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Candidacidal Activity of Xylitol and Sorbitol

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Purpose: It has been reported that xylitol and sorbitol affect antifungal activities by enhancing antimicrobial activities of other substances. The purpose of this study was to investigate the direct candidacidal activities of xylitol and sorbitol at a wide range of concentration.

Methods: Xylitol and sorbitol solubilized with simulated salivary buffer at a range of 0.8 μM to 1.05 M were used. *Candida albicans* strains, ATCC strains 10231, 11006, and 18804 were used for the candidacidal assay. The candidacidal activities of xylitol and sorbitol were determined by comparing the numbers of colony forming units between in the presence and absence of xylitol or sorbitol and calculating the percent loss of cell viability.

Results: There were some differences in the candidacidal activities according to the types of sugar alcohols and *C. albicans* strains. The candidacidal activity of more than 10% was observed when a final concentration of 32.9 mM in xylitol or sorbitol was maintained and that of about 20% was observed when a final concentration of 131 mM was maintained. Even at a high concentration of 1.05 M, the candidacidal activity of xylitol or sorbitol was about 20%.

Conclusions: Xylitol and sorbitol at the concentrations used in commercial oral health care products had some levels of candidacidal activities.

Key Words: *Candida albicans*; Candidacidal activity; Sorbitol; Xylitol

INTRODUCTION

Xylitol and sorbitol are the most widely used sugar alcohols. Due to their hypo- and non-acidogenicity, they have been utilized for anticariogenic purposes. It has been known that xylitol is a better agent in reducing the incidence of dental caries than sorbitol. Xylitol is known to exert caries-reducing effects by inhibiting growth, metabolism, and polysaccharide production of mutans streptococci.[1-4]

Xylitol has no direct antibacterial properties, but it has anti-adhesive effects on microorganisms. Thus, xylitol has been suggested to decrease the amount of dental plaque by decreasing the adhesivity of plaque.[1,3] The combination of chlorhexidine and xylitol was more effective than each single treatment in controlling dental biofilm.[5] Due to its similar effects on *Streptococcus pneumonia*, xylitol has been used for the prevention and treatment of acute otitis media.[6] Xylitol feeding also caused a shift in fecal microbial population from Gram-negative to Gram-positive bacteria in human volunteers as well as rodents.[7]

Sugar alcohols also affect antifungal activity. *Candida albicans* cultured in media supplemented with xylitol showed changed susceptibility to lysozyme.[8] The antifungal activity of *Lactobacillus* was enhanced in the presence of xylitol and sorbitol.[9,10] Xylitol and sorbitol also enhanced the fungicidal effect of benzethonium chloride in vitro *C. albicans* biofilm.[11] A decrease in the number of yeast in human stool was observed after a single 30 g oral dose of xylitol.[7] Whereas the dietary intake of sucrose induces *C. albicans* growth in the gastrointestinal tract, xylitol intake has been

reported as a possible inhibitor of *C. albicans*.[12] In the presence of xylitol, a decrease in adhesion of *C. albicans* in the oral cavity has also been reported, suggesting inhibitory activities on fungal infections such as thrush.[13]

The consumption of a xylitol diet has been reported to increase salivary peroxidase activity[14] and xylitol and sorbitol enhanced the enzymatic activities of both bovine lactoperoxidase and salivary peroxidase significantly in vitro assays.[15] However, the candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase system were neither enhanced nor inhibited significantly by xylitol or sorbitol.[15] Although there have been some reports on the indirect effects of xylitol and sorbitol on *Candida*, it has been remained to be answered whether xylitol and sorbitol have direct candidacidal activities. The purpose of this study was to investigate the direct candidacidal activities of xylitol and sorbitol at a wide range of concentration.

MATERIALS AND METHODS

1. Xylitol, Sorbitol, and *Candida albicans* Strain

Xylitol and sorbitol (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) solubilized with simulated salivary buffer (SSB; 0.021 M Na_2HPO_4/NaH_2PO_4, containing 36 mM NaCl and 0.96 mM $CaCl_2$)[16] were used in the experiments. Three kinds of *C. albicans* strains, ATCC strains 10231, 11006, and 18804 were used for the candidacidal assay. At high concentration experiments, only *C. albicans* 11006 strain was used.

