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Low Cariogenicity of Maltosyl-erythritol, Major Transglycosylation Product of Erythritol, by *Bacillus stearothermophilus* Maltogenic Amylase

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Abstract Maltosyl(G2)-erythritol, produced by the transglycosylation reaction of erythritol with maltotriose by *Bacillus stearothermophilus* maltogenic amylase, was not utilized either as a substrate for lactic acid production or for water-insoluble glucan synthesis. An inhibition assay of dextransucrase and mutansucrase showed that the dental caries suppression effect of G2-erythritol was greater than that of erythritol.

Key words: Maltosyl-erythritol, transglycosylation, *Streptococcus sobrinus*, *Streptococcus mutans*, dextransucrase, mutansucrase

The consumption of sucrose is one of the principal dietary factors involved in dental caries [19]. Mutans streptococci are one of the microorganisms responsible for dental caries, where cariogenicity depends on the availability of sucrose [2, 7, 16, 23]. An effective method for preventing dental caries is to substitute other sweetening substances for sucrose [12, 19]. Nonfermentability by oral microorganisms, nonacidogenicity, and the absence of glucan formation in *in vitro* rat caries tests are the criteria used to assess the noncariogenic property of a substance [22]. Erythritol is a four-carbon sugar alcohol, also referred to as a polyol as all its oxygen atoms are in hydroxyl groups. The sweetness of erythritol is about 65% of that of sucrose. However, erythritol is noncariogenic and nonacidogenic.

Transglycosylation has been used to improve certain characteristics of food carbohydrates. *Bacillus stearothermophilus* maltogenic amylase (BSMA) includes both hydrolytic activity and transglycosylation activity [1]. Maltosyl-erythritol, which has an α-1,4 glycosidic linkage between maltose and erythritol, is the major product transglycoslated by BSMA. Accordingly, the current study was undertaken to assess the *in vitro* cariogenicity, acidogenicity and glucan formation of maltosyl-erythritol.

The following reaction was carried out for the production of maltosyl-erythritol. One gram of maltotriose and 7.3 g of erythritol were mixed in 10 ml of 50 mM sodium citrate buffer (pH 6.0) and boiled until all solutes were dissolved. After pre-incubation at 55°C for 10 min, 0.2 unit (U) of BSMA per mg of maltotriose were added to the mixture. One unit (U) of enzyme was defined as the amount of enzyme producing 1 μmol of maltose per min. The reaction was stopped by boiling for 5 min after incubation at 55°C for the required time. Maltosyl-erythritol was separated from the above reaction mixture by gel filtration chromatography using a Bio-Gel P-2 column (2.6×95 cm).

To examine the inhibitory effect of maltosyl-erythritol on the acid fermentation of sucrose, *Streptococcus sobrinus* NRRL 14555 was cultured at 37°C for 18 h in a brain heart infusion (BHI) broth, then 0.25 ml of 10 mM erythritol, 10 mM maltosyl-erythritol, or 10 mM glucose was added to the reaction mixture [10]. In the culture system, 2.5×10^6 of pre-cultured *Streptococcus sobrinus* was inoculated into 30 ml of the BHI broth, the pH was adjusted to 7.0, and the bacterium was cultured for 61 h at 37°C until the substrate was almost consumed. Changes in the pH of the reaction mixture were continuously measured for 60 min using a pH meter.

To determine the formation of glucan, erythritol or maltosyl-erythritol was added to the BHI broth containing 50 mM sucrose to a final concentration of 50 mM, then the broth (5 ml) was placed in a small test tube. Pre-cultured *S. sobrinus* (2.5×10^6) was inoculated into the BHI broth and the bacterium was cultured at 37°C for 24 h by fixing cultivation. Thereafter, the supernatant of the culture solution was discarded, and the remainder was washed with

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distilled water. The glucans synthesized in the mixture were dissolved in 0.5 M NaOH, and the degree of water-insoluble glucan formation was determined by measuring absorbance at 550 nm [4].

Dextransucrase and mutansucrase were isolated from *Streptococcus sobrinus* NRRL 14555 and *Streptococcus mutans*, respectively, grown in 3 l of the BHI medium (0.5% yeast extract and 3.7% BHI) by stirred batch cultures, while the pH and temperature were controlled at 7.0 and 37°C, respectively [21]. After fermentation, the culture was harvested, and the cells were removed by centrifugation and then concentrated using a hollow fiber membrane (30 K cut-off, Millipore, Japan). The concentrated enzyme solution was dialyzed overnight against 8 l of 20 mM phosphate buffer (pH 6.5) containing 50 mM NaCl. The dialyzate was loaded onto a 1.0×15 cm DEAE-cellulose column equilibrated with the same buffer. The column was then washed with 200 ml of the same buffer, followed by 500 ml of 20 mM phosphate buffer (pH 6.5) containing 200 mM NaCl, and finally eluted with a linear NaCl gradient (800 ml, 0.2-0.1 M) over a period of 6 h. The dextransucrase activity was determined by analyzing the rate of fructose released from sucrose (200 mM in a 20 mM imidazole HCl-buffer, pH 6.5, 23°C). The quantity of fructose on the TLC plate was analyzed using a densitometer [13].

To determine the inhibitory activity of maltosyl-erythritol on dextransucrase and mutansucrase, 0.25 ml of 100 mM sucrose in 25 mM imidazole-HCl buffer (pH 6.5) was mixed with 0.25 ml of dextransucrase or mutansucrase and 0.25 ml of 34 mM maltosyl-erythritol or erythritol, and the mixtures were then incubated at 28 or 37°C for 10 to 50 min (at intervals of 10 min). To stop the reaction, 0.05 ml of 50 mM NaOH was added to the reaction mixture. The relative inhibition of the enzymes was determined by measuring the amount of fructose released by thin layer chromatography. An equal volume of the buffer in place of sugar was added to the control.

