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Effect of Xylitol on Growth of Nasopharyngeal Bacteria In Vitro

TERO KONTIOKARI,[1]* MATTI UHARI,[1] AND MARKKU KOSKELA[2]

Department of Pediatrics[1] and Division of Microbiology,[2] University of Oulu, Fin-90220 Oulu, Finland

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Xylitol is known to reduce caries by inhibiting the growth of *Streptococcus mutans*. We hypothesized that xylitol could also affect the growth of other nasopharyngeal bacterial flora, which could be important when considering respiratory infections caused by these bacteria. We studied this in vitro by adding xylitol to the medium and observed that 1 and 5% xylitol reduced markedly the growth of alpha-hemolytic streptococci, including *S. pneumoniae*. It reduced slightly the growth of beta-hemolytic streptococci but not that of *Haemophilus influenzae* or *Moraxella catarrhalis*. The inhibitory growth pattern was similar to that previously seen with *S. mutans*, which may indicate a similarity in the enzymatic processing of five-carbon sugars such as xylitol. This sugar alcohol is a widely used sweetener, and the concentrations used in our experiments are easily achieved in the oral cavity. If xylitol reduces the growth of *S. pneumoniae* in the nasopharynx, it could also reduce the carriage of this pathogen and thus have clinical significance in the prevention of pneumococcal diseases.

Being a five-carbon sugar alcohol, xylitol is not fermented by most oral microorganisms, such as *Streptococcus mutans* (3), although most *S. mutans* strains are able to transport xylitol into the cell via the fructose phosphotransferase system (1a). Once in the cell, it is phosphorylated to xylitol-5-phosphate, which then has to be expelled from the cell (16). This futile energy-consuming xylitol cycle is thought to be responsible for the inhibition of *S. mutans* growth observed both in vitro and in vivo when the bacteria are exposed to xylitol (16).

It has been shown on several occasions that regular consumption of xylitol reduces the incidence of caries, although the mechanisms are not clearly understood (2, 6, 9, 14, 15). The most significant effect so far demonstrated is its ability to reduce the growth and acid production of *S. mutans*. Research into caries has not shown any other major changes in the oral flora, except occasionally a reduction in other oral streptococci (1a, 10, 13, 18).

We hypothesized that xylitol could also affect the growth of other bacteria in the nasopharyngeal flora, which could be important when considering respiratory infections caused by these bacteria. *S. pneumoniae*, in particular, could make use of xylitol in a manner similar to that of *S. mutans*. To test our hypothesis, we evaluated the growth of some strains of nasopharyngeal bacteria when xylitol was added to the medium.

MATERIALS AND METHODS

Bacterial strains. Ten strains of *S. pneumoniae* and *Haemophilus influenzae* and five strains of *Moraxella* (*Branhamella*) *catarrhalis* were isolated from consecutive routine patient middle ear effusion samples or maxillary sinus aspirates. Five strains of *S. pyogenes* and *S. mitis* and four strains of group G streptococci were isolated from throat samples of asymptomatic children. Pneumococci, *H. influenzae*, *M. catarrhalis*, and beta-hemolytic streptococci were selected because of their ability to cause upper respiratory tract infections, and *S. mitis* was included as an alpha-hemolytic streptococcus of the normal nasopharyngeal flora. *S. pneumoniae*, *M. catarrhalis*, beta-hemolytic streptococci, and *S. mitis* were subcultured on sheep blood agar plates, and *H. influenzae* was subcultured on chocolate blood agar plates. *S. mutans* NCTC 10449, kindly supplied by E. Söderling (Department of Biochemistry, Institute of Dentistry, University of Turku, Turku, Finland), was used as a positive control strain. The strain had been obtained in the logarithmic phase of growth in Trypticase soy broth and was stored deep frozen at −80°C until used.

