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Fermentative Production of Polyols and Utilization for Food and Other Products in Japan

Takafumi KASUMI
Department of Applied Microbiology, National Food Research Institute
(Tsukuba, Ibaraki, 305 Japan)

Abstract
Polyols are widely distributed in nature, for example in lichens, mushrooms, fruits, lens semen, and some fermented foods such as wine, Japanese sake, or soy sauce. Among them glycerol, sorbitol and mannitol are industrially producted at present and used as chemical of food ingredients due to their specific characteristics. Erythritol, a C4-sugar alcohol, was considered to be used as ingredient of food, chemicals and pharmaceuticals. Moreover erythritol can be chemically synthesized or biochemically produced. However, since a mass production process of erythritol has not been development yet, the chemical, physical and physiological properties have not been studied thoroughly. In this paper polyol fermentation from as a substrate will be described, with emphasis placed on the screening and isolation of microorganisms which produce erythritol in high yield. Furthermore physicochemical and physiological properties of erythritol, and its utilization for food and other products will be discussed.

Discipline: Food/Biotechnology
Additional key words: erythritol, *Aureobasidium* sp.

Introduction

Polyols (sugar alcohols) are distributed in plants, animals, and microorganisms. Glycerol is normally present as lipid constituent, sorbitol (glucitol) is found in fruits such as strawberry, and mannitol is also present in various kinds of algae (Table 1). Polyols industrially produced at present include glycerol, xylitol, sorbitol, mannitol and some other specific compounds like maltitol or lactitol. Most of them except for glycerol are chemically converted from corresponding sugars by hydrogenation using metal catalyst such as raney-nickel, while glycerol is formed from by-products of soap or fatty acids. They are used as ingredients of chemicals, fire powder, resins, foods, pharmaceuticals, cosmetics, etc.

Erythritol is also one of the sugar alcohols derived from tetrose (C4 sugar). It is found in lichens, mushrooms, fruits, animal semen, lens, and some fermented foods such as wine, Japanese sake, or soy sauce derived from microbial metabolism. It was considered that erythritol could be utilized as food material as well as ingredient of chemicals, pharmaceuticals and medicines as in the case of the other polyols. However, since no method of mass-production of erythritol has been developed, the chemical, physical and physiological properties have not been studied thoroughly. There are two main methods for preparing erythritol: chemical synthesis and fermentative process. Erythritol can be prepared through the reduction of *meso*-tartarate, or oxidation of 4, 6-0-ethylidene-D-glucose. One of the disadvantages of these chemical procedures is that since the derivatives of organic acid or sugar must be used as starting materials, the process is complicated and expensive. On the other hand, the fermentative method seems more practical because the process is very simple, and a less expensive material such as glucose can be used as substrate. Some kinds of fungi and yeasts like *Moniliella*[1], *Trigonopsis*[5] or *Torulopsis*[5] were reported to produce and accumulate erythritol in the media containing glucose or sucrose. Especially, Torula yeast (*Moniliella tomentosa*) isolated by Hajny in 1964[1] was reported to give a 41% erythritol yield (conversion ratio of sugar consumed), and pilot-scale fermentation was investigated subsequently. However, the yield of

Table 1. Natural distribution of sugar alcohols (polyols)

Polyols		Distribution
Glycerol	CH_2OH $HCOH$ CH_2OH	Lipids, Plants, Insects, Fermented foods, distributed widely in nature
Erythritol	CH_2OH $HCOH$ $HCOH$ CH_2OH	Algae, Lichen, Fungi, Fruits, Fermented foods, Human urine, Semen
Sorbitol	CH_2OH $HCOH$ $HOCH$ $HCOH$ $HCOH$ CH_2OH	Fruits, Plants
Mannitol	CH_2OH $HOCH$ $HOCH$ $HCOH$ $HCOH$ CH_2OH	Algae, Bacteria, Fungi, Plants

erythritol and osmotolerance of yeast cells which are essential for industrial application, were still too low. Erythritol fermentation by using these microorganisms had not been developed practically.