2. Candidacidal Activity of Xylitol and Sorbitol

The candidacidal activities of xylitol and sorbitol were investigated by a stepwise way. First, a preliminary experiment was performed at a final concentration of 131 mM of xylitol and sorbitol used in our previous study[15] and in some commercial oral health products. The second candidacidal experiments were done at a medium concentration level which ranges from 6.6 to 131 mM. Then, the third experiments were done at a low concentration level which ranges from 0.8 μM to 0.66 mM. The final experiments were performed at high concentrations ranging from 131 mM to 1.05 M.

For candidacidal assays, one colony of *C. albicans* grown on Sabouraud dextrose agar (SDA) was inoculated into 10 mL Sabouraud dextrose broth and incubated with shaking at 37°C for 18 hours. Cells were harvested, washed, and resuspended to 1×10^5 cells per mL in SSB. Twenty microliters of cell suspension was added to 40 μL of xylitol or sorbitol. The samples were incubated with shaking at 37°C for 1 hour. After the incubation, the mixtures were diluted 10-fold, and 50 μL (167 cells) of the diluted cells were plated onto SDA plates in triplicate and grown overnight at 37°C. The influence on candidacidal activity was determined by comparing the number of colonies (colony forming units, CFUs) on experimental (with xylitol or sorbitol) and control (without xylitol or sorbitol) plates. The percent loss of cell viability was calculated as 1 minus the ratio of the number of colonies on the experimental plates to that on the control plates. The experiments were performed 8 times.

3. Statistics

The Friedman test, Wilcoxon signed rank test, and Mann-Whitney U test were used to analyze differences between variables. p-values less than 0.05 were considered statistically significant.

RESULTS

1. Candidacidal Activity of Xylitol and Sorbitol at a Concentration of 131 mM

The candidacidal activities (%killing) of xylitol and sorbitol at a concentration of 131 mM were 16.1%-26.6% and 14.7%-19.1%, respectively. There were some differences in the candidacidal activity according to *C. albicans* strain, but there were no differences in the activities between xylitol and sorbitol (Fig. 1).

2. Candidacidal Activity of Xylitol and Sorbitol at a Medium Concentration Range

There were some variations in the candidacidal activities according to the types of sugar alcohols and the *C. albicans* strains, but xylitol and sorbitol at these concentrations showed some levels of candidacidal activity and the levels of activities were about 10%-20%. Although the statistical significances according to the concentrations of xylitol or sorbitol were not always found, the level of activity

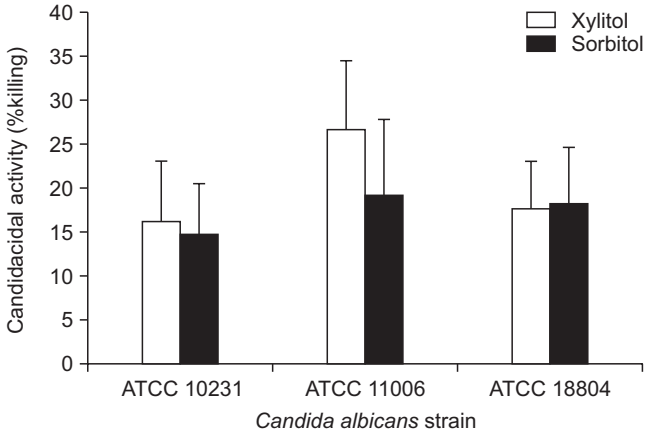

Fig. 1. Candidacidal activities (%killing) of xylitol and sorbitol at a concentration of 131 mM. The candidacidal activity (%killing) was calculated as 1 minus the ratio of the number of colonies (colony forming units) on the experimental plates (with xylitol or sorbitol) to that on the control (without xylitol or sorbitol) plates. The experiments were performed 8 times.

was dose-dependent. The candidacidal activities at 131 mM showed significant differences compared to those at 32.9 mM or 6.6 mM of xylitol or sorbitol in 11006 strain and of xylitol in 10231 strain (Table 1).

3. Candidacidal Activity of Xylitol and Sorbitol at a Low Concentration Range

Candidacidal activities of xylitol and sorbitol at these low concentrations were minimal and showed about 0%–5%. Although there was a significant difference in the case of xylitol and ATCC 10231 strain, no significant differences were found according to the concentration of xylitol and sorbitol at these low concentrations (Table 2).

