression, and characterization of an anti-Prelog stereospecific carbonyl reductase from Gluconobacter oxydans DSM2343. Enzyme Microb. Technol. 2015, 70, 18–27.

(9) (a) Burger, C.; Kessler, C.; Gruber, S.; Ehrenreich, A.; Liebl, W.; Weuster-Botz, D. L-erythulose production with a multideletion strain of Gluconobacter oxydans. Appl. Microbiol. Biotechnol. 2019, 103, 4393–4404. (b) Zou, X.; Lin, J.; Mao, X.; Zhao, S.; Ren, Y. Biosynthesis of 1-erythrose by Assembly of Two Key Enzymes in Gluconobacter oxydans. J. Agric. Food Chem. 2017, 65, 7721–7725.
Cloning of a gene for D-sorbitol dehydrogenase from Matsuura, M.; Takata, Y.; Noguchi, Y.; Saito, Y.; Yamashita, M.

Food Chem. 2013, 145, 100–107. (c) Yasuki, T.; Terada, Y.; Ozaki, S.; Kataoka, N.; Akakabe, Y.; Adachi, O.; Matsutani, M.; Matsushita, K. Aldopentoses as new substrates for the membrane-bound, pyrroloquinoline quinone-dependent glycerol (polyl) dehydrogenase of Gluconobacter sp. Appl. Microbiol. Biotechnol. 2018, 102, 3159–3171.

(19) Prust, C.; Hoffmeister, M.; Liesegang, H.; Wieser, A.; Fricke, W. F.; Ehrenreich, A.; Gottschalk, G.; Denpeenmeier, U. Complete genome sequence of the acetic acid bacterium Gluconobacter oxydans. Nat. Biotechnol. 2005, 23, 195–200.

(20) (a) Greenfield, S.; Claus, G. W. Nonfunctional tricarboxylic acid cycle and the mechanism of glutamate biosynthesis in Acetobacter suboxydans. J. Bacteriol. 1972, 112, 1295–1301. (b) Kiefer, L.; Bringer, S.; Bott, M. Metabolic engineering of Gluconobacter oxydans 621H for increased biomass yield. Appl. Microbiol. Biotechnol. 2017, 101, 5453–5467.

(21) Macauley-Patrick, S.; McNeil, B.; Harvey, L. M. By-product formation in the L-sorbitol to L-sorbose biotransformation by Gluconobacter suboxydans ATCC 621 in batch and continuous cultures. Process Biochem. 2005, 40, 2113–2122.

(22) Peters, B.; Junker, A.; Kraus, M.; Müllhüter, B.; Kostner, D.; Mientus, M.; Liebl, W.; Ehrenreich, A. Deletion of pyruvate decarboxylase by a new method for efficient markerless gene deletions in Gluconobacter oxydans. Appl. Microbiol. Biotechnol. 2013, 97, 2521–2530.

(23) (a) Peters, B.; Mientus, M.; Kostner, D.; Junker, A.; Liebl, W.; Ehrenreich, A. Characterization of membrane-bound dehydrogenases from Gluconobacter oxydans 621H via whole-cell activity assays using multideletion strains. Appl. Microbiol. Biotechnol. 2013, 97, 6397–6412. (b) Mientus, M.; Kostner, D.; Peters, B.; Liebl, W.; Ehrenreich, A. Characterization of membrane-bound dehydrogenases of Gluconobacter oxydans 621H using a new system for their functional expression. Appl. Microbiol. Biotechnol. 2017, 101, 3189–3200.

(24) Gao, L.; Zhou, J.; Liu, J.; Du, G.; Chen, J. Draft genome sequence of Gluconobacter oxydans WSH-003, a strain that is extremely tolerant of saccharides and alditols. J. Bacteriol. 2012, 194, 4455–4456.

(25) Adachi, O.; Toyama, K.; Shinagawa, E.; Matsuhashi, K.; Ameayama, M. Purification and characterization of particulate alcohol dehydrogenase from Gluconobacter suboxydans. Biosci. Biotechnol. Biochem. 1978, 42, 2045–2056.

(26) Adachi, O.; Toyama, K.; Shinagawa, E.; Matsuhashi, K.; AmeAyama, M. Purification and characterization of membrane-bound L-sorbose dehydrogenase from Gluconobacter suboxydans in membrane preparations. J. Biochem. 1980, 44, 503–515.