Isolation, characterization and improvement of microorganisms producing erythritol

1) Screening and isolation of microorganisms

First of all, microorganisms were screened and isolated from various sources in Japan using agar plate containing 50% glucose, 0.3% yeast extract, 0.3% malt extract and 0.5% peptone. About 700 cultures of exudate yeast and more than 1,000 newly isolated yeast strains with a high glucose tolerance were then surveyed for polyol production in liquid media composed of 20–30% glucose, 0.5% yeast extract, 0.2% KH_2PO_4 and 0.1% urea (in case of exudate yeast) in shaking culture at 30°C for several days. Table 2 lists the exudate yeasts and newly isolated strains selected through the screening test described above[7]. More than 85% of the wild strains isolated produced arabinitol and glycerol, and only a few strains produced erythritol. Some of the exudate yeasts produced ca. 40% arabinitol and glycerol, but they could not grow on the media containing more than 20% glucose. Three strains selected from the new isolates arbitrarily designated as T-18, O-88 and T-115, respectively, could grow even in 30% glucose and produced a large amount of polyols. Main products were glycerol (T-18), arabinitol (O-88), and erythritol (T-115) as shown in Table 3. Among the erythritol-producing strains, T-115, a novel microorganism taxonomically identified as a species of *Aureobasidium* was found to be superior in terms of erythritol yield, osmotolerance and

Table 2. Polyol production by various microorganism

Microorganism	Polyols	Number of strains
Exudate yeast	Arabinitol	215
	Glycerol	120
	Mannitol	4
	Mannitol + Erythritol	2
Newly isolated strain	Arabinitol + Glycerol	28
	Glycerol + Mannitol	22
	Erythritol + Glycerol	4
	Arabinitol + Mannitol + Glycerol	3

Table 3. Polyol production by isolated yeast strains

Strain	Culture time (day)	Polyol yield (%)			
		Glycerol	Erythritol	Arabinitol	Mannitol
T-18	12	42, 4	–	–	23, 7
T-36	7	40, 3	6, 9	–	10, 3
T-39	11	21, 8	–	–	42, 4
O-88	14	5, 3	–	59, 5	–
T-113	7	2, 7	–	53, 6	–
T-115	8	3, 5	44, 2	–	–
T-124A	7	3, 9	43, 0	–	–
T-45	6	5, 2	34, 3	–	–

growth rate compared with the microorganisms hitherto reported to produce erythritol. Typical mycological and fermentative characteristics of *Aureobasidium* sp. are as follows[8]:

Vegetative cells; elliptic 4–7 × 4–15 μm,
Propagation; multipolar budding,
Mycelia; form true hyphae,
Streak culture; not glistening, color turns from yellowish-cream to brown with time,
Fermentative sugars; glucose, sucrose, maltose,
Assimilative sugars; glucose, sucrose, maltose, ribose,
Yield of erythritol; (conversion ratio of glucose consumed) 45% in 20% (W/W) glucose media, 37% in 30% (W/W) glucose media,
Optimum temperature and pH for erythritol production; 34–38°C, pH 5–6.

2) Improvement of wild strain

For large scale fermentation, improvement of some of the characteristics of this wild strain was still necessary. Especially, a vigorous foaming property was unsuitable for the aerobic culture conditions, and osmo-tolerance in media with a high glucose concentration was too low for practical application. Then attempts were made to improve *Aureobasidium* sp. by mutation through UV or gamma-ray irradiation or mutagen NTG (N-methyl-N'-nitro-N-nitrosoguanidine) treatment. The mutant strain obtained and shown in Plate 1 displayed

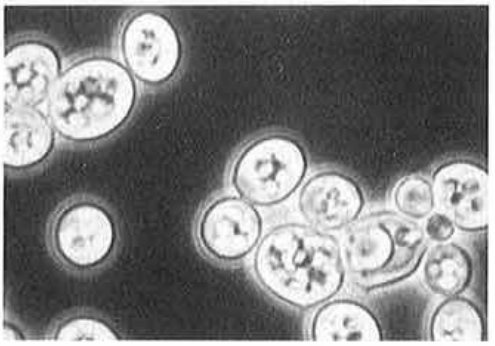

Plate 1. Photomicrograph of mutant strain of *Aureobasidium* sp.