When considering the results of medium and low concentrations together, the %killing of more than 10% is observed

Table 1. Candidacidal activities (%killing) of xylitol and sorbitol at a medium concentration range (n=8)

Candida albicans strain		6.6 mM (Group I)	32.9 mM (Group II)	65.7 mM (Group III)	131 mM (Group IV)	Significance[a]	Significance between groups[b]
ATCC 10231	Xylitol	13.5±10.5	11.8±8.3	16.0±13.7	25.6±10.4	0.044*	(I, IV) (II, IV)*
	Sorbitol	12.2±14.6	22.8±10.6	18.3±10.0	22.7±5.0	NS	NS
ATCC 11006	Xylitol	7.8±13.6	13.1±5.8	16.1±11.7	22.3±6.6	0.043*	(II, IV)*
	Sorbitol	1.0±8.6	12.9±11.7	11.1±11.4	15.2±12.5	0.010*	(I, II) (I, IV)*
ATCC 18804	Xylitol	7.9±12.9	18.3±12.6	18.0±16.9	18.2±12.6	NS	NS
	Sorbitol	9.1±13.8	10.0±9.2	11.2±10.8	12.5±10.5	NS	NS

NS, not significant.
Values are presented as mean±standard deviation.
The candidacidal activity (%killing) was calculated as 1 minus the ratio of the number of colonies (colony forming units) on the experimental plates (with xylitol or sorbitol) to that on the control (without xylitol or sorbitol) plates.
[a]Statistical significance was evaluated using the Friedman test.
[b]Statistical significance was evaluated using the Wilcoxon signed rank test.
*p<0.05.

Table 2. Candidacidal activities (%killing) of xylitol and sorbitol at a low concentration range (n=8)

Candida albicans strain		0.8 μM (Group I)	6.6 μM (Group II)	65.7 μM (Group III)	0.66 mM (Group IV)	Significance[a]	Significance between groups[b]
ATCC 10231	Xylitol	0.7±12.2	12.1±18.8	15.5±10.0	6.1±23.3	0.026*	(I, III)*
	Sorbitol	4.1±20.4	−3.6±26.0	−0.9±20.9	4.5±16.9	NS	NS
ATCC 11006	Xylitol	−11.3±17.2	4.3±20.1	3.5±9.7	1.6±10.8	NS	NS
	Sorbitol	−5.2±10.4	3.8±9.9	−6.5±23.3	4.9±18.1	NS	NS
ATCC 18804	Xylitol	3.1±14.5	4.4±12.7	5.8±14.3	3.3±11.6	NS	NS
	Sorbitol	1.1±9.9	−4.3±23.2	−6.4±26.7	11.2±18.9	NS	NS

NS, not significant.
Values are presented as mean±standard deviation.
The candidacidal activity (%killing) was calculated as 1 minus the ratio of the number of colonies (colony forming units) on the experimental plates (with xylitol or sorbitol) to that on the control (without xylitol or sorbitol) plates.
[a]Statistical significance was evaluated using the Friedman test.
[b]Statistical significance was evaluated using the Wilcoxon signed rank test.
*p<0.05.

when a final concentration of 32.9 mM (5.0 mg/mL of xylitol and 6.0 mg/mL of sorbitol) is maintained. The %killing of about 20% is observed when a final concentration of 131 mM (20 mg/mL of xylitol and 24 mg/mL of sorbitol) is maintained (Fig. 2, 3).

4. Candidacidal Activity of Xylitol and Sorbitol at a High Concentration Range

There were no significant differences in the candidacidal activities at these high concentrations according to the concentration of xylitol and sorbitol. The candidacidal activities of xylitol and sorbitol were almost the same at these high concentrations and the %killing results were about 20%. Even at the concentration of 1.05 M (160 mg/mL of xylitol and 192 mg/mL of sorbitol), the candidacidal activities were 18.2% in xylitol and 21.7% in sorbitol (Table 3).

