JOURNAL OF BIOSCIENCE AND BIOENGINEERING
Vol. 97, No. 2, 89–94. 2004

REVIEW

Izumoring: A Novel and Complete Strategy for Bioproduction of Rare Sugars

TOM BIRGER GRANSTRÖM,[1] GORO TAKATA,[1] MASAAKI TOKUDA,[1] AND KEN IZUMORI[1]*

Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa 761-0795, Japan[1]

Received 29 October 2003/Accepted 12 November 2003

Starch, whey or hemicellulosic waste can be used as a raw material for the industrial production of rare sugars. D-Glucose from starch, whey and hemicellulose, D-galactose from whey, and D-xylose from hemicellulose are the main starting monosaccharides for production of rare sugars. We can produce all monosaccharides; tetroses, pentoses and hexoses, from these raw materials. This is achieved by using D-tagatose 3-epimerase, aldose isomerase, aldose reductase, and oxidoreductase enzymes or whole cells as biocatalysts. Bioproduction strategies for all rare sugars are illustrated using ring form structures given the name Izumoring.

[**Key words:** Izumoring, rare sugars, aldose, ketose, polyol, ketohexose, ketopentose, ketotetrose, D-tagatose 3-epimerase]

I. HISTORY

Sugar production was established in the seventh century AD. Since then, the sugar industry has grown immensely. In the year 2002, global sugar production exceeded 133 million tons per year (1). The earliest examples of starch hydrolysis are as old as ancient cooking, brewing or wine making. The modern history of enzymes dates back to 1833 when the isolation of an amylase complex from germinating barley was reported by Anselme Payen and Jean-François Persoz. However, it was not until the early 20th century that scientists were able to explain what is happening to starch and what is causing it. The first patent on the production of D-fructose from D-glucose by D-xylose isomerase was issued in 1960 (2).

Increasing amounts of sugars in food, sweets, soft drinks, and so on has raised some concerns about their health effects. The number of people suffering from diabetes, obesity, cancer, and cardiovascular diseases is increasing every year in the developing world according to the WHO (press release, March 3, 2003). However, the success of xylitol has shown that alternatives to existing dominant sweeteners are possible. Xylitol is a five carbon polyol (pentitol) that has beneficial health properties. For example, it can prevent tooth decay and acute otitis media in children, when used regularly (3, 4). It is also a polyol, and is produced industrially and represents an alternative to current conventional sweeteners.

* Corresponding author. e-mail: izumori@ag.kagawa-u.ac.jp
phone: +81-(0)87-891-3290 fax: +81-(0)87-891-3021

The International Society of Rare Sugars (ISRS) has defined rare sugars as monosaccharides and their derivatives that rarely exist in nature. According to this definition, xylitol is a rare sugar. In our laboratory, we have been studying the production of rare sugars using microbial and enzymatic reactions. We have devised a scheme to display all the rare sugars in a ring-form called an “Izumoring”. Using this display, we can illustrate their relation to each other. In addition, we can describe the biochemical reactions needed to produce them and the connection between the D- and L-configurations of these sugars. There are three different Izumorings, one for tetroses, one for pentoses, and one for hexoses.

II. BIOCATALYSTS FOR PRODUCTION OF RARE SUGARS

It is advantageous to use cheap natural resources, such as starch, wood or whey, as starting materials for the production of rare sugars. D-Glucose is obtained from starch whereas D-xylose can be derived from the hemicellulosic fraction of wood. Whey contains lactose, which can be hydrolysed into D-glucose and D-galactose. We have studied the production of various rare sugars using whole cells and enzymes. The main biocatalysts are enzymes such as D-tagatose 3-epimerase, various oxidoreductases, polyol dehydrogenases, and aldose isomerases. D-Tagatose 3-epimerase (DTE) catalyses the epimerisation of all ketohexoses and ketopentoses at the C-3 position, producing the corresponding ketoses (5). The amino acid sequence of this enzyme was determined and no homology to other DTE enzymes was reported (6). We used this enzyme for the mass pro-

duction of various rare ketohexoses such as D-psicose from D-fructose (7, 8), D-sorbose from D-tagatose (9), L-fructose from L-psicose, and L-tagatose from L-sorbose (10).