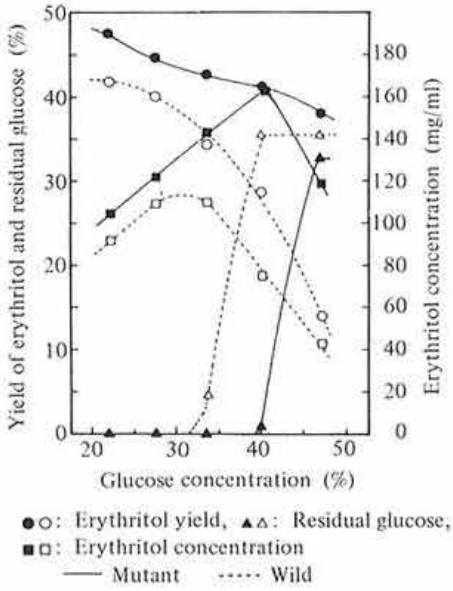

Fig. 1. Effect of glucose concentration on erythritol production

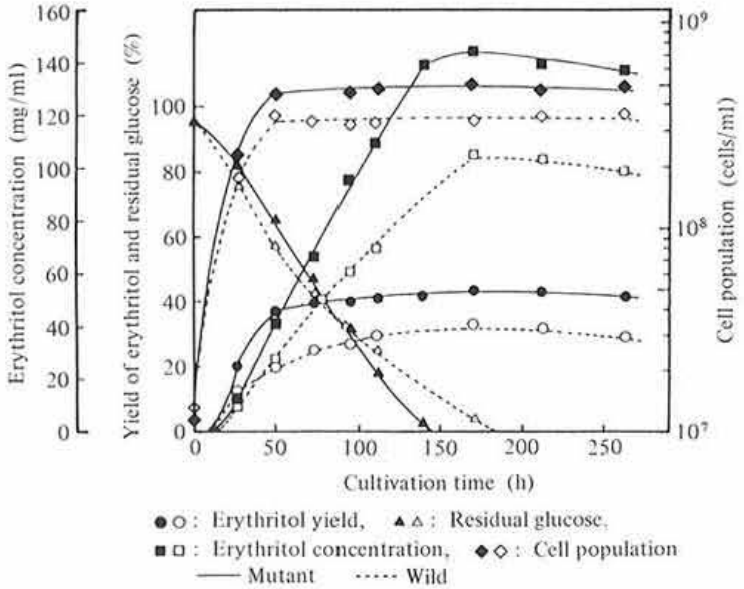

Fig. 2. Time course of cultivation

superior characteristics compared with the original one. Fig. 1 shows the effect of the glucose concentration on erythritol production by the wild and mutant strains in shaking culture. In the case of the wild strain, both yield and concentration (or productivity) of erythritol decreased when the initial glucose concentration in the liquid media exceeded 30%, whereas in the mutant strain the erythritol concentration became maximum even at a 40% glucose concentration. Fig. 2 illustrates the time course of cultivation. The erythritol yield of the mutant strain and the productivity were 10% and 20–30% higher than those of the wild strain, respectively. Some characteristics of the mutant strain of *Aureobasidium* sp. are as follows[2];

(a) Non-foaming under aeration and non-aggregative.
(b) High osmo-tolerance; fermentation occurs even in a saturated glucose solution (83.3%) and erythritol is produced.
(c) Conversion ratio of erythritol is 43–52% and production of erythritol is more than 2g/l in 40–50% glucose media.
(d) Only a small amount of glycerol is produced as a by-product, and under particular conditions no by-products are detected.
(e) Optimum fermentation temperature is 35–37°C, the highest temperature among the erythritol-producing microorganisms so far identified.

