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Effect of Xylitol on various Oral bacteria

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Xylitol is a five-carbon sugar alcohol that reduces the incidence of caries by inhibiting the growth of oral streptococci, including *Streptococcus mutans*. Since xylitol is transported via the fructose phosphotransferase system, we hypothesized that it could also affect the growth of other oral bacteria strains. We tested the effects of xylitol against non-periodontopathogenic oral bacteria frequently found in healthy subjects as well as periodontopathogens including *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia*. With 5% xylitol, *Streptococcus vestibularis* and *Gemella morbillorum* showed marked growth inhibition. With 10% xylitol, all of the tested periodontopathogens and *Actinomyces naeslundii* showed marked growth inhibition, whereas the growth inhibition of *Neisseria mucosa, Neisseria sicca* and *Veillonella parvula* was mild only. Xylitol is a widely used sweetener and the concentration used in our experiment is easily achieved in the oral cavity. If xylitol reduces the growth of periodontopathogens more preferentially, it could also reduce the prevalence of these pathogens and have clinical utility in the prevention or treatment of periodontal disease.

Key words: Oral bacteria, Periodontopathogen, Xylitol

Introduction

Xylitol is a naturally occuring five-carbon sugar alcohol and has been widely used as a sweetener. Xylitol is not fermented by oral microorganisms, such as Streptococcus mutans [1]. However, most of oral streptococci are able to transport xylitol into the cell via the fructose phosphotransferase system [2]. Once in the cell, xylitol is phosphorylated to xylitol-5-phosphate and subsequently expelled from the cell, which requires energy [3, 4]. The energy-consuming cycle is responsible for the inhibition of S. mutans growth observed both in vitro and in vivo by xylitol [3].

Although the inhibitory mechanisms of xylitol are not fully understood, regular consumption of xylitol has been reported to reduce the incidence of caries [5-7]. The most significant effect of xylitol against S. mutans is to reduce the growth and acid production [8] and to decrease the adherence [9]. Research into caries has not shown any other major changes in oral flora, except occasionally a reduction in other oral streptococci [5, 10].

Periodontitis is a chronic inflammatory disease induced by infection of major periodontopathogens, such as

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Porphyromonas gingivalis, Tannerella forsythia, and *Treponema denticola* [11, 12]. We have reported that xylitol inhibited inflammatory responses in macrophages infected with both live *P. gingivalis* (unpublished data) and *P. gingivalis* LPS [13]. However, direct inhibitory effects of xylitol against periodontopathogens are not studied well.

In this study, we hypothesized that xylitol could also affect the growth of bacteria in oral cavity, which could further influence oral flora population. To test our hypothesis, we evaluated the growth of several oral bacteria mostly found in healthy subjects and periodontopathogens when xylitol was added to the bacterial culture medium.

Materials and Methods

Bacterial strains

The bacterial strains used in this study and their culture conditions are listed in table 1.

S. vestibularis, G. morbillorum, A. naeslundii, N. mucosa, N. sicca and *V. parvula* were selected as normal oral bacteria mostly found in healthy subjects [14, 15]. *P. gingivalis, P. intermedia, A. actinomycetemcomitans, F. nucleatum, C. ochracea, C. sputigena, T. denticola* and *P. denticola* were selected as periodontotpathogens. The strains had been obtained from ATCC and KCTC. All the strains were freshly cultured for the experiments.

Media and cultivation of bacteria

For growth inhibition measurements, each tested strain was cultured in the recommended media as described in Table 1. Brain heart infusion (BHI, Difco, Detroit, MI, USA), Gifu Anaerobic medium (GAM, Nissui Seiyaju, Tokyo, Japan) and new oral spirochete broth (NOS) were prepared for culture medium. Xylitol (Sigma, St. Louis, MO, USA) was added to the basic medium to the appropriate concentrations and sterilized by filtration (Saltorius, Goettingen, Germany). The test medium contained 5%, 10% or 15% (wt/vol) xylitol, while the corresponding control medium was free of xylitol. Each strain was cultured aerobically or aerobically in the appropriate basic medium at 37ŶC. The dilution ratios are also described in Table 1. Each strain was diluted into 96 well plate (Corning, Williamsburg, NY, USA) containing test medium with various xylitol concentrations. The test plates were incubated at 37 °C for 1 to 4 days. The optical density (OD) of each well was measured at a wavelength of 650 nm with an ELISA reader (Molecular device, Sunnyvale, CA, USA) against the standard medium,