Oxidoreductases catalyze oxidation-reduction between ketoses and polyols. We have been able to use oxidoreductase reactions using microbial cells as catalysts instead of enzymes. D-Tagatose was produced from galactitol (11) and L-tagatose from galactitol (12). Using the reverse reaction of polyol dehydrogenase, various rare hexitols were prepared from ketohexoses by microbial reactions. These include D-talitol production from D-tagatose (13), allitol from D-psicose (14), D-talitol from D-psicose (15), and D-iditol from D-sorbose (16).

Various aldose isomerases transform ketohexoses to the corresponding aldohexoses. Bhuiyan *et al*. (17, 18) reported L-mannose and D-allose production from L-fructose and D-psicose, respectively. D-Xylose isomerase is used in the conversion of D-glucose into D-fructose in the industrial production of high fructose corn syrup (HFCS). However, Pastinen *et al*. (19) found that D-xylose isomerase can be used in the production of various rare aldohexoses from ketohexoses, such as D-altrose, and D-allose from D-psicose, as well as D-gulose and D-idose from D-sorbose. They have also shown that active cross-linked D-xylose isomerase crystals can be used for the separation of enzyme inhibitors such as xylitol and D-sorbitol from impure mixtures of D-arabinitol, D-mannitol, ribitol, and monosaccharides (20).

III. IZUMORING FOR HEXOSES

Figure 1 presents the arrangement and interconnections between all ketohexoses and all hexitols. It shows four epi-

