특집 : 새로운 감미료의 산업적 이용과 전망

Production of Rare Monosaccharides Using Microorganisms and Their Enzymes

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Abstract

Microbial or enzymatical methods are suitable for production of rare monosaccharides. Using oxidation and reduction ability of microorganisms, various rare ketoses and polyols can be produced, for example D-tagatose from galactitol by *Enterobacter agglomerans* strain 221e, L-tagatose from galactitol by *Klebsiella pneumoniae* strain 40b, L-psicose from allitol by *Gluconobacter frateurii* IFO 3254, D-talitol from D-tagatose by *Aureobasidium pullulans* strain 113B, allitol from D-psicose by *Enterobacter agglomerans* strain 221e and so on. We can produce various rare aldoses and ketoses using aldose isomerases, for example L-galactose from L-tagatose by D-arabinose isomerase, and L-ribose from L-ribulose by L-ribose isomerase, and so on. D-Tagatose 3-epimerase of *Pseudomonas* sp. ST-24 is very useful for preparation of various rare ketoses, for example D-psicose from D-fructose, D-sorbose from D-tagatose, L-fructose from L-psicose and so on. Using polyol dehydrogenases, aldose isomerases and D-tagatose 3-epimerase, we can design the suitable pathway for production of a certain rare monosaccharide from a suitable substrate.

INTRODUCTION

We can classify monosaccharides into two groups, the first one contains natural type monosaccharides which are widely distributed in nature, for example D-glucose, D-fructose, D-mannose, D-galactose, D-sorbitol and so on, and the second one contains rare type monosaccharides which are not abundant in nature, for example L-glucose, D-psicose, L-galactose, D-gulose, allitol and so on. Rare sugars are very expensive and most of them are unavailable as the commercial product. So, it is almost impossible for us to obtain enough amount of rare monosaccharides even for laboratory experiments. Accordingly, few studies have been done on utilization of rare monosaccharides.

Using microbial or enzymatical process to produce rare monosaccharides is more effective than that of chemical ones. Because usually, in the chemical reaction, the yield of the product is low, the by-products which are impurities often found in the product, and it needs many steps for completion of reactions. On the other hand, microbial or enzymatical reactions are suitable for the production of rare sugars, in which the yield is high and there is a few by-products in the reaction product.

To produce enough amount of rare monosaccharides, we have to use natural substrates which are abundant in nature as starting materials. This means, in the first step of the study, we are devoted to find the enzyme that is active on natural monosaccharides producing rare products. After obtaining a rare sugar from a natural one, we can transform the rare sugar to the other new one. There are two types of the enzymes that we can use for production of rare monosaccharides. The first type belongs to the enzymes which have wide substrate specificity on various carbohydrates having similar structures as its real substrates of the enzyme. One of them is the ribitol dehydrogenase of *Enterobacter agglomerans* strain 221e that was isolated from soil(1). The ribitol dehydrogenase is active on many polyols; ribitol, allitol,

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xylitol, L-sorbitol, L-iditol, galactitol and so on. When galactitol, which is easily prepared by reduction of D-galactose, is added to the enzyme reaction mixture containing the ribitol dehydrogenase, galactitol is oxidized to D-tagatose that is a rare ketohexose by the polyol dehydrogenase. The second one is the enzymes whose real substrate is a rare sugar. One of them is D-tagatose 3-epimerase that was found in *Pseudomonas* sp. ST-24(2). The epimerase is active on a rare D-tagatose producing D-sorbose that is another rare ketohexose. The meaning of the existence of this enzyme whose real substrate is a rare sugar, D-tagatose, is still lin mystery.

We have been studying on the production of various rare monosaccharides using oxidation reduction activities of microorganisms, aldose isomerases and D-tagatose 3-epimerase. A few studies on rare monosaccharide production using microbial or enzymatical methods have been done so far. So, in this mini review, we introduce some results done in our laboratory.

PRODUCTION OF RARE KETOSES AND POLYOLS USING OXIDATION AND REDUCTION ABILITY OF MICROORGANISMS

Polyol dehydrogenases catalyze oxidation of a polyol to a ketose and reduction of a ketose to a polyol at the C-2 position requiring NAD(P) or NAD(P)H as a coenzyme, respectively. Usually, we have to use intact cells for production of polyols and ketoses using polyol dehydrogenases. The most famous production of a ketose from a polyol using oxidation activity of a microorganism is "L-sorbose fermentation"(3), and L-sorbose, which is prepared from D-sorbitol by intact cell reaction of *Gluconobacter suboxydans*, is raw materials for industrial production of vitamin C. The same method used for L-sorbose fermentation is useful for production of rare ketoses from polyols. The reversible reaction, reduction of ketoses to polyols, can be used to produce rare polyols from rare ketoses.