3) Pathway of erythritol fermentation

Erythrose is considered to be a precursor of erythritol. Erythrose-4-phosphate, subsequently converted to erythrose by a phosphatase, is produced through a coupled reaction in the Embden-Meyerhof-Parnas cycle and pentose phosphate cycle as indicated in Fig. 3. Erythrose is then reduced to erythritol by erythrose reductase. Erythrose reductase seems to be a key enzyme in erythritol fermentation and to be different from aldose reductase (EC 1.1.1.12) or polyol dehydrogenase so far identified based on the substrate specificity[3]. Aldose reductase was reported to display a relatively wide affinity for various aldoses, while erythrose reductase shows an affinity only for erythrose, glyceraldehyde and dihydroxyacetone as indicated in Table 4. Molecular weight, pI, optimum reaction temperature and pH of erythrose reductase from *Aureobasidium* sp. are 37,000, 4.8, 45°C and 6.5, respectively[3]. The oxidative activity was less than 0.1% of the reductive one.

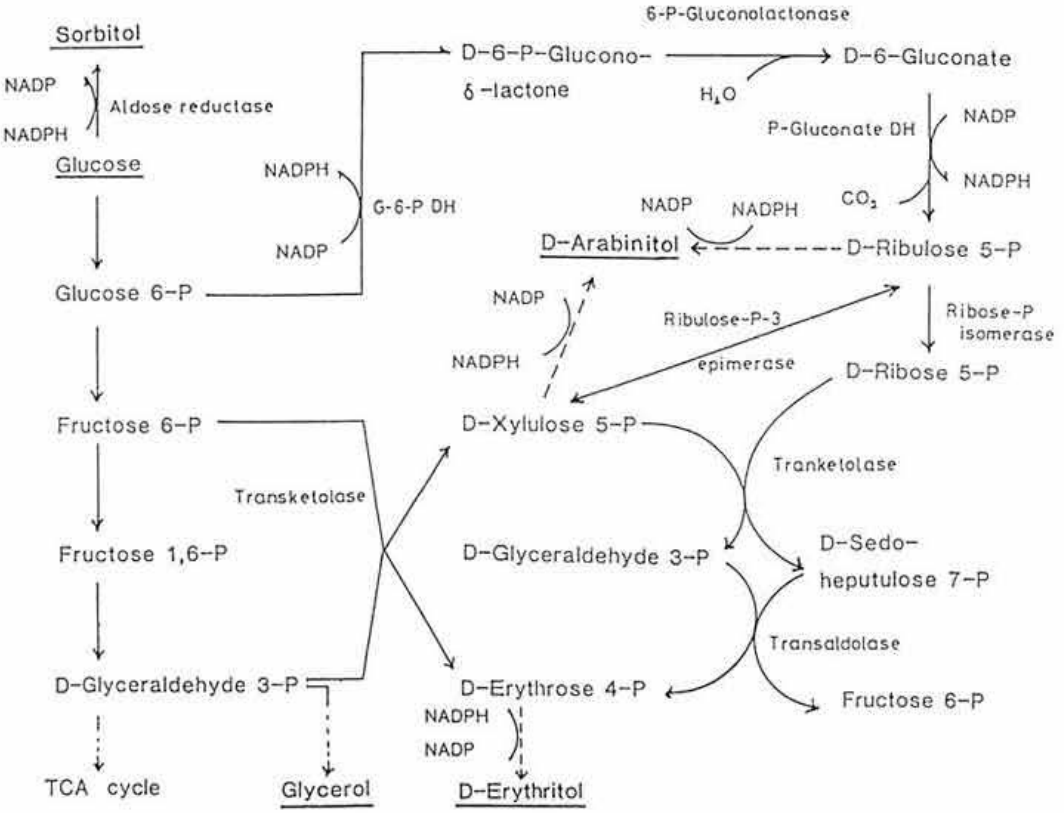

Fig. 3. Possible pathway of polyol formation

Table 4. Comparison of substrate specifity of sugar-oxidoreductases

	Erythrose reductase	Glycerol dehydrogenase	Aldose reductase	Polyol dehydrogenase
D-Erythrose	100	100	100	100
D-Glyceraldehyde	66	135	104	111
Dihydroxyacetone	20	5	–	0
D-Xylose	1	–	63	56
D-Ribose	1	7	59	24
D-Arabinose	0	3	34	0
D-Glucose	0	0.5	10	7