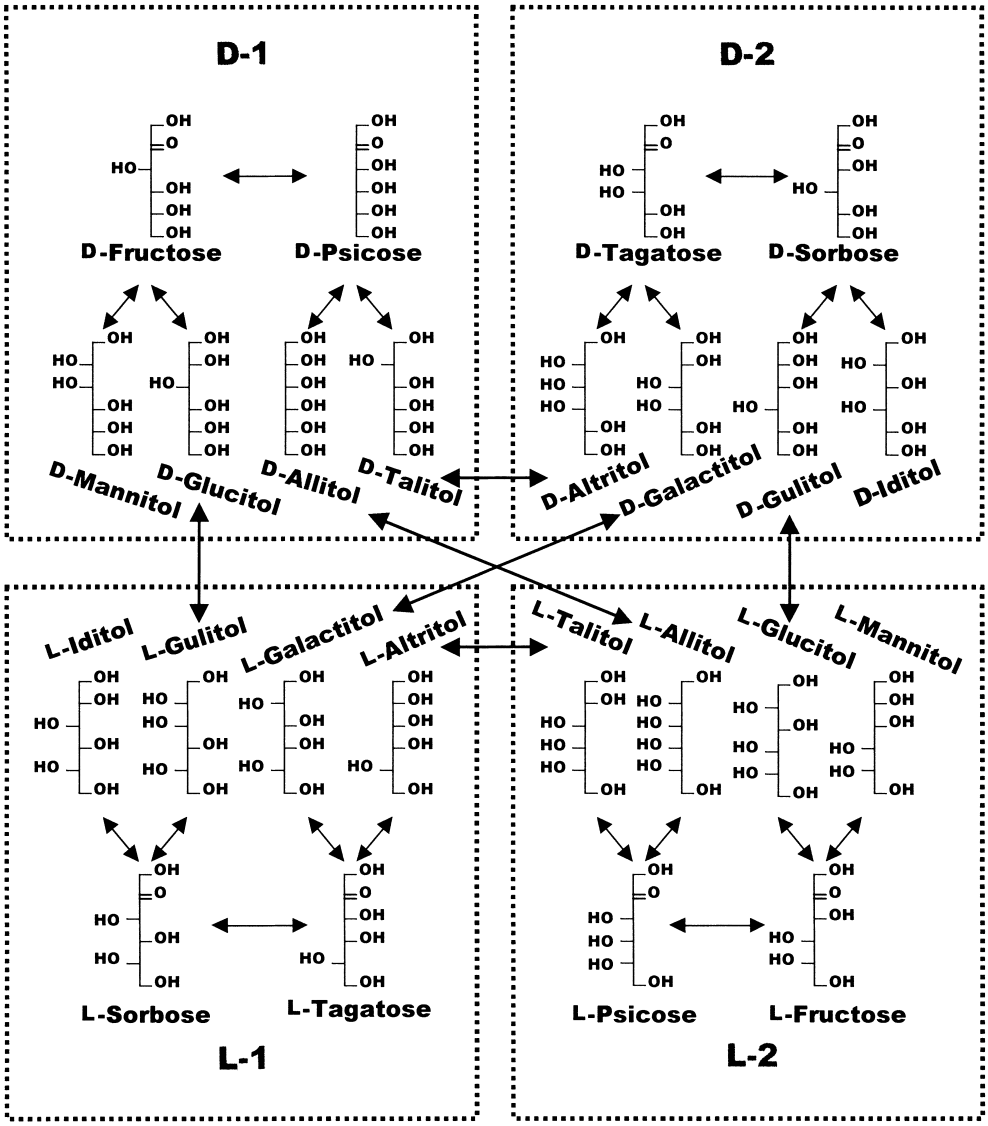

FIG. 1. Arrangement and interconnections between ketohexoses and hexitols. Ketohexoses and hexitols were divided into four groups, D-1, D-2, L-1, and L-2 and the groups were linked with six hexitols. One group consisted of two ketohexoses and four hexitols. Four epimerization reactions and 16 oxidoreductase reactions are shown by arrows. The six arrows between the four groups are bridges connecting them with two identical hexitols.

merization reactions between ketohexoses and 16 oxidoreductase reactions to ketohexoses and hexitols. We can classify eight ketohexoses into four groups depending on the epimerase reaction. A ketohexose is reduced by two oxidoreductases at the C-2 position producing two different hexitols. The hexitols produced from the eight ketohexoses are also classified into four corresponding groups depending on the ketoses used as substrates. The groups D-1 and D-2 contain all the D-series ketohexoses and hexitols. Consequently, the groups L-1 and L-2 contain L-series ketohexoses and hexitols (Fig. 1). Although two ketohexoses and four hexitols in each group are directly linked with D-tagatose 3-epimerase and oxidoreductase, it seems that these groups, D-1, D-2, L-1, and L-2, are completely independent. However, if we superimpose D-glucitol on L-gulitol when the latter is rotated 180° in the plane of the paper, we see that these two hexitols are identical to each other. Therefore, it was concluded that there are definite bridges between the four groups. Group D-1 is connected directly with group L-1 via D-glucitol (L-gulitol). Similarly, D-allitol, D-talitol, D-galactitol, D-gulitol, and L-talitol are identical to L-allitol, D-altritol, L-galactitol, L-glucitol, and L-altritol, respectively (Fig. 1).

Construction of the ketohexose ring was carried out in the following way. A scheme was drawn for the production of ketohexoses based upon the network composed of the four groups comprising all of the ketohexoses and hexitols mentioned above. The main idea of this strategy was to link production of all the ketohexoses and hexitols using D-tagatose 3-epimerase and oxidoreductases. As shown in Fig. 2 (the bottom ring), a circular map was drawn in the form of a ring based on a cycle composed of eight ketohexoses and four hexitols connected by the two enzymes. The ring is divided into three different vertical zones illustrated with different colors, *i.e.*, L-zone (pink), DL-zone (yellow), and D-zone (blue). The point of symmetry of D- and L-sugars is located at the center of the ring. The D-series and L-series compounds are placed in the D- and L-zone, respectively. Those compounds that have both D- and L-symmetry belong to the DL-zone in the center of the ring. The ring was composed of D-glucitol (L-gulitol) → D-fructose → D-psicose → D-talitol (D-altritol) → D-tagatose → D-sorbose → D-gulitol (L-glucitol) → L-fructose → L-psicose → L-talitol (L-altritol) → L-tagatose → L-sorbose → L-gulitol (D-glucitol). Galactitol (D-,L-galactitol) and allitol (D-,L-allitol) are placed at the center of the ring because both of these hexitols are optically inactive among the 18 compounds (8 ketohexoses and 10 hexitols). The remaining four hexitols, D-mannitol, D-iditol, L-mannitol, and L-iditol, are placed at the outer ring. Furthermore, aldohexoses can be converted to the corresponding ketohexoses by an aldose isomerase reaction, and also converted to the corresponding hexitols by a hydrogenation reaction. Accordingly, all 16 aldohexoses can be connected with ketohexoses and hexitols in the Izumoring. We can draw a symmetric Izumoring comprising of 16 aldohexoses, 8 ketohexoses, and 10 hexitols (Fig. 2, the biggest ring). All the compounds are connected to each other by enzyme reactions or by hydrogenation reactions as illustrated in Fig. 2. This symmetric ring was named by a student in our laboratory as an Izumoring. This Izumoring is not a metabolic scheme like the Krebs TCA cycle *in vivo*, but rather a complete design for the bioproduction of ketohexoses and hexitols as well as aldohexoses.