1. D-Tagatose production from galactitol by *Enterobacter agglomerans* strain 221e

The first report of the microbial production of D-tagatose from galactitol was made in 1984 from our laboratory using *Arthrobacter globiformis*(4). Fig. 1 shows the reaction of the D-tagatose production from galactitol. The same ability of oxidation of galactitol to D-tagatose was also found in *Mycobacterium smegmatis* grown on L-sorbose(5). However, the drawbacks of these reports were requirement of long reaction time for the product formation and the consumption of the product when a low concentration of substrate was used. We tried to isolate a potent D-tagatose producer which requires considerably shorter reaction time and converts high concentration of substrate. An organism that can convert galactitol to D-tagatose was isolated from soil and identified as *Enterobacter agglomerans* strain 221e(6). The strain had very high activity to convert galactitol to D-tagatose when the cells were grown on 1% glycerol and 1% erythritol. The conversion rate was about 92% when 2% galactitol was used. The sodium alginate-immobilized cells and cells kept at -20°C also showed high conversion activity. We tried to find the polyol dehydrogenase which is working on oxidation of galactitol and found the ribitol dehydrogenase(1). As mentioned in the introduction, the ribitol dehydrogenase of the *Enterobacter agglomerans* strain 221e has wide substrate specificity. The ribitol dehydrogenase is active on many polyols; ribitol, allitol, xylitol, L-sorbitol, L-iditol, galactitol and so on. As the microbe has no activity of metabolizing galactitol and D-tagatose, the conversion rate was high.

Galactitol used as substrates for the production of D-tagatose was prepared from lactose(7). *Mycobacterium smegmatis* SMDU produced galactitol from D-galactose in a cultivation medium containing 1% tryptic soy broth and 1% substrate. The transformation rate was highest (70% or more) in the presence of D-glucose as a carbon

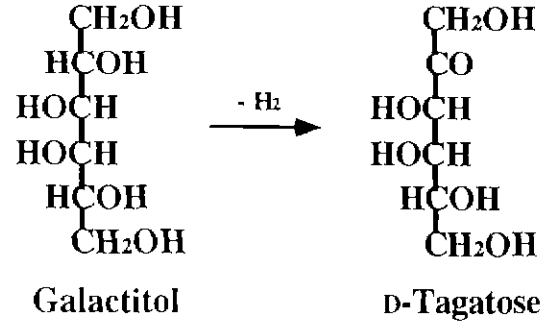

Fig. 1. Oxidation of galactitol to D-tagatose.

source. Similar transformation results were obtained when β-D-galactosidase treated whey was used in the medium instead of D-glucose and D-galactose. After lactose is split by β-D-galactosidase, whey can be used directly without further separation or purification for the production of galactitol.

2. L-Tagatose production from galactitol by *Klebsiella pneumoniae* strain 40b

An organism isolated from soil, *Klebsiella pneumoniae* strain 40b, was found to convert galactitol to L-tagatose(8). The cells grown on 1% xylitol showed maximum conversion activity and the addition of glycerol to the reaction mixture accelerated the conversion rate. The conversion rates were about 90%, 80% and 70% with 0.5%, 1.0% and 1.5% galactitol concentration, respectively. Since the strain does not utilize galactitol and L-tagatose, almost 100% of galactitol consumed was converted to L-tagatose. This is the first report of production of L-tagatose from galactitol by microbial cells.

3. Allitol production from D-psicose by *Enterobacter agglomerans* strain 221e

Enterobacter agglomerans strain 221e which had ability to oxidize galactitol to D-tagatose transformed D-psicose to allitol at a faster rate in the presence of D-glucose in the reaction mixture when the entrance of oxygen was restricted(9). The transformation rates were about 97.0, 95 ad 62.5%, respectively when 0.5, 1.0 and 2.0% substrates were used. No consumption of substrate or product was observed in any case. We can prepare D-psicose, which was used as the substrate for the production of allitol, by D-tagatose 3-epimerase from D-fructose as mentioned below.

4. L-Psicose production from allitol by *Gluconobacter frateurii* IFO 3254

Another L-ketohexose, L-psicose, was found to be produced from allitol by *Gluconobacter frateurii* IFO 3254 (10). The transformation was carried out at 30°C with shaking using the washed cells and the cells grown on tryptic soy broth containing 1% glycerol were found to have the best conversion potential. The conversion rate was about 98% when 10% allitol was used. It seems that the same polyol dehydrogenase that is active on conversion of D-sorbitol to L-sorbose in L-sorbose fermentation is working on the oxidation of allitol to L-psicose.

