

## Inhibitory Effects of D-mannose on *Streptococcus mutans* in the Presence of Sucrose

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This study aimed to examine the inhibitory effect of rare sugars on *Streptococcus mutans* (*S. mutans*) in the presence of sucrose. Xylitol and three rare sugars (D-xylose, D-lyxose and D-mannose) were used in this study. *S. mutans* KCTC 3065 was cultured in Brain Heart Infusion (BHI) medium containing xylitol, D-xylose, D-lyxose, or D-mannose in the presence of sucrose, and the effect on *S. mutans* growth was assessed by measuring solution turbidity at different time points after inoculation. To assess effects on pH, sucrose was added at different concentrations, and solution pH was measured at different time points after inoculation. All sugars significantly inhibited the growth of *S. mutans* in the presence of sucrose. Especially, D-lyxose and D-mannose exhibited significantly greater inhibition than that of xylitol. Furthermore, unlike D-lyxose, D-mannose significantly inhibited the decrement of pH, and its effect was greater than that of xylitol. Taken together, D-mannose has strong inhibitory effect on *S. mutans* in the presence of sucrose.

**Key Words:** *Streptococcus mutans*, D-mannose, D-lyxose, Xylitol, Dental caries

### INTRODUCTION

The facultatively anaerobic bacterium *Streptococcus mutans* (*S. mutans*) plays an important role in the development of dental caries. *S. mutans* adheres to the pellicle of teeth and synthesizes insoluble glucan by transferring glucosyl moieties from various sugars, which promotes the formation of dental plaque (1). In dental plaque, *S. mutans* produces organic acids through carbohydrate metabolism. These organic acids induce demineralization of the tooth surface and result in dental caries. Xylitol, which is roughly

as sweet as sucrose, can prevent dental caries by inhibiting the growth of and acid production by *S. mutans* (2~4).

However, this preventive effect is inhibited by other monosaccharides, such as D-fructose or D-glucose, which can be metabolized by *S. mutans* (5, 6). In this study, the inhibitory effects of xylitol and three rare sugars (D-xylose, D-lyxose, and D-mannose) on *S. mutans* in the presence of sucrose were determined. Xylitol was used as a control monosaccharide because it is well known to inhibit the growth and acid production of *S. mutans*.

D-xylose, a rare aldopentose, can be converted to xylitol by D-xylose reductase in many yeasts such as *Candida*

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*tropicalis* (7, 8). D-lyxose is a C-2 epimer of D-xylose, and is used as a starting material for antitumor and immunostimulatory agents (9). D-lyxose can be produced from xylulose by lyxose isomerase (10). This rare pentose is not commonly utilized by microorganisms, and its metabolism is poorly understood because of its limited availability. D-mannose, a rare six-carbon sugar, is used for treating cystitis and as a feedstuff additive for preventing bacterial infection (11). However, unlike xylitol or D-xylose, its role in dental caries is unclear. Hence, the aim of this study was to examine and compare the inhibitory effect of these rare sugars on *S. mutans*, particularly in the presence of sucrose.

## MATERIALS AND METHODS

### Reagents

All sugars were purchased from Sigma-Aldrich Korea (Seoul, Korea) with a stated purity of >99 wt%. Brain Heart Infusion (BHI) medium supplemented with dextrose was prepared for activation of *S. mutans*. However, assessing the effects of the rare sugars using a rich-sugar medium was problematic, so BHI medium without dextrose was used instead of BHI medium with dextrose in bacterial growth assay. These media were purchased from MB Cell Korea (Seoul, Korea).