Table 1. Bacteria species and their culture conditions

Bacteria species	Strain	Culture		
		Media	Condition	Dilution
Gemella morbillorum	JCM 12968	GAM	Anaerobic	1:100
Streptococcus vestibularis	ATCC 27853	BHI	Aerobic	1:100
Actinomyces naeslundii	KCTC 9013	BHI	Aerobic	1:100
Neisseria sicca	KCTC 5415	GAM	Anaerobic	1:100
Veillonella parvula	KCTC 5019	GAM	Anaerobic	1:100
Neisseria mucosa	KCTC 5414	GAM	Aerobic	1:100
A ggregatibacter actinomycetemcomitans	ATCC 33384	GAM	Aerobic	1:100
Prevotella intermedia	ATCC 25611	GAM	Anaerobic	1:30
Fusobacterium nucleatum subpp. nucleatum	KCTC 5549	GAM	Anaerobic	1:10
Fusobacterium nucleatum subpp. vincentii	KCTC 5105	GAM	Anaerobic	1:100
Capnocytophaga ochracea	KCTC 3693	GAM	Anaerobic	1:5
Capnocytophaga sputigena	KCTC 5789	GAM	Anaerobic	1:10
Porphyromonas gingivalis	ATCC 33277	GAM	Anaerobic	1:100
Treponema denticola	KCTC 15104	NOS	Anaerobic	1:5
Prevotella denticola	KCTC 5455	GAM	Anaerobic	1:10

with the measurements being performed during the bacterial growth. The OD results were calculated as the mean of at least four measurements.

Results

Effect of xylitol on the growth of non-periodontogenic oral bacteria

First, the growth inhibitory effect of xylitol on oral bacteria frequently found in healthy subjects was tested. For oral bacteria found in healthy subjects, *S. vestibularis, G. morbillorum, A. naeslundii, N. mucosa, N. sicca,* and *V. parvula* were selected. They were grown to late log phase and $1x10^7$ CFU of each species were inoculated into fresh media with various xylitol concentration.

In the presence of 5% xylitol, marked growth inhibition (e.g. more than 50% inhibition) was observed in *S. vestibularis* and *G. morbillorum*. *A. anaeslundii* growth was greatly inhibited in the presence of 10% xylitol. The growth of *N. mucosa, N. sicca* and *V. parvula* was inhibited depending on xylitol concentration. At 15% xylitol, only *N. sicca* showed mild growth while the others showed almost no bacterial growth (Fig. 1).

Effect of xylitol on the growth of periodontopathogens

Next, the inhibitory effects of xylitol on periodontopathogens were measured. *P. gingivalis, P. intermedia, A. actinomycetemcomitans, F. nucleatum subsp. vincentii, F. nucleatum subsp. nucleatum, C. ochracea, C. sputigena, T. denticola* and *P. denticola* were selected as periodontopathogens. In the presence of 5% xylitol, marked growth inhibition was detected only in *P. intermedia*, while other periodontopathogens tested showed mild growth inhibition (e.g. less than 50% inhibition). However, most of bacteria tested showed marked growth inhibition in 10% xylitol. *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum subsp. vincentii,* and *P. denticola* showed marked bacterial growth inhibition in 10% xylitol. Bacterial growth was nearly completely inhibited by 10% xylitol for *F. nucleatum subsp. nucleatum*, *C. ochracea, C. sputigena,* and *T. denticola.* At 15% xylitol, all the periodontopathogens tested showed complete bacterial growth inhibition (Fig. 2).