This ring scheme reveals the interesting fact that there are four entrances to transfer into the L-hexose configuration from the D-hexose configuration. Figure 2 shows that these entrance hexitols that belong to the D-,L-series area are D-glucitol (L-gulitol), galactitol (D-,L-galactitol), allitol (D-,L-allitol), and D-gulitol (L-glucitol). Four L-series ketohexoses are produced from these four hexitols. Some of these L-series sugars are particularly interesting. For example, L-sorbose is used in the industrial production of vitamin C, whereas L-galactose was found to act as an intermediate in the biosynthetic pathway for vitamin C in higher plants (21). Galactitol (D-,L-galactitol) can be used to produce L-tagatose or D-tagatose, the latter being an increasingly attractive sweetener for the food industry. Earlier, Shimonishi *et al*. (12) reported the production of L-tagatose using *Klebsiella pneumoniae* 40b, whereas Huwig *et al*. (22) used galactitol dehydrogenase enzyme from *Rhodobacter spaeroides*. L-Psicose is produced from allitol (D-,L-allitol) and it is studied for its advantageous properties in the medical field. Takeshita *et al*. (23) succeeded in the production of L-psicose using *Gluconobacter frateurii* IFO 3254.

A ketohexose synthesis strategy using the Izumoring clearly suggests a way for designing a production process for any ketohexose from D-fructose that can be produced from D-glucose. For example, L-fructose has some interesting properties as a source of energy (24, 25) and as an inhibitor for glycoproteins (26). Following the route of the Izumoring from D-fructose to L-fructose we can facilitate the production method for L-fructose via four reactions. The first step is epimerization of D-fructose to D-psicose by D-tagatose 3-epimerase (7, 8). The second step is reduction of D-psicose to allitol (D-,L-allitol) by oxidoreductase (14, 27). The third reaction is transformation of allitol (D-,L-allitol) to L-psicose by oxidoreductase (23). The final step is epimerization of L-psicose to L-fructose by D-tagatose 3-epimerase (10).

IV. IZUMORINGS FOR PENTOSES AND TETROSES

The construction of Izumorings for pentoses and tetroses follows the same pattern as that for hexoses (Fig. 2, the top ring and middle one). In the case of pentoses there are eight aldopentoses, four ketopentoses, and four pentitols. The entrance from a D-pentose configuration to an L-pentose configuration occurs through xylitol (D,L-xylitol) and ribitol (D,L-ribitol). D-Xylose can be used as a starting material for the synthesis of all pentoses. D-Xylose reductase that reduces D-xylose into xylitol has been purified from *Candida guilliermondii* by Granström *et al*. (28). It was shown to reduce D- and L-forms of arabinose, ribose and lyxose, in addition to D,L-xylose. Bhuiyan *et al*. (29) reported the preparation of L-lyxose from ribitol using microbial and enzymatic reactions. First ribitol was oxidized to L-ribulose by *Acetobacter acetii* IFO 3281. L-Ribulose was epimerized to L-xylulose by D-tagatose 3-epimerase and finally isomerized to L-lyxose by L-rhamnose isomerase. Kylmä *et al*. (30) was