### Bacterial strains and culture conditions

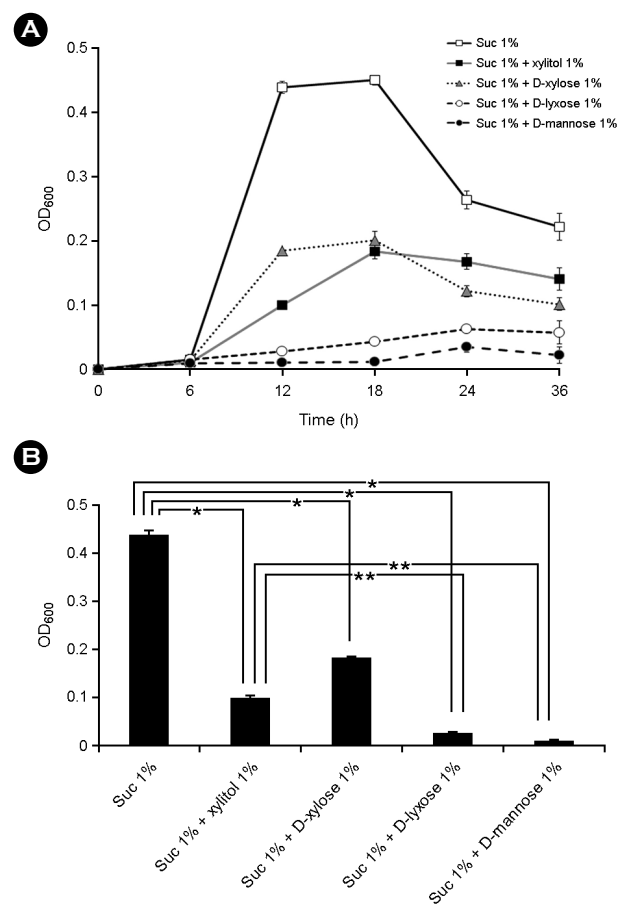
*S. mutans* KCTC 3065 was stored at  $-80^{\circ}\text{C}$  until use. *S. mutans* was grown in 8 ml of BHI medium (with dextrose) at  $37^{\circ}\text{C}$  in static culture in an air-sealed tube for 24 h. BHI medium (5 ml; without dextrose) containing 50 mg (1% w/v) sucrose was prepared in five air-sealed tubes. With the exception of the control, which contained only sucrose, 50 mg (1% w/v) of xylitol, D-xylose, D-lyxose, or D-mannose were added to the other four tubes. These solutions were used for bacterial growth assay. Because most of the soft drinks that are recognized as a major cause of dental caries contain almost 10% sugar (12), 1%, 5% and 10% sucrose were used for the pH test to examine the inhibitory effect on acid production of *S. mutans* under various conditions.

Before the inoculation, *S. mutans* cultured in BHI medium

(with dextrose) was washed twice with 0.5 ml of Sodium Phosphate buffer (pH 6.4). A *S. mutans* suspension (50  $\mu\text{l}$ ) was inoculated into 5 ml BHI medium (without dextrose), and the air-sealed tubes were statically cultured at  $37^{\circ}\text{C}$ .

### Bacterial growth assay

Bacterial growth was assessed by measuring solution turbidity. In the presence of a high concentration of sucrose, *S. mutans* formed aggregates containing insoluble glucan that were non-homogeneously distributed, especially in the control which contained only sucrose. So, turbidity was measured only in the groups containing 1% sucrose. To



**Figure 1.** The inhibition of *S. mutans* growth by rare sugars in the presence of sucrose. (A) The turbidity of the solutions at 6, 12, 18, 24, and 36 h after inoculation. (B) The ability of rare sugars to inhibit *S. mutans* growth at 12 h after inoculation. \* $p < 0.05$  for the mean values vs. the control group (only contained sucrose). \*\* $p < 0.05$  for the mean values vs. the xylitol group.

**Table 1.** The effect of various rare sugars on pH change of the growth medium containing sucrose at 12, 24, and 36 h after inoculation

Solution	pH		
	12 h	24 h	36 h
Sucrose 1%	4.75±0.185	4.24±0.092	4.07±0.036
Sucrose 1% + xylitol 1%	5.11±0.070	4.31±0.093	4.09±0.032
Sucrose 1% + D-xylose 1%	4.75±0.140	4.20±0.045	4.04±0.023
Sucrose 1% + D-lyxose 1%	4.97±0.131	4.26±0.050	4.06±0.023
Sucrose 1% + D-mannose 1%	6.62±0.168	4.63±0.206	4.20±0.055
Sucrose 5%	4.86±0.114	4.25±0.047	4.08±0.031
Sucrose 5% + xylitol 1%	5.19±0.125	4.31±0.057	4.11±0.040
Sucrose 5% + D-xylose 1%	4.86±0.213	4.23±0.076	4.08±0.020
Sucrose 5% + D-lyxose 1%	5.04±0.167	4.28±0.040	4.10±0.021
Sucrose 5% + D-mannose 1%	6.73±0.079	4.73±0.131	4.24±0.061
Sucrose 10%	4.93±0.136	4.25±0.057	4.11±0.025
Sucrose 10% + xylitol 1%	5.26±0.107	4.39±0.085	4.17±0.047
Sucrose 10% + D-xylose 1%	4.91±0.110	4.27±0.075	4.09±0.029
Sucrose 10% + D-lyxose 1%	5.06±0.139	4.35±0.047	4.13±0.021
Sucrose 10% + D-mannose 1%	6.79±0.115	5.03±0.120	4.33±0.071