4) Large scale fermentation

Using this mutant strain, large scale fermentation in a 10 m^3 reactor tank was tested mainly from the point of view of cost reduction. Satisfactory results were obtained in terms of yield of erythritol, decrease of amount of coloring materials and by-products like glycerol or ethanol, aeration conditions in the reactor tank. Continuous fermentation of erythritol in a 3 l jar fermentor was also investigated. As a result, higher productivity (5.1 g/l h) and yield (ca. 50% conversion in 40% glucose media) were achieved over one month compared with batch fermentation. Industrial production of erythritol from glucose and related compounds was obtained and since the mass supply of erythritol became possible, the characteristics of erythritol could be studied in detail.

Characterization and utilization of erythritol

1) Physical and chemical properties

Erythritol is a fine crystal with a level of sweetness being 70–80% of that of sugar, which is comparable to that of sorbitol or maltitol. Plate 2 shows a photomicrograph of crystallized erythritol. Sweetness is plain and distinct because the after-taste is very weak, which is different from that of other non-sugar sweeteners such as steviosides or aspartame. Erythritol gives a chilly sensation in the mouth due to the development of an endoergonic reaction. Heat of solution is three times larger than that of sorbitol (Fig. 4). Erythritol is much less hygroscopic than sugar, showing almost no absorbency when preserved even at 90% relative humidity. It is so stable to heat treatment that no decomposition and colorization were observed at 200°C for 1 h.

2) Physiological properties

Absorption, metabolism and excretion of erythritol were examined by using rats. According to Oku et al.[6], erythritol is easily absorbed through the small intestine, and more than 90% is excreted intact into the urine in 24 h. As shown in Fig. 5, only a small portion of erythritol is excreted into the feces and the rest is transferred to the large intestine, where it is fermented by intestinal microorganisms and finally excreted as CO_2, CH_4, or short chain fatty

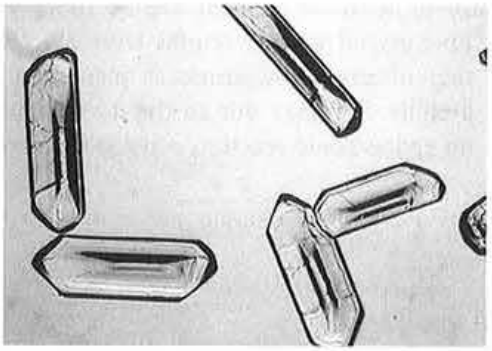

Plate 2. Photomicrograph of crystallyzed erythritol

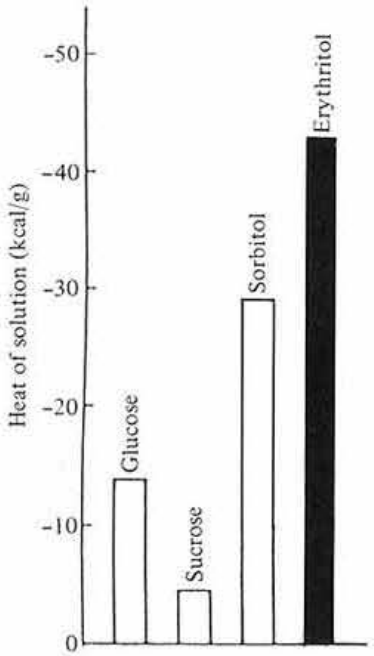

Fig. 4. Heat of solution of erythritol

acids. These results indicate that the energy value of erythritol is less than one tenth of that of digestible saccharides such as glucose or starch. Estimated energy values of polyols are listed on Table 5. Cariogenic properties were assessed by Kawanabe et al.[4]. Erythritol was not assimilated by the group of *Streptococcus mutans* and other oral mirobes which were considered to be the cause of dental caries, and consequently no insoluble glucan or lactic acid was formed.

Physical, chemical and physiological properties of erythritol hitherto determined are as follows:

(a) Fine crystal with sweetness level 75–80% of that of sugar (Sweetness is plain and gives a chilly sensation due to the development of an endoergonic reaction when it is dissolved; −180 J/g).