Comparison of bacterial growth inhibition at late log phase

To compare the growth inhibitory effect of xylitol at various concentrations, each bacterial growth inhibition was determined at the late log phase (Fig. 3). In the presence

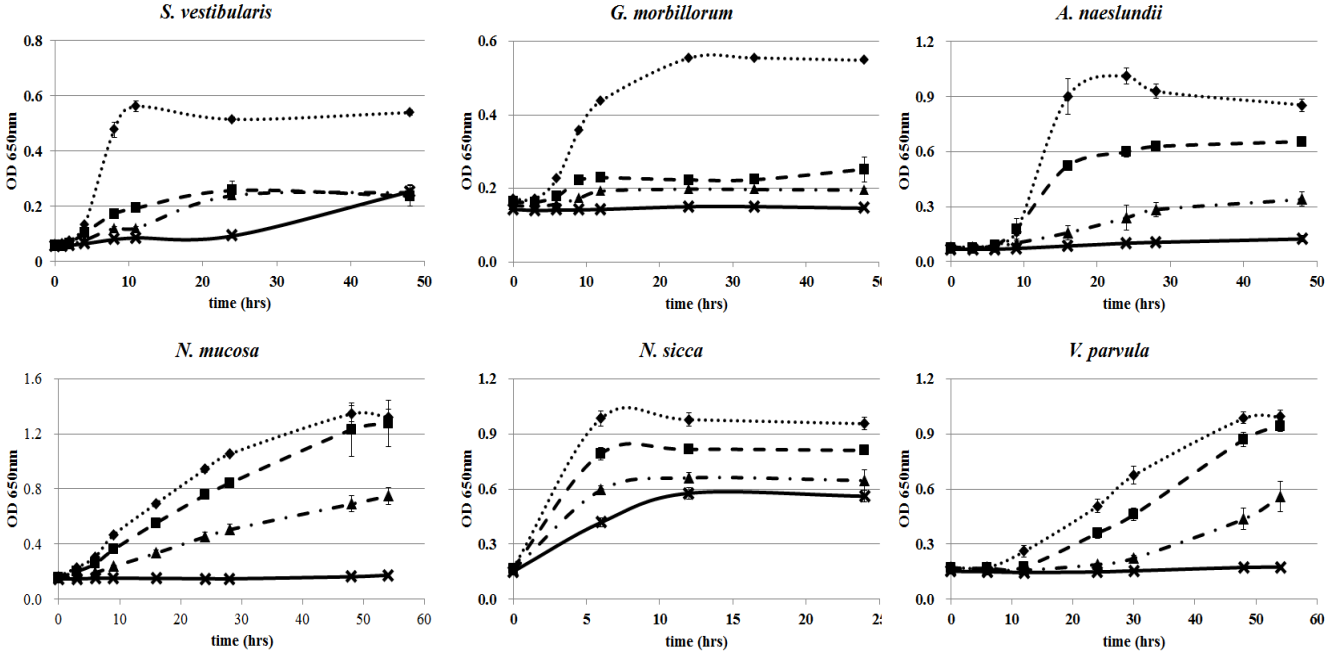

Figure 1. Growth of normal oral bacteria in terms of OD counts, in media, 5% xylitol, 10% xylitol, and 15% xylitol. The OD of each tube was measured at a wavelength of 650 nm with a spectrophotometer against the standard medium, with the measurements being performed during the bacterial growth. The OD results were calculated as the means of at least three measurements. Symboles :♦, no xylitol; ■, 5% xylitol; ▲, 10% xylitol; ✖, 15% xylitol.

