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Potential Suppression of Dental Caries by Maltosyl-Mannitol Produced by *Bacillus stearothermophilus* Maltogenic Amylase

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Abstract Maltosyl (G2)-mannitol, produced by the transglycosylation of mannitol with maltotriose by *Bacillus stearothermophilus* maltogenic amylase, was not found to support lactic acid production by *Streptococcus sobrinus* NRRL 14555. Furthermore, the synthesis of water-insoluble glucans from maltosyl-mannitol by *S. sobrinus* NRRL 14555 was much lower than that from xylitol or mannitol. Consequently, these results suggest that maltosyl-mannitol could be used as a noncariogenic sugar substitute in food products.

Key words: Maltosyl-mannitol, transglycosylation, *Bacillus stearothermophilus* maltogenic amylase (BSMA), acid production, water-insoluble glucan

Nonfermentability by oral microorganisms, non-acidogenicity, and the inhibition of glucan formation *in vitro*, as well as the results of rat caries tests, are the criteria normally used to assess the noncariogenicity of a substance [15]. However, cariogenicity also depends on the availability of sucrose [3, 14]. Thus, substituting other sweeteners for sucrose is an effective method of preventing dental caries [9]. Mannitol is not fermented as rapidly as sucrose, and not easily utilized for the synthesis of the polysaccharide that causes the accumulation of dental plaque on tooth surfaces by mutans streptococci, one of the microorganisms responsible for dental caries [7]. Mannitol is noncariogenic and nonacidogenic, and thus reduces the incidence of dental caries, which are promoted by acidic conditions that develop in the mouth after eating carbohydrates and proteins. Mannitol is also only partially metabolized by humans and does not induce hyperglycemia, making it a useful sweetener for diabetics [12, 16].

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Enzymatic transglycosylation is a well-known process for modifying the characteristics of food materials such as rutin, steviosides, and rudusoside to increase their solubility or reduce their bitter taste [2, 11]. *Bacillus stearothermophilus* maltogenic amylase (BSMA) has both hydrolytic and transglycosylation activities [1], and maltosyl-mannitol, which has an α-1,6-glycosidic linkage between maltose and mannitol, is the major product of the BSMA transglycosylation reaction. Accordingly, the current study was undertaken to assess the dental caries suppression by maltosyl-mannitol. The reaction mixture for the production of maltosyl-mannitol consisted of 10% (w/v) maltotriose as the donor and 30% (w/v) mannitol as the acceptor in a 50 mM sodium citrate buffer (pH 6.0). The mixture was then boiled until all the solute was dissolved, incubated at 55°C for 10 min, and 0.2 U of BSMA added per mg of maltotriose. One unit (U) of enzyme was defined as the amount of enzyme producing 1 μmol/min of maltose from β-cyclodextrin. After 12 h of incubation at 55°C, the reaction was stopped by boiling the mixture for 5 min. The maltosyl-mannitol was separated from the reaction mixture by gel filtration chromatography using a Bio-Gel P-2 (Bio-Rad Laboratories, Hercules, CA, U.S.A.) column (2.6× 95 cm) and recycling preparative HPLC (JAI model LC-918, Japan Analytical Industry, Tokyo, Japan) equipped with a W251 column (2×50 cm, Japan Analytical Industry), as shown in Fig. 1.

To compare the utilization of maltosyl-mannitol with that of other sugar substitutes in lactic acid fermentation, *Streptococcus sobrinus* NRRL 14555 was cultured at 37°C for 18 h in a brain heart infusion (BHI) broth [8]. In the culture system, 2.5×10^6 cells of the precultured *S. sobrinus* were inoculated into 3 ml of the BHI broth. The pH was adjusted to 7.0, and the same quantity of cells added to a new BHI broth containing 2% xylitol, mannitol, sucrose, a mixture of maltose and mannitol, or maltosyl-

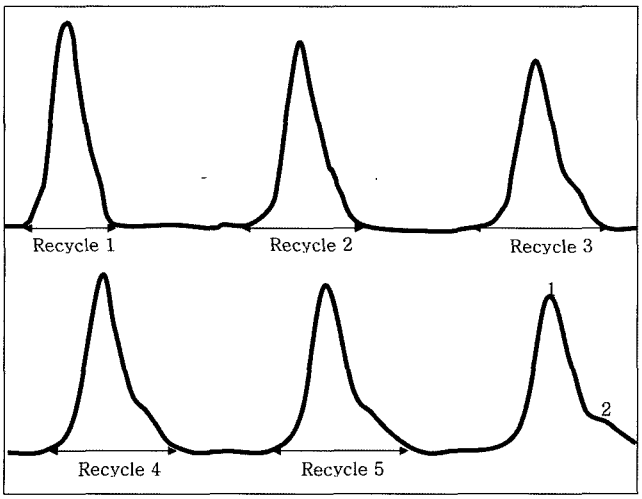

Fig. 1. Purification of maltosyl-mannitol by recycling preparative HPLC.
1, Maltosyl-mannitol; 2, glucosyl-mannitol.