5. D-Talitol production from D-tagatose by *Aureobasidium pullulans* strain 113B

D-Tagatose was reduced to D-talitol by a microbe, *Aureobasidium pullulans* strain 113B isolated from soil (11). The strain transformed D-tagatose to D-talitol at faster rate in the presence of glycerol in the reaction mixture. The transformation rates were 93.0, 72.0 and 68% respectively when 0.5, 1.0 and 2.0% substrate was used. Cells grown on D-glucose were found to have the most potential for obtaining maximum transformation. In a flask reaction, about 0.58g D-talitol crystals were recovered from 1g of D-tagatose after various product purification treatments.

As mentioned above, using oxidation and reduction ability of microorganisms, we can produce various rare ketoses and polyols.

PRODUCTION OF RARE ALDOSES AND KETOSES USING ALDOSE ISOMERASES

D-Xylose isomerase is active not only on D-xylose but also on D-glucose producing D-fructose which is the most sweet monosaccharide. Indeed, isomerization of the D-glucose syrup to an equilibrium mixture of D-glucose and D-fructose, known as high-fructose corn syrup(HFCS), is one of the most important industrial production of a sugar sweetener. HFCS is used as an economical substitute for sucrose particularly in soft drinks. However, various aldose isomerase are obtained so far in our laboratory which are active on rare monosaccharides. Thus, we can use these isomerases for the production of rare sugars.

D-Arabinose isomerase, which was obtained from a soil bacterium *Aerobacter aerogenes*, is active on L-galactose producing L-tagatose and the reaction is reversible. So, we can use this isomerase for production of a rare aldohexose, L-galactose from L-tagatose. In another study, *Pseudomonas* sp. strain LL172 was found to be constitutive for L-rhamnose isomerase that catalyzes

Table 1. General properties of L-ribose isomerase purified from *Acinetobacter calcoaceticus* strain DL-28

Optimum pH	9.0
pH stability	7.0~9.0
Optimum temperature	30°C
Thermal stability	<30°C
Substrates	L-ribose(*Km*=44mM), D-lyxose, D-talose, D-mannose, L-allose and L-gulose
Inducer	L-ribose[1)]
Molecular weight	
Gel filtration(Sephadex G-150)	120,000
SDS-PAGE	32,000
pI value	5.1

[1)]in case of parental strain

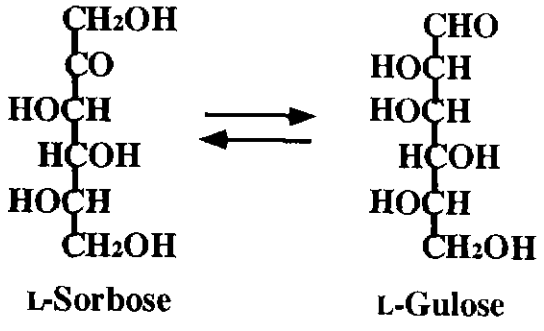

Fig. 2. Production of L-gulose from L-sorbose by L-ribose isomerase.

the reversible isomerization between D-allose and D-psicose. In the same way, we can prepare D-allose from D-psicose.

Recently, a new pentose isomerase, L-ribose isomerase, was found in *Acinetobacter* sp. strain DL-28(12). The general properties of the purified L-ribose isomerase are shown in Table 1. We can use this isomerase for production of L-ribose from L-ribulose, D-lyxose from D-xylulose, L-allose from L-psicose and L-gulose from L-sorbose. Fig. 2 shows the isomerization of L-sorbose to L-gulose.

In our laboratory, we are now in progress of the production of various rare monosaccharides using D-arabinose isomerase, L-rhamnose isomerase and L-ribose isomerase.

PRODUCTION OF RARE KETOSES USING D-TAGATOSE 3-EPIMERASE

D-Tagatose 3-epimerase was found accidentally in our laboratory during the course of the study on production of D-tagatose from galactitol. When a bacterium, *Pseudomonas* sp. ST-24, that had ability to transform galactitol to D-tagatose was incubated in the medium containing galactitol for a long time, a new product was recognized. The new product was isolated from the reaction mixture and determined as D-sorbose. We called the production of D-sorbose from galactitol as "D-sorbose fermentation"(13). D-Sorbose was produced not only from galactitol but also D-tagatose. The pathway of D-sorbose production from galactitol involved oxidation of galactitol to D-tagatose and epimerization of D-tagatose to D-sorbose. Finally, we found a new epimerase that is active on all ketohexoses and ketopentoses at C-3 position(2). This epimerase is the first enzyme that is active on free monosaccharides. The new epimerase was induced by D-tagatose, it had highest activity against D-tagatose and the Km value for D-tagatose was smallest. So, we named the epimerase as D-tagatose 3-epimerase. The general properties are shown in Table 2. The epimerase is very useful for production of rare ketoses.