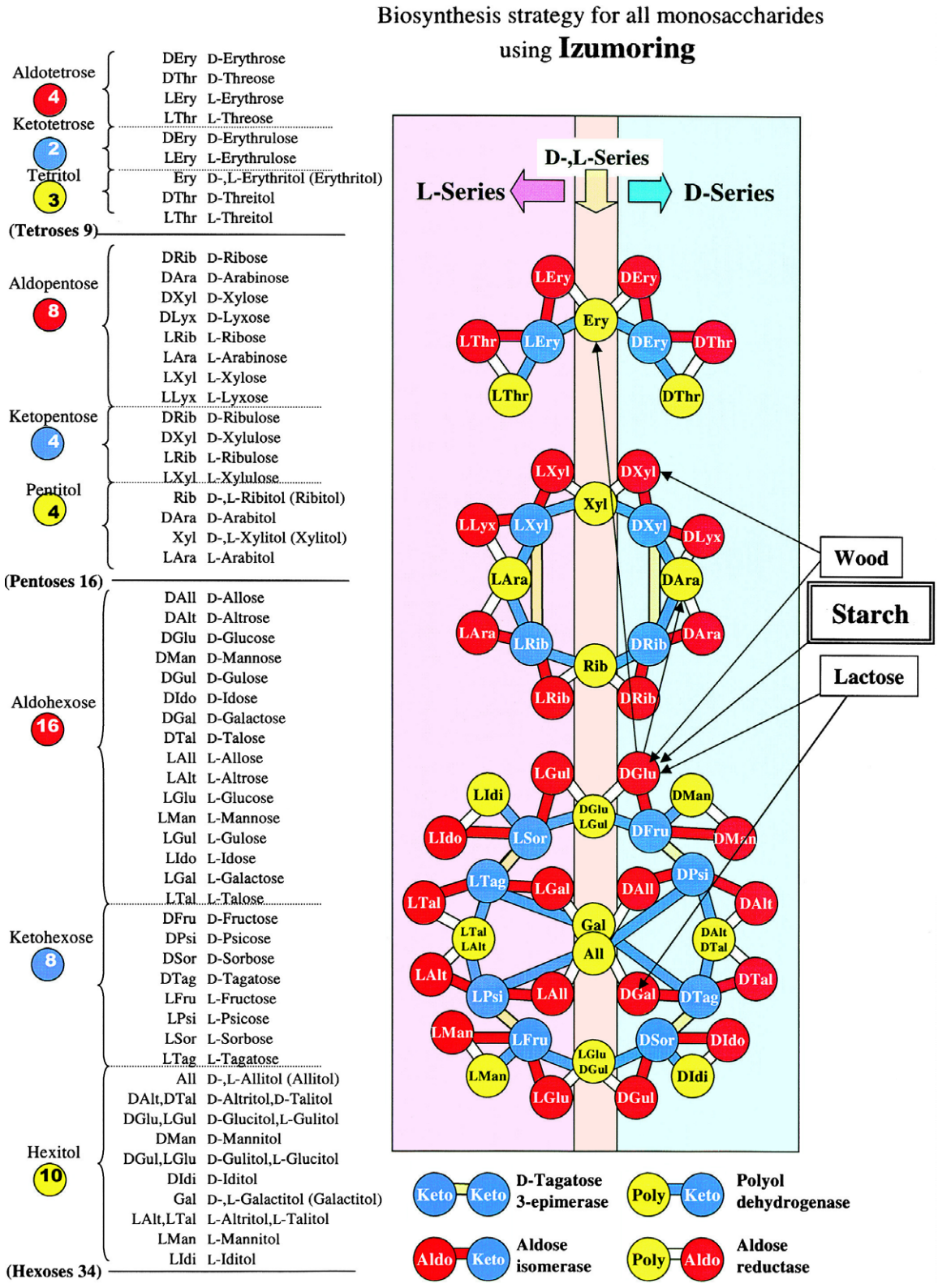

FIG. 2. Izumorings for tetroses, pentoses and hexoses are presented in tree form. The D-, DL-, and L-sugars are divided in the three different vertical zones. The Izumoring tree illustrates the strategy to design the production processes of all monosaccharides from readily available raw materials; starch, wood and lactose.

able to determine a specific production rate of 1.2 $g \cdot g^{-1} \cdot h^{-1}$ in the bioreactor for L-ribulose conversion from ribitol using resting cells of *A. acetii* IFO 3281.

The Izumoring for tetroses consists of four aldotetroses, two ketotetroses, and three tetritols. The entry point from a D-configuration to an L-configuration goes through erythritol. Erythritol is currently used as a bulk sweetener in low calorie foods such as cookies, candies, and yoghurts. Mizanur *et al*. (31) used erythritol as a starting material for preparing the rare and expensive aldotetrose L-erythrose. Erythritol was first converted to the ketotetrose L-erythrulose by *Gluconobacter frateurii* IFO 3254 and then isomerized to L-erythrose with L-ribose isomerase enzyme. Vuolanto *et al*. (32) showed that D-xylose isomerase is able to isomerase and epimerize all the different D- and L-tetroses.