mannitol. The pH change in the reaction mixture was measured using a digital pH meter at regular intervals for 16 h at 37°C [13].

To investigate the formation of water-insoluble glucans, the precultured *S. sobrinus* (2.5×10^6/ml) was inoculated into 3 ml of the BHI broth containing 2% sucrose, mannitol, xylitol, or maltosyl-mannitol, and cultured at 37°C for 24 h in a glass vial. The culture broth supernatant was discarded, and the remainder washed with distilled water. The synthesized glucans were then dissolved in 0.5 M NaOH, and the degree of water-insoluble glucan formation assayed by monitoring the absorbance at 550 nm [10, 13].

The fermentation of sugars by oral bacteria produces acid, which is unfavorable to tooth enamel below a critical pH. Thus, a substance is considered to be nonacidogenic

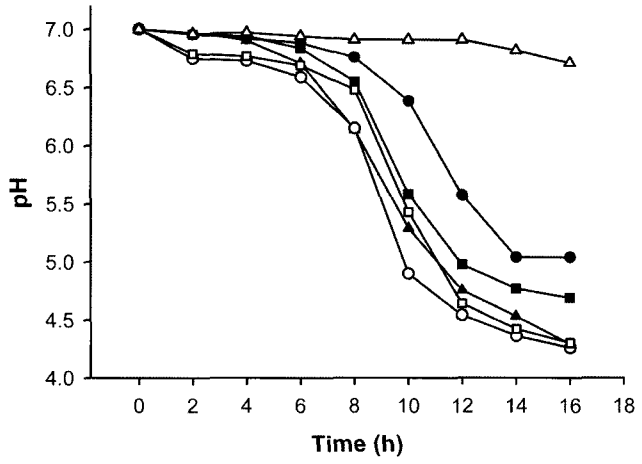

Fig. 2. Effects of xylitol (●), mannitol (■), sucrose (▲), maltose (○), a mixture of maltose and mannitol (□), and maltosyl-mannitol (△) on acid production.

Table 1. Relative amount of insoluble glucans produced from various carbohydrates by *S. sobrinus* NRRL 14555.

Carbohydrate	Relative amount of produced glucan (Abs at 550 nm)
Sucrose	0.387
Mannitol	0.223
Xylitol	0.121
Maltosyl-mannitol	0.051

Values are the average of three determinations.

when the pH value in humans, as measured by plaque pH telemetry, is equal to or greater than 5.7 [4].

The use of alternative sweeteners has been suggested as an effective method for the prevention of caries [9]. Therefore, this study compared the lactic acid production by *S. sobrinus* NRRL 14555 from maltosyl-mannitol with that from sucrose and various sugar substitutes, such as mannitol, xylitol, and maltose (Fig. 2). The maltosyl-mannitol did not support acid production until after 16 h of incubation. However, when maltose, mannitol, xylitol, or a mixture of maltose and mannitol was used as the substrate for lactic acid production, the substrates were all rapidly utilized after 8 h. The pH values produced by the incubation of maltose and sucrose were also lower (about 4.3) than those produced by the incubation of polyalcohols after 16 h. Therefore, these results indicate that the degradation of polyalcohols by mutans streptococci occurred at a much slower rate than that of sucrose [5], and maltosyl-mannitol was superior to the other polyalcohol sugar substitutes in this respect.

Sticky water-insoluble glucans mediate the accumulation of mutans streptococci bacteria on tooth surfaces, causing the aggregation of bacteria as dental plaque, which finally leads to dental caries [6, 17]. As shown in Table 1, the relative amount of water-insoluble glucans produced by *S. sobrinus* NRRL 14555 from maltosyl-mannitol was much lower than that produced from mannitol or sucrose and was about 40% of that produced from xylitol.

Consequently, these results reveal that maltosyl-mannitol does not serve as a substrate for the growth of *S. sobrinus* and can be useful as a noncariogenic sugar substitute.

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