(27) Shinagawa, E.; Matsuhashi, K.; Adachi, O.; AmeAyama, M. Purification and characterization of L-sorbose dehydrogenase from membrane of Gluconobacter suboxydans var. a. Agric. Biol. Chem. 1982, 46, 135–141.

(28) Matsuhashi, K.; Fujii, Y.; Ano, Y.; Toyama, H.; Shinjoh, M.; Tomiyama, N.; Miyazaki, T.; Sugisawa, T.; Hoshino, T.; Adachi, O. L-sorbose production is catalyzed by a quinoprotein glycerol dehydrogenase, major polyol dehydrogenase, in Gluconobacter species. Appl. Environ. Microbiol. 2003, 69, 1959–1966.

(29) Adachi, O.; Ano, Y.; Moonmangmee, D.; Shinagawa, E.; Toyama, H.; Theeragool, G.; Lotong, N.; Matsuhashi, K. Crystal structure and properties of NAPDH-dependent L-sorbose reductase from Gluconobacter melanogenus IFO 3294. Biosci. Biotechnol. Biochem. 1999, 63, 2137–2143.

(30) Adachi, O.; Fujii, Y.; Ano, Y.; Moonmangmee, D.; Toyama, H.; Shinagawa, E.; Theeragool, G.; Lotong, N.; Matsuhashi, K. Membrane-bound L-sorbose alcohol dehydrogenase in L-sorbose acid bacteria catalyzes L-ribulose formation and NAD-dependent ribitol dehydrogenase is independent of the oxidative fermentation. Biosci. Biotechnol. Biochem. 2001, 65, 115–125.

(31) Zhang, H.; Yun, J.; Zabed, H.; Yang, M.; Zhang, G.; Qi, Y.; Guo, Q.; Qi, X. Production of xylitol by expressing xylitol dehydrogenase and alcohol dehydrogenase from Gluconobacter oxydans. Protein Expression Purif. 2018, 145, 100–107.
thailandicus and co-biotransformation of whole cells. Bioresour. Technol. \textbf{2018}, 257, 223–228.

(32) Sugiyama, M.; Suzuki, S.; Tonouchi, N.; Yokozeki, K. Cloning of the xylitol dehydrogenase gene from \textit{Gluconobacter oxydans} and improved production of xylitol from \textit{D}-arabitol. Biosci. Biotechnol. Biochem. \textbf{2003}, \textit{67}, 584–591.

(33) Matsushika, A.; Watanabe, S.; Kodaki, T.; Makino, K.; Inoue, H.; Murakami, K.; Takimura, O.; Sawayaama, S. Expression of protein engineered NADP⁺-dependent xylitol dehydrogenase increases ethanol production from xylene in recombinant \textit{Saccharomyces cerevisiae}. Appl. Microbiol. Biotechnol. \textbf{2008}, \textit{81}, 243–255.

(34) De Muynck, C.; Pereira, C. S. S.; Naessens, M.; Parmentier, S.; Soetaert, W.; Vandamme, E. J. The genus \textit{Gluconobacter oxydans}: Comprehensive overview of biochemistry and biotechnological applications. Crit. Rev. Biotechnol. \textbf{2007}, \textit{27}, 147–171.

(35) (a) Klasen, R.; Bringer-Meyer, S.; Sahm, H. Incapability of \textit{Gluconobacter oxydans} to produce tartaric acid. Biotechnol. Bioeng. \textbf{1992}, \textit{40}, 183–186. (b) Rauch, B.; Pahlke, J.; Schweiger, P.; Deppenmeier, U. Characterization of enzymes involved in the central metabolism of \textit{Gluconobacter oxydans}. Appl. Microbiol. Biotechnol. \textbf{2010}, \textit{88}, 711–718. (c) Gupta, A.; Qazi, G. N.; Verma, V. Transposon induced mutation in \textit{Gluconobacter oxydans} with special reference to its direct-glucose oxidation metabolism. FEMS Microbiol. Lett. \textbf{1997}, \textit{147}, 181–188.

(36) Krajewski, V.; Simić, P.; Mouncey, N. J.; Bringer, S.; Sahm, H.; Bott, M. Metabolic engineering of \textit{Gluconobacter oxydans} for improved growth rate and growth yield on glucose by elimination of gluconate formation. Appl. Environ. Microbiol. \textbf{2010}, \textit{76}, 4369–4376.