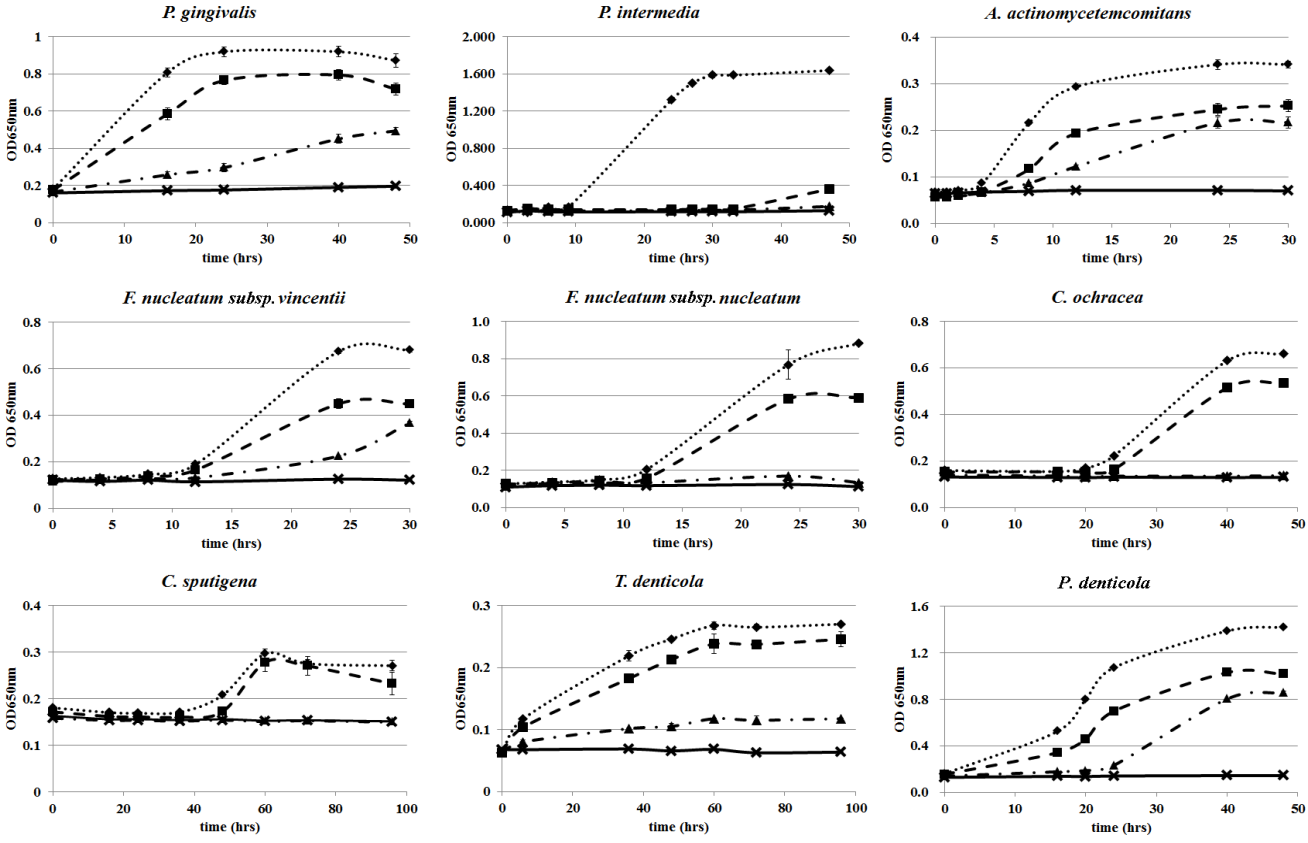

Figure 2. Growth of periodontopathogens in terms of OD counts, in media, 5% xylitol, 10% xylitol, and 15% xylitol. The OD of each tube was measured at a wavelength of 650 nm with a spectrophotometer against the standard medium, with the measurements being performed during the bacterial growth. The OD results were calculated as the means of at least three measurements. Symboles :♦, no xylitol; ■, 5% xylitol; ▲, 10% xylitol; ✖, 15% xylitol.

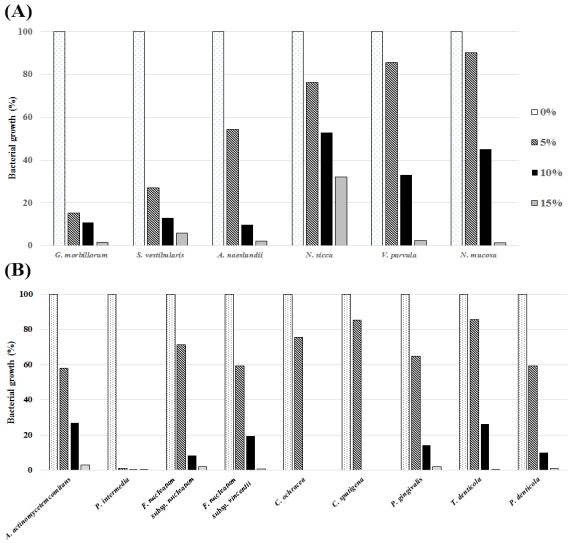

Figure 3. (A) Inhibition of the normal oral bacterial growth was determined at the late log phage. (B) Inhibition of the periodontopathogen growth was determined at the late log phage. Bacterial growth percentage was calculated by (OD 650 nm without xylitol- OD 650nm with xylitol) x100 / (OD 650 nm read without xylitol).