V. ALTERNATIVE SWEETENER — XYLITOL

Xylitol, a rare sugar, is industrially produced by the chemical reduction of pure D-xylose, obtained from hard-wood hydrolysates, in the presence of a Raney nickel catalyst (Finnish patent no. 589388). This process requires several purification steps because only pure D-xylose can be used for chemical reduction (33). Xylitol production with yeasts has been studied extensively as an alternative to the chemical reduction process (34, 35). In fully aerobic conditions D-xylose is used for cell respiration and biomass growth, but under oxygen limited conditions the yeast cells produce xylitol. This is due to the fact that in these conditions the intracellular amount of NADH is elevated due to insufficient respiration. Consequently, NADH acts as an inhibitor of xylitol dehydrogenase, redirecting xylose flux into xylitol (Fig. 3). Computer simulations and *in vitro* enzyme assays have shown that NAD(P)H and ATP are regenerated through intracellular substrate cycling and ethanol/glycerol production in order to compensate for increased demand of these cofactors under oxygen limited conditions (36, 37).

Xylitol is one of most significant alternatives to conventional sweeteners. It is mainly used as an additive in commercial products such as chewing gums, mints, sweets, and tooth paste. Xylitol has an insulin independent metabolism, and it can prevent dental caries and acute otitis media in children (3, 4). In 2002, its worldwide annual production was estimated to be about 15,000 t. The advantage of using whole cells instead of chemical reduction in xylitol production lies in the fact that crude industrial side streams could be used as a raw material. Even though part of the D-xylose goes into cell maintenance metabolism, the xylitol yield is significantly higher in the biotechnological process than in chemical reduction.

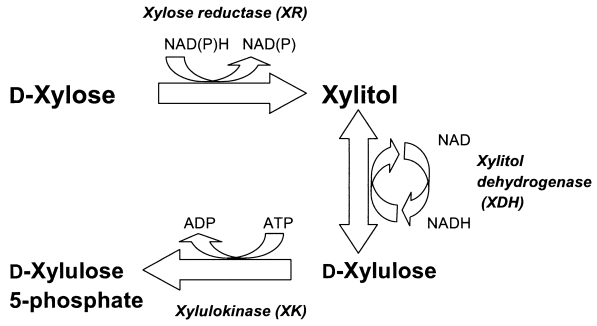

FIG. 3. The first three enzymes of the D-xylose metabolic pathway in yeast. NAD(P)H–dependent xylose reductase (XR) reduces D-xylose into xylitol. Consequently, xylitol is oxidized to D-xylulose by NAD-dependent xylitol dehydrogenase (XDH). Finally, D-xylulose is phosphorylated into D-xylulose 5-phosphate by xylulokinase (XK). From there D-xylulose 5-phosphate enters the pentose phosphate pathway (PPP).

VI. FUTURE OF RARE SUGARS

The production methods for various rare sugars require multidiscipline approaches that include fermentation technology, molecular biology, enzyme technology, and organic chemistry. The first task was the construction of the three Izumorings, following which the key biocatalysts have been isolated and purified (38). Next, we are concentrating on developing methods for the mass production of rare sugars from readily available raw materials such as starch, hemicellulosic waste, and whey. Eventually, our aim is to make all of the rare sugars included in the three Izumorings available for research purposes. In the future, we expect to find as of yet unidentified novel characteristics from these rare sugars which will have a profound effect on the everyday life and health of people. Currently, low caloric alternatives to the present dominant sweeteners such as sugar, and HFCS whose effects on human health have been questioned, are being sought. Rare sugars, such as D-psicose, which we can easily produce from D-fructose using D-tagatose 3-epimerase, will most definitely have a role in this development. In addition, various novel physiological functions of rare sugars have been discovered. For example, D-allose has a potent inhibitory effect on the production of reactive oxygen species (39), and thus, may be used for medical purposes. The potential for a wide range of applications is a characteristic of rare sugars.

ACKNOWLEDGMENTS

The staff of the Laboratory of Applied Enzymology at Kagawa University is appreciated for their constructive comments; without their long-term cooperation and help, this strategy could not have been developed. We would like to express our sincerest gratitude to Prof. Matti Leisola of the Helsinki University of Technology for his vision and long term collaboration with our laboratory. M. Sato and T. Miyake are thanked for their helpful discussions. This study was supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

1. **Knapp, R.:** Sugar: world markets and trade. Circular Series FS 2–02. Foreign Agricultural Service, US Department of Agriculture, Washington, D.C. (2002).
2. **Jensen, V. J. and Rugh, S.:** Industrial-scale production and application of immobilized glucose isomerase. Methods Enzymol., **136**, 356–370 (1987).
3. **Mäkinen, K. K.:** Dietary prevention of dental caries by xylitol — clinical effectiveness and safety. J. Appl. Nutr., **44**, 16–28 (1992).