Values are presented as mean ± standard deviation

determine solution turbidity, the optical density at 600 nm was measured by spectrophotometry Spectra MAX 190 (Molecular Devices, TX, US) at 6, 12, 18, 24, and 36 h after inoculation.

### Measurement of pH

Acid production by *S. mutans* was measured at 12, 24, and 36 h after inoculation using a pH meter pH-200L (Istek, Seoul, Korea). The initial pH of the BHI medium without dextrose in the presence of various sugars was 7.4.

### Statistical analysis

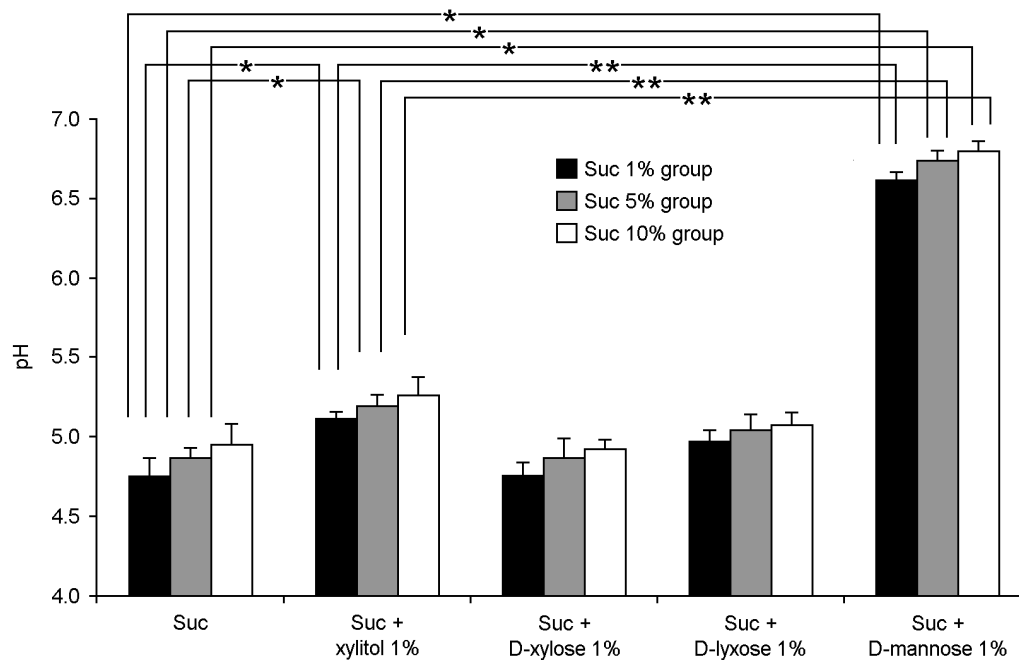
All experiments were performed in triplicate. Each value represents the mean ± SD of triplicate experiments. Statistical significance was determined by *t*-test. *P*-values < 0.05 were accepted as significant.

## RESULTS

### Bacterial growth assay

The turbidity of *S. mutans* suspensions is shown in Fig. 1A. *S. mutans* grew rapidly in the control medium, which contained only 1% sucrose. The turbidity of *S. mutans* suspensions in D-mannose was lowest among the sugars throughout the experiment. The turbidity in D-lyxose was lower than that in xylitol. Xylitol, D-xylose, D-lyxose, and D-mannose groups significantly inhibited the growth of *S. mutans* ( $p < 0.05$ ) compared to the control group (Fig. 1B). D-lyxose and D-mannose exhibited significantly greater inhibition of bacterial growth than xylitol ( $p < 0.05$ ).