of 5 % xylitol, *G. morbillorum, S. vestibularis* and *P. intermedia* showed bacteria growth inhibition more than 60%, suggesting high susceptibility to xylitol. In the presence of 10% xylitol, *A. naeslundii* and all the periodontopathogens tested showed marked bacterial growth inhibition, suggesting moderate susceptibility to xylitol.

Discussion

While most of studies focus on the inhibitory effect of xylitol on *S. mutans* which is the major cariogenic bacteria in oral cavity, there are only limited number of reports that include other oral bacteria [8, 16]. Thus, in this study, we examined the inhibitory effect of xylitol on both oral bacteria frequently found in healthy subject and periodontopathogens.

Our results indicate that xylitol markedly reduced the growth of various oral bacteria in a dose dependent

manner and these inhibitory effects were statistically significant throughout the exponential growth phase. Among oral bacteria mostly found in healthy subjects, *S. vestibularis* and *G. morbillorum* showed marked growth inhibition (more than 50%) with 5% xylitol during logarithmic phase. *A. naeslundii* showed marked growth inhibition with 10% xylitol and mild growth inhibition (20~ 50%) with 5% xylitol. *N. mucosa, N. sicca,* and *V. parvula* showed resistance to 5% xylitol and mild growth inhibition with 10% xylitol suggesting high resistance to xylitol.

Among periodontopathogens which frequently found in periodontitis, *P. intermedia* showed almost complete growth inhibition with 5% xylitol, suggesting high sensitivity to xylitol. With 5% xylitol, the growth inhibition of other periodontopathogens tested was less than 50%. However, with 10% xylitol, marked growth inhibition was observed in all the periodontopathogens tested. With 10% xylitol, more selective growth inhibitory effect on periodontopathogens is expected, which indicates that long-lasting xylitol delivery influences oral microbiota population.

Xylitol is widely used in candy industry. Chewing gum and hard candy are considered ideal vehicles for providing a long-lasting effect in oral cavity. In our preliminary study, xylitol concentration up to 15% was well tolerated by volunteers (unpublished data). The xylitol concentrations of 5, 10, and 15% used in this study can be easily achieved, at least temporarily, in the saliva and on the mucous membranes.

The inhibitory mechanisms of xylitol on the growth of oral bacteria are unclear. Comparing the growth curves and bacterial growth inhibition at late log phase suggests the possibility of some common futile xylitol cycle between the bacterial species. The bacterial growth rate or bacterial genus seems to be of minor importance for the xylitol sensitivity.

During the transition from periodontal healthy state to periodontal disease, a well characterized oral microbial shift is frequently described in periodontitis [17]. Several bacterial complexes associated with periodontitis have been identified. Three bacterial species which are designated as 'red-complex' are *P. gingivalis*, *T. forsythus*, and *T. denticola*. They have been well recognized for the strong association with periodontitis [11]. Other bacteria which have been also suggested to be involved in periodontitis include *A. actinomytemcomitans, C. ochracea, C. sputigena, P. denticola*, *P. intermedia*, and *F. nucleatum* [12, 18]. The most effective and most widely used treatment is physical removal of pathogenic dental plaque biofilm by scaling and root planning. However, restoring the microbial shift to healthy microbial state or preventing the pathogenic microbial transition should be better than the treatment after the occurrence of the disease.

In this study, we have only tested the effect of xylitol against single bacterial species at each experiment. Several non-periodontopathogenic oral bacteria showed relatively higher resistance 10% xylitol suggesting the possibility of selectively restoring healthy oral microbial population.

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