DISCUSSION

Sugar alcohols have been applied for food and pharmaceutical industries and dentistry. In food industry, sugar alcohols have been used for confectioneries and chewing gums. In pharmaceutical industry, sugar alcohols have been formulated for otitis media and upper respiratory infections, and used as a sweetener in syrups and tablets. Most publications in dentistry regarding sugar alcohols were about antibacterial and anticariogenic effects. Sugar alcohols, especially xylitol, inhibit growth, metabolism, and polysaccharide production of mutans streptococci.[1-4] Because of their anticariogenic effects and sweet taste which can stimulate salivary secretion, xylitol and sorbitol have been included in saliva substitutes for patients with dry mouth whose susceptibility to oral candidiasis was increased. However, there

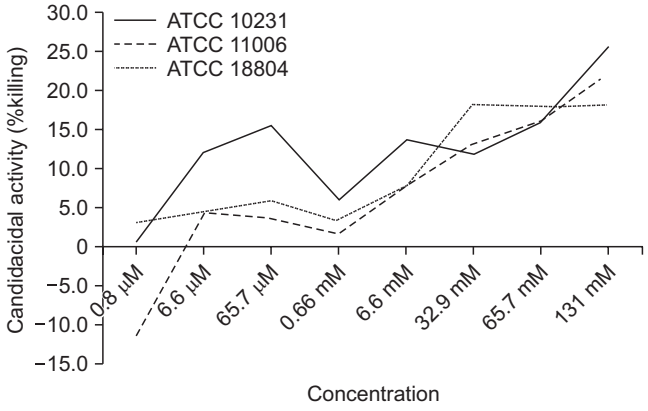

Fig. 2. Candidacidal activities (%killing) of xylitol at different concentration levels. The Fig. 2 was made of the data from Tables 1 and 2. The candidacidal activity (%killing) was calculated as 1 minus the ratio of the number of colonies (colony forming units) on the experimental plates (with xylitol) to that on the control (without xylitol) plates. The experiments were performed 8 times.

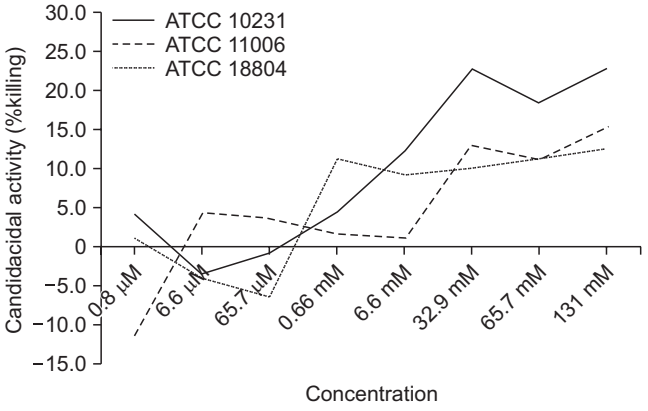

Fig. 3. Candidacidal activities (%killing) of sorbitol at different concentration levels. The Fig. 3 was made of the data from Tables 1 and 2. The candidacidal activity (%killing) was calculated as 1 minus the ratio of the number of colonies (colony forming units) on the experimental plates (with sorbitol) to that on the control (without sorbitol) plates. The experiments were performed 8 times.

Table 3. Candidacidal activities (%killing) of xylitol and sorbitol at a high concentration range (n=8)

Candida albicans strain		131 mM (Group I)	263 mM (Group II)	526 mM (Group III)	1.05 M (Group IV)	Significance[a]	Significance between groups[b]
ATCC 11006	Xylitol	16.3±9.2	21.5±10.8	15.7±9.0	18.2±10.8	NS	NS
	Sorbitol	18.8±5.6	24.7±10.7	20.6±8.0	21.7±9.4	NS	NS

NS, not significant.

Values are presented as mean±standard deviation.

The candidacidal activity (%killing) was calculated as 1 minus the ratio of the number of colonies (colony forming units) on the experimental plates (with xylitol or sorbitol) to that on the control (without xylitol or sorbitol) plates.

[a]Statistical significance was evaluated using the Friedman test.

[b]Statistical significance was evaluated using the Wilcoxon signed rank test.

have been limited numbers of studies on antifungal activities of sugar alcohols.