Our preliminary study showed that early stationary phase in the growth of *S. mutans*, in the presence of sucrose, appeared at 12 h after inoculation. Because early stationary phase shows the peak growth of *S. mutans* and most bacterial cells in late stationary phase become smaller (13), the data



**Figure 2.** The effect of various rare sugars on pH change of the growth medium containing sucrose at 12 h after inoculation. \* $p < 0.05$  for the mean values vs. the control group (only contained sucrose). \*\* $p < 0.05$  for the mean values vs. the xylitol group.

obtained at 12 h after inoculation was compared in Fig. 1B, as a criterion for comparison of the growth of *S. mutans*.

### Measurement of pH

The decrement of pH in the control medium, and xylitol, D-xylose, D-lyxose, and D-mannose-containing media was measured in the presence of sucrose (Table 1).

Likewise bacterial growth assay, the data obtained at 12 h after inoculation was compared in Fig. 2, as a criterion for comparison of the acid production of *S. mutans*. As shown in Fig. 2, only D-mannose significantly inhibited the pH decrement at all sucrose concentrations ( $p < 0.05$ ). Xylitol significantly inhibited the pH decrement in the presence of 1% and 5% sucrose ( $p < 0.05$ ). Interestingly, D-mannose showed significantly greater inhibition of pH decrement compared to xylitol at all sucrose concentrations ( $p < 0.05$ ). D-xylose and D-lyxose did not show significant inhibition of pH decrement irrespective of sucrose concentration.

**Table 2.** Summary of the inhibitory effects on *S. mutans* by rare sugars in the presence of sucrose

	Bacterial growth	Acid production
Xylitol	++	+
D-xylose	++	-
D-lyxose	+++	-
D-mannose	+++	++

- : no inhibition,  $p > 0.05$   
 + : slight inhibition,  $0.01 < p < 0.05$   
 ++ : moderate inhibition,  $0.0001 < p < 0.01$   
 +++: strong inhibition,  $p < 0.0001$

## DISCUSSION

As shown in Fig. 2, the significant inhibitory effect on acid production by xylitol disappeared in the presence of 10% sucrose ( $p > 0.05$ ). Although xylitol has anti-caries activity, numerous studies have evaluated whether the anti-caries effect of xylitol is superior to those of other sugars,

such as sorbitol (14~16). In addition, daily use of xylitol did not result in a statistically or clinically significant reduction in 33-month caries increment among adults with an elevated risk of caries development (17).

To my knowledge this is the first study to investigate the inhibition of *S. mutans* by addition of D-mannose to sucrose, instead of using polyol such as xylitol. Even when D-mannose was used approximately 1/10 of sucrose, it significantly inhibited the growth and acid production of *S. mutans*. This study revealed that D-mannose would be a potent anticaries material especially in the presence of general sweeteners such as sucrose. In addition, the sweetness of D-mannose (especially,  $\alpha$ -anomer, used in this study) is almost 60% compared to that of sucrose (18~20), suggesting that the sweetness of D-mannose is similar with that of D-glucose.

However, further studies are necessary to determine the inhibitory mechanisms of D-mannose as well as D-lyxose. Whether D-mannose, in the presence of sucrose, alters the expression of various *S. mutans* enzymes, such as sucrase, should be determined. Although sucrase in *S. mutans* may not be the same as that in the human body (21), it is likely that D-mannose can inhibit the activity of this enzyme in *S. mutans*. The reason that D-lyxose did not suppress acid production by *S. mutans*, unlike D-mannose, despite its inhibition of growth, is also unclear. Investigating the different effects on acid production of *S. mutans* induced by these two rare sugars could be of help in understanding the inhibition mechanism of D-mannose.

Although further studies of the mechanisms underlying the effect of D-mannose are necessary, D-mannose seems to be beneficial for oral health in that it prevents the growth and acid production of *S. mutans* significantly in the presence of sucrose.

This study aimed to examine and compare the inhibitory effect of rare sugars on *S. mutans* in the presence of sucrose. The inhibitory effects of the rare sugars on the growth and acid production of *S. mutans* is summarized in Table 2. The growth of *S. mutans* was inhibited by xylitol-, D-xylose-, D-lyxose-, and D-mannose in the presence of sucrose. Especially, D-lyxose and D-mannose exhibited significantly greater inhibition on the growth of *S. mutans* than that of

xylitol. Xylitol and D-mannose inhibited acid production by *S. mutans* in the presence of sucrose. D-mannose showed significantly greater inhibition of pH decrement compared to xylitol. Taken together, D-mannose has strong inhibitory effect on *S. mutans* in the presence of sucrose.

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