S. mutans and certain other plaque microorganisms, which rapidly produce large amounts of acids from sucrose and other low molecular-weight carbohydrates, are associated with the development of dental caries [14, 20]. Plus, lactic acid, the predominant acid formed in dental plaque [9], is the main cause of enamel demineralization.

The use of alternative sweeteners has been suggested as an effective measure to prevent caries [6, 8, 15, 17, 25]. Maltosyl-erythritol is a unique sweetening agent, which involves an enzymatic transglycosylation reaction where erythritol is the acceptor and maltotriose is the donor [24]. Thus, to assess whether this substance can be utilized as a substitute for sucrose in preventing caries, its cariogenicity was tested.

The fermentation of sugars by oral bacteria produces acid, which is unfavorable to tooth enamel below a critical pH. Generally, a substance is considered to be nonacidogenic when the critical pH value in humans, as measured by plaque pH telemetry, is equal to or greater than pH 5.7 [3].

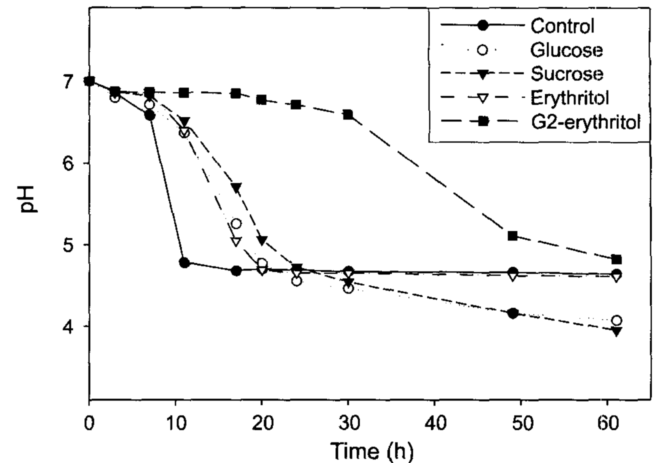

Fig. 1. pH changes in cultures of *Streptococcus sobrinus*.

In the current study, when examining the inhibitory effect of maltosyl-erythritol on the acid fermentation of sucrose, maltosyl-erythritol did not support acid production by *Streptococcus sobrinus* until after 30 h of incubation. Maltosyl-erythritol was consumed very slowly as a substrate for acid production for 30 h, and thereafter, the substrate was rapidly used as the pH dropped to 5.1. When glucose, sucrose, and erythritol were used as the substrate for lactic acid production, all substrates were rapidly used for 20 h, and the pH for erythritol remained constant thereafter at around 4.8, while the pH values for glucose and sucrose was lower (about 4.2) at 60 h (Fig. 1).

A sticky water-insoluble glucan is produced from sucrose by the glucosyltransferase from *Streptococcus mutans* [5]. The production of insoluble glucan by certain oral micro-organisms, including *S. mutans*, forms plaque on the tooth surface, thereby inhibiting the diffusion of locally produced acid to oral cavities and constituting a barrier to the inward diffusion of saliva into plaque. Consequently, the cleansing action is lost and acid neutralization diminishes. Sucrose also causes the aggregation of *S. mutans* and certain other

Table 1. Relative inhibition of insoluble glucan formation from sucrose by *S. sobrinus*.

Reaction mixture containing carbohydrates*	Abs 550 nm	Relative inhibition (%)
Control**	0.375	0.0
Erythritol	0.372	0.8
G2-Erythritol	0.096	74.5

*Reaction mixture contained 50 mM sucrose and 50 mM test carbohydrate.
**Control reaction mixture only contained 50 mM sucrose without test carbohydrate.

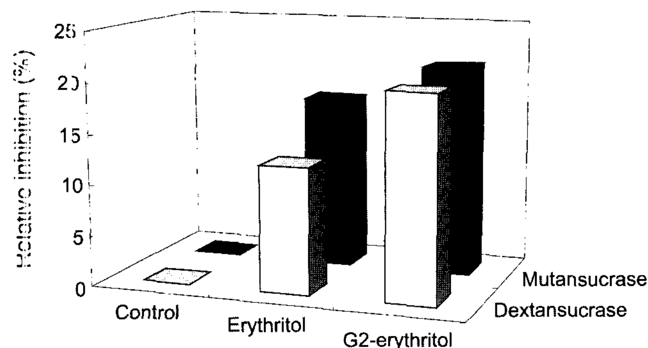

Fig. 2. Inhibition percentage for dextransucrase and mutansucrase activity (IU/ml).

microflora, which favor the initial formation of plaque. Finally, sucrose is fermented and metabolized into lactic as well as other acids that demineralize enamel [10].

The amount of insoluble glucan produced by the glucosyltransferase from *S. sobrinus* was only 1/75 of that produced from sucrose alone (Table 1). This result demonstrated that maltosyl-erythritol strongly inhibited the formation of insoluble glucan.

The enzyme dextransucrase or mutansucrase is a glycosyl transferase that plays a central role in the development of dental caries [18]. The dental caries suppression effect of maltosyl-erythritol was compared with that of erythritol. Figure 2 shows that the inhibitory effect of maltosyl-erythritol against enzymes was greater than that of erythritol. In particular, in the case of dextransucrase, it was about 2 times higher than that of erythritol. In conclusion, the current results suggest that maltosyl-erythritol is non-cariogenic and has desirable properties as a tooth-friendly functional food ingredient.

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