The reported effects of sugar alcohols, especially xylitol, on *C. albicans* were mainly about indirect mechanisms. The susceptibility of *C. albicans* to lysozyme was different according to the types of carbohydrates supplemented in culture media. *C. albicans* cultured in sucrose or galactose were more resistant to the lytic activity of lysozyme and those in lactose were the most susceptible to the killing effects of lysozyme. The results obtained for maltose, xylitol, and glucose were intermediate. It has been suggested that these effects were because of extracellular polysaccharides formed by *Candida* in carbohydrate-supplemented culture media.[8] The antifungal activities of *Lactobacillus* also depended on the growth medium. In the presence of xylitol and sorbitol, antifungal activities of *Lactobacillus* were enhanced and these effects were suggested to be due to the synthesis of specific metabolites and/or peptides with antifungal properties.[9,10] Similarly, the growth and invasion of *C. albicans* in the gastrointestinal tract were influenced by the types of carbohydrate supplementation. Xylitol intake inhibited the growth of *C. albicans* in the gastrointestinal tract compared to sucrose intake.[12]

Xylitol can also increase enzymatic activity of salivary peroxidase which has an antifungal activity. It has been reported that salivary peroxidase activity was increased several times in individuals receiving a strict xylitol diet for 2 years. This increased enzymatic activity was regarded as a result of the increased secretion of salivary peroxidase.[14] According to the in vitro experiments, xylitol and sorbitol enhanced the enzymatic activities of both bovine lactoperoxidase and salivary peroxidase significantly. These effects were greater for salivary peroxidase than bovine lactoperoxidase. The suggested mechanism was complex formation of sugar alcohols with polyvalent cations in the active site of peroxidase, which could lead the thermodynamic properties to be favorable, then increase the enzymatic activities.[15] However, the candidacidal activities of the peroxidase system and the glucose oxidase-mediated peroxidase system were not enhanced by xylitol or sorbitol,[15] and this discrepancy between enzymatic and candidacidal activities was not fully explained.

Sugar alcohols such as xylitol, sorbitol, and erythritol enhanced the fungicidal effect of benzethonium chloride in vitro *C. albicans* biofilms, and it has been suggested that low-molecular-weight sugar alcohols diffuse into the biofilm produced by the candidal cells, weaken the attachment of the biofilm, and enhance the fungicidal effect of benzethonium chloride rather than having direct fungicidal activities.[11] In fact, no fungicidal effects were observed with sugar alcohols themselves without benzethonium chloride in these biofilm experiments.[11] Interestingly, however, there is sparse information regarding direct fungicidal effects of sugar alcohols in a solution-phase assay.

In the present study, direct candidacidal activities of xylitol and sorbitol were examined in the solution-phase assays. Although there were some differences in the candidacidal activities according to the types of sugar alcohols and *C. albicans* strains, the candidacidal activity of more than 10% was observed when a final concentration of 32.9 mM (5.0 mg/mL of xylitol and 6.0 mg/mL of sorbitol) was maintained and that of about 20% was observed when a final concentration of 131 mM (20 mg/mL of xylitol or 24 mg/mL of sorbitol) was maintained. Therefore, it is likely that xylitol and sorbitol at the concentrations used in commercial oral health care products have some levels of candidacidal activities. The present study did not investigate the mechanism of candidacidal activities in sugar alcohols, but suggested antifungal mechanisms in other oral antimicrobials could be involved. The possible mechanisms were direct binding with yeast cell-wall mannans followed by an influence on yeast viability,[17,18] the activation or de-regulation of autolytic enzymes,[17,18] the interaction with fungal components followed by resultant de-regulation of the influx and efflux of cellular constituents,[17] the enzymatic hydrolysis of N-glycosidic bonds that link polysaccharides and structural proteins of the yeast cell wall,[19,20] and the modulation of secreted aspartyl proteinase (Sap), a virulence factor of *C. albicans*.[21] Based on these suggested mechanisms, further research is needed to investigate the candidacidal mechanisms of sugar alcohols.

There were some limitations to extrapolate these experimental results to the in vivo situation. There are many powerful antifungal host proteins in human saliva and supplemented sugar alcohols could not be so helpful. There are also many interactions between supplemented sugar

alcohols and host molecules, which might change biological properties of sugar alcohols. However, lack of antimicrobial components often results from hyposalivation conditions. Therefore, individuals with dry mouth usually need some commercialized oral health care products compensating for the decreased antimicrobial functions.[22] In this case, sugar alcohols could stimulate residual capacity of salivary glands and add antifungal activities as well as anticariogenic activities into residual saliva.

In conclusion, xylitol and sorbitol at the concentrations used in commercial oral health care products had some levels of candidacidal activities which might help patients with increased susceptibility to oral candidiasis.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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