4. **Uhari, M., Kontiokari, T., Koskela, M., and Niemelä, M.:** Xylitol chewing gum in prevention of acute otitis media: double blind randomised trial. Br. Med. J., **313**, 1180–1184 (1996).
5. **Itoh, H., Okaya, H., Khan, A. R., Tajima, S., Hayakawa, S., and Izumori, K.:** Purification and characterization of D-tagatose 3-epimerase from *Pseudomonas* sp. ST-24. Biosci. Biotech. Biochem., **58**, 2168–2171 (1994).
6. **Ishida, Y., Kamiya, T., Itoh, H., Kimura, Y., and Izumori, K.:** Cloning and characterization of the D-tagatose 3-epimerase gene from *Pseudomonas cichorii* ST-24. J. Ferment. Bioeng., **83**, 529–534 (1997).
7. **Itoh, H., Sato, T., and Izumori, K.:** Preparation of D-psicose from D-fructose by immobilized D-tagatose 3-epimerase. J. Ferment. Bioeng., **80**, 101–103 (1995).
8. **Takeshita, K., Suga, A., Takada, G., and Izumori, K.:** Mass production of D-psicose from D-fructose by a continuous bioreactor system using immobilized D-tagatose 3-epimerase. J. Biosci. Bioeng., **90**, 453–455 (2000).
9. **Itoh, H., Sato, T., Takeuchi, T., Anisur, R. K., and Izumori, K.:** Preparation of D-sorbose from D-tagatose by immobilized D-tagatose 3-epimerase. J. Ferment. Bioeng., **79**, 184–185 (1995).
10. **Itoh, H. and Izumori, K.:** Enzymatic production of L-tagatose and L-fructose from L-sorbose and L-psicose, respectively. J. Ferment. Bioeng., **81**, 351–353 (1996).
11. **Izumori, K., Miyoshi, T., Tokuda, S., and Yamabe, K.:** Production of D-tagatose from dulcitol by *Arthrobacter globiformis*. Appl. Environ. Microbiol., **46**, 1055–1057 (1984).
12. **Shimonishi, T., Okumura, Y., and Izumori, K.:** Production of L-tagatose from galactitol by *Klebsiella pneumoniae* strain 40b. J. Ferment. Bioeng., **79**, 620–622 (1995).
13. **Muniruzzaman, S., Kobayashi, H., and Izumori, K.:** Production of D-talitol from D-tagatose by *Aureobasidium pullulans* strain 113B. J. Ferment. Bioeng., **78**, 346–350 (1994).
14. **Muniruzzaman, S., Tokunaga, H., and Izumori, K.:** Conversion of D-psicose to allitol by *Enterobacter agglomerans* strain 221e. J. Ferment. Bioeng., **79**, 323–327 (1995).
15. **Sasahara, H., Mine, M., and Izumori, K.:** Production of D-talitol from D-psicose by *Candida famata* R28. J. Ferment. Bioeng., **85**, 84–88 (1998).
16. **Sasahara, Y. and Izumori, K.:** Production of D-iditol from D-sorbose by *Rhodotorula rubra* RY10 isolated from miso paste. J. Biosci. Bioeng., **87**, 548–550 (1999).
17. **Bhuiyan, S. H., Itami, Y., and Izumori, K.:** Immobilisation of L-rhamnose isomerase and its application in L-mannose production from L-fructose. J. Ferment. Bioeng., **84**, 558–562 (1997).
18. **Bhuiyan, S. H., Itami, Y., Rokui, Y., Katayama, T., and Izumori, K.:** D-Allose production from D-psicose using immobilised L-rhamnose isomerase. J. Ferment. Bioeng., **85**, 539–541 (1998).
19. **Pastinen, O., Schoemaker, H. S., and Leisola, M.:** Xylose isomerase catalysed novel hexose epimerization. Biocatal. Biotransform., **17**, 393–400 (1999).
20. **Pastinen, O., Visuri, K., and Leisola, M.:** Xylitol purification by cross-linked glucose isomerase crystals. Biotechnol. Tech., **12**, 557–560 (1998).
21. **Wheeler, G. L., Jones, M. A., and Smirnoff, N.:** The biosynthetic pathway of vitamin C in higher plants. Nature, **393**, 365–369 (1998).
22. **Huwig, A., Emmel, S., Jkel, G., and Glifforn, F.:** Enzymatic synthesis of L-tagatose from galactitol with galactitol dehydrogenase from *Rhodobacter spaeroides*. Carbohydr. Res., **305**, 337–339 (1998).