(b) Heat-stable (No decomposition and colorization occurred at 200°C for 1 h).

(c) More crystallizable and less hygroscopic than sucrose (No hygroscopicity observed at 90% R.H.).

(d) Extremely low energy value (Absorbed erythritol is rapidly excreted intact into the urine at the rate of 90% in 24 h. Energy value is estimated to be below 1.67 kJ/g, being much lower than that of other sugar alcohols such as sorbitol (12.5 kJ/g) and xylitol (15 kJ/g)).

(e) Non-toxic (LD_{50} Rat p.o is 13 g/kg, acute and chronic toxicities, and mutagenesis were not observed at all).

(f) Non cariogenic (Not assimilated by group of *Streptococcus mutans*).

(g) Weaker laxative action than that of other sugar alcohols like sorbitol and maltitol, which have been used as sweeteners.

(h) Sweetness-improving effect (Improves sweetness of stevioside or aspartame).

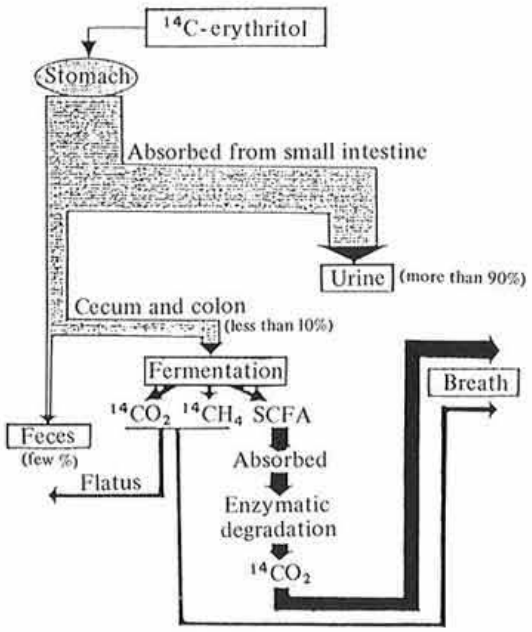

Fig. 5. Summary of metabolism and elimination of ^{14}C-erythritol

Table 5. Estimated energy values of polyols

Polyol	Nutrition board (Netherlands) kJ/g	(kcal/g)	Int. symposium[a] (Kyoto, Japan) kJ/g	(kcal/g)
Xylitol	15	(3.59)	=	(=)
Sorbitol	12.5	(2.99)	=	(=)
Mannitol	8.5	(2.03)	=	(=)
Maltitol	12	(2.87)	>8.37	(>2.0)
Lactitol	8.5	(2.03)	8.37	(2.0)
Erythritol	–	–	>1.67	(>0.4)

a): International symposium on caloric evaluation of carbohydrates, 1990.
–; No data, =; Not re-evaluated.

The properties described above are suitable for a new sweetener. Since erythritol is distributed in fruits, wine, Japanese sake and soy sauce in a range of 100–1,000 mg/l, it is absorbed by a large number of people both in Japan and in other countries. Several artificial low calorie sweeteners are presently used such as sodium saccharin, aspartame, stevioside, etc. Although the sweetness of these compounds is high compared with that of sucrose, their application is limited mainly due to their taste, low stability against acidic pH and heat. On the other hand, erythritol is a fairly stable new sweetener with physical properties (sweetness, crystallization, etc.) similar to those of sucrose and hardly absorbed in the human body.

Due to these outstanding properties, the utilization of erythritol is rapidly increasing in commercial food processing as a very low calorie and non-cariogenic sweetener for chocolate, tablet candy, chewing gum, cookies, soft drinks and table-top sweeteners. In addition, erythritol can be used for improving the taste of the artificial sweeteners previously described, for preparing less-hygroscopic foods and attenuating the stimulation of ethanol in distilled spirits.

In the chemical industry, erythritol could be used as material for certain kinds of resins as paints. Furthermore, pharmaceutical and medical products are now being developed using erythritol as the

ingredient. The utilization of erythritol in various fields is likely to increase as the characteristics of erythritol are being studied in more detail.

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