1. D-Psicose production from D-fructose

D-tagatose 3-epimerase immobilized on Chitopearl beads of BCW 2503 was used in the preparation of D-psicose from D-fructose(14). When D-fructose solution (10%) was passed through a column packed with immobilized D-tagatose 3-epimerase about 20% of the D-fructose was converted to D-psicose. This column was continuously used for 10 days at 45°C and about 90g of D-psicose were obtained from D-fructose(500g). Through coupling with the immobilized epimerase and D-xylose isomerase, D-psicose could be prepared directly from D-glucose(Fig. 3).

Table 2. General properties of D-tagatose 3-epimerase purified from *Pseudomonas* sp. ST-24

Optimum pH	7.5
pH stability	7.0~11.0
Optimum temperature	60°C
Thermal stability	<60°C
Substrates	D-tagatose(*Km*=55mM), D-sorbose, D-psicose, D-fructose, L-tagatose, L-sorbose, L-psicose, L-fructose, D-ribulose, L-ribulose. D-xylulose, and L-xylulose
Inducer	L-tagatose
Molecular weight	
Gel filtration	68,000
SDS-PAGE	33,000
pI value	4.6

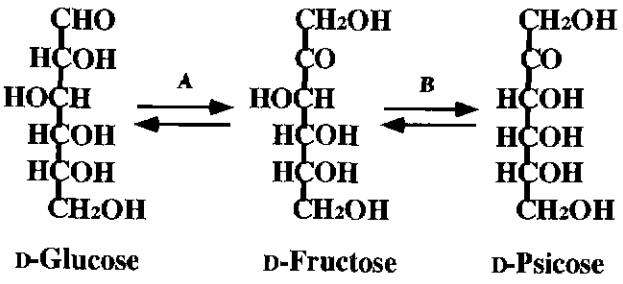

Fig. 3. Conversion of D-glucose to D-psicose using D-xylose isomerase and D-tagatose 3-epimerase.
A: D-xylose isomerase B: D-tagatose 3-epimerase

2. D-Sorbose production from D-tagatose

D-Tagatose prepared from galactitol by *Enterobacter agglomerans* strain 221e was used as a substrate of the immobilized epimerase producing D-Sorbose. In a high concentration(30%) of substrate, the reaction progressed without substrate inhibition. Two grams of D-sorbose crystals could be obtained from 3g D-tagatose. Furthermore, in a batch reaction repeated five times, about 70% of D-tagatose was converted to D-sorbose each time(15).

3. L-Fructose production from L-psicose and L- tagatose production from L-sorbose

In the same manner of the production of D-psicose and D-sorbose, L-tagatose and L-fructose was produced from L-sorbose and L-psicose, respectively. The production yield was 20% for L-tagatose from L-sorbose and 65% for L-fructose from L-psicose(16).

DISCUSSION

In this paper, we have described mainly on the production of rare hexoses done in our laboratory. However, polyol dehydrogenases, aldose isomerases and D-tagatose 3-epimerase are also active on pentoses. So, we can use the methods mentioned above for the production of rare pentoses.

As described in the introduction, to produce enough amount of rare monosaccharides, we have to use natural substrates which are abundant in nature and cheap as starting materials. D-Glucose from starch or D-galactose from lactose should be used as raw materials for the production of rare hexoses, and D-xylose from hemicellulose for the production of rare pentoses. Using polyol dehydrogenases, aldose isomerases and D-tagatose 3-epimerase, we can design the suitable pathway for production of a certain rare hexose from a suitable substrate (17). For instance, a pathway design of L-glucose synthesis from D-glucose is as follows: 1. Isomerization of D-glucose to D-fructose by an aldose isomerase, 2. Epimerization of D-fructose to D-psicose by the epimerase, 3. Reduction of D-psicose to allitol by a polyol dehydrogenase, 4. Oxidation of allitol to L-psicose by a polyol dehydrogenase. 5. Epimerization of L-psicose to L-fructose by the epimerase and 6. Isomerization of L-fructose to L-glucose by an aldose isomerase. Similar attempts can easily be made to design the pathway for synthesis of all other rare hexoses using polyol dehydrogenases, aldose isomerases and D-tagatose 3-epimerase.

A rare ketohexose, D-tagatose, is getting much attention as a low-calorie carbohydrate sweetener and bulking agent(18-20). In near future, other rare monosaccharides will be noticed not only as raw materials of natural products but also as low-calorie sweeteners and functional sweeteners.

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