23. **Takeshita, K., Shimonishi, T., and Izumori, K.:** Production of L-psicose from allitol by *Gluconobacter frateurii* IFO 3254. J. Ferment. Bioeng., **81**, 212–215 (1996).
24. **Klein, M., Hoffmann, L., Block, H., and Herrmann, U.:** Investigation of energetic utilization of L-fructose in rats. Nahrung, **37**, 305–320 (1993).
25. **Livesey, G. and Brown, J. C.:** Whole body metabolism is not restricted to D-sugars because energy metabolism of L-sugars fits a computational model in rats. J. Nutr., **125**, 3020–3029 (1995).
26. **Muniruzzaman, S., Pan, Y. T., Zeng, Y., Atkins, B., Izumori, K., and Elbein, A. D.:** Inhibition of glycoprotein processing by L-fructose and L-xylulose. Glycobiology, **6**, 795–803 (1996).
27. **Takeshita, K., Ishida, Y., Takada, G., and Izumori, K.:** Direct production of allitol from D-fructose by a coupling reaction using D-tagatose 3-epimerase, ribitol dehydrogenase and formate dehydrogenase. J. Biosci. Bioeng., **90**, 545–548 (2000).
28. **Granström, T., Wu, X., Airaksinen, U., and Leisola, M.:** *Candida guilliermondii* grows on rare pentoses — implications on production of pure xylitol. Biotechnol. Lett., **24**, 507–510 (2002).
29. **Bhuiyan, S. H., Ahmed, Z., Utamura, M., and Izumori, K.:** A new method for the production of L-lyxose from ribitol using microbial and enzymatic reactions. J. Ferment. Bioeng., **86**, 513–516 (1998).
30. **Kylmä, A. K., Granström, T., and Leisola, M.:** Growth characteristics and oxidative capacity of *Acetobacter acetii* IFO 3281: implications for L-ribulose production. Appl. Microbiol. Biotechnol., **63**, 584–591 (2004) .
31. **Mizanur, R. M. D., Takeshita, K., Moshino, H., Takada, G., and Izumori, K.:** Production of L-erythrose via L-erythrulose from erythritol using microbial and enzymatic reaction. J. Biosci. Bioeng., **92**, 237–241 (2001).
32. **Vuolanto, A., Pastinen, O., Schoemaker, H., and Leisola, M.:** C-2 Epimer formation of tetrose, pentose and hexose sugars by xylose isomerase. Biocatal. Biotrans., **20**, 235–240 (2002).
33. **Härkönen, M. and Nuojua, P.:** Eri tekijöiden vaikutus ksyloosin katalyyttiseen hydraukseen ksylitoliksi, osa 1 (The effects of different factors on catalytic hydration of xylose into xylitol, part 1). Kemia–Kemi, **6**, 445–447 (1979).
34. **Lachke, A. H. and Jeffries, T. W.:** Levels of the enzymes of the pentose phosphate pathway in *Pachysolen tannophilus* Y-2460 and selected mutants. Enzyme Microb. Technol., **8**, 353–359 (1986).
35. **Granström, T., Ojamo, H., and Leisola, M.:** Chemostat study of xylitol production by *Candida guilliermondii*. Appl. Microbiol. Biotechnol., **55**, 36–42 (2001).
36. **Granström, T., Aristidou, A. A., and Leisola, M.:** Metabolic flux analysis of *Candida tropicalis* growing on xylose in oxygen limited chemostat. Metab. Eng., **4**, 248–256 (2002).
37. **Granström, T. and Leisola, M.:** Controlled transient changes reveal differences in metabolite production in two *Candida* yeasts. Appl. Microbiol. Biotechnol., **58**, 511–516 (2002).
38. **Izumori, K.:** Bioproduction strategies for rare hexoses. Naturwissenschaften, **89**, 120–124 (2002).
39. **Murata, A., Sekiya, K., Watanabe, Y., Yamaguchi, F., Hatano, N., Izumori, K., and Tokuda, M.:** A novel inhibitory effect of D-allose on production of reactive oxygen species from neutrophils. J. Biosci. Bioeng., **96**, 89–91 (2003).