Media and cultivation of bacteria. For growth inhibition measurements, *S. mutans* was cultured in brain heart infusion medium (Difco Laboratories, Detroit, Mich.) supplemented with hemin and NAD (SR 158; Oxoid, Unipath Ltd., Hampshire, England). To ensure optimal growth of the other bacteria, 10% (vol/vol) human serum was added. Xylitol (Sigma Chemical Co., St. Louis, Mo.) was added to the basic medium in the appropriate concentrations and sterilized by filtration (Minisart NML 0.2-μm-pore-size filter; Millipore Corp., Bedford, Mass.). The test medium contained 1 or 5% (wt/vol) xylitol, while the corresponding control medium was free of xylitol. Each strain was cultured aerobically in the appropriate basic medium at 37°C up to the exponential phase of growth. Three hundred microliters of this suspension was transferred into 3 ml of medium without xylitol (control) or containing 1 or 5% xylitol. The test tubes were incubated aerobically at 37°C for 1 to 4 days. Each test was carried out in triplicate.

Measurements. The optical density (O.D.) of each tube was measured at a wavelength of 650 nm with an SFM 35 spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) against the appropriate basic medium. The measurements were performed at 1- to 3-h intervals during the logarithmic phase of growth, and the samples were shaken properly before each measurement. The O.D. results were calculated as means of three measurements. To verify the relationship between O.D. and the total number of viable cells, viability counts were done by the standard dilution method on sheep blood or chocolate blood (*H. influenzae*) agar plates at two O.D. points during the logarithmic phase of growth.

Statistical analyses. One-way analysis of variance was used to test the differences in O.D. between the groups at each measurement time. When there was a significant difference, the mean values for the groups were further analyzed with the unpaired *t* test.

RESULTS

The presence of 1 and 5% xylitol resulted in 35 and 72% inhibition of *S. pneumoniae* growth, respectively, at 2 h of incubation and 39 and 51% inhibition at 6 h (Fig. 1). The control strains began to break up during the 24 h of cultivation, a phenomenon that is typical of *S. pneumoniae* as cultures age and is assumed to be mediated by autolytic enzymes. There was a statistically significant difference between the groups at 2 to 6 h of incubation ($P < 0.001$), i.e., during the logarithmic phase of growth, but the difference was no longer significant at 17 h ($P = 0.74$).

The growth of *S. mitis* was inhibited similarly: growth inhibition of 59 and 72% at 16 h of incubation and 32 and 53% at 18 h (Fig. 2). The difference between the groups was statisti-

* Corresponding author. Mailing address: Department of Pediatrics, University of Oulu, Fin-90220 Oulu, Finland. Phone: 358 81 3155101. Fax: 358 81 3155559.

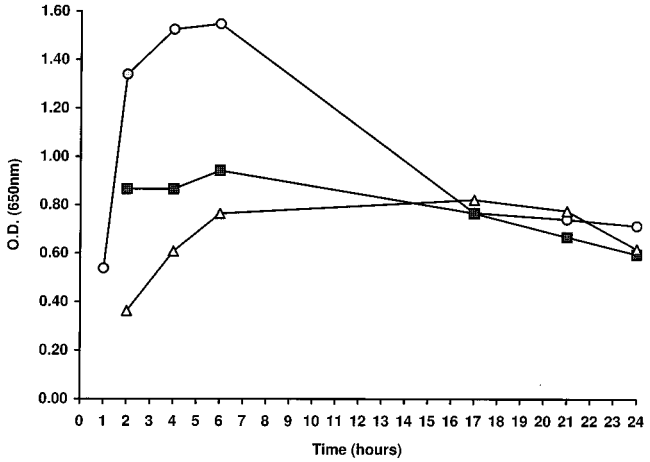

FIG. 1. In vitro growth of *S. pneumoniae*. Addition of 1 and 5% xylitol resulted in 35 and 72% growth inhibition, respectively at 2 h of incubation and 39 and 51% inhibition at 6 h. The difference between the groups treated with xylitol and the control group was statistically significant throughout the exponential growth phase (hours 2, 4, and 6; $P < 0.001$). The bacterial strains in the control group began to break up at the end of the logarithmic growth phase, a phenomenon typical of rapidly dividing *S. pneumoniae*. Symbols: ○, no xylitol; ■, 1% xylitol; △, 5% xylitol.

cally significant at 14 to 24 h of incubation ($P = <0.001$ to 0.04), except at 24 h, at which time the difference between the control and 1% xylitol group was no longer significant ($P = 0.68$).

Group A and G beta-hemolytic streptococci were analyzed together because of their similar growth patterns. There was no difference between the groups until the end of the logarithmic phase, when a slight inhibition of 10% by 5% xylitol was observed ($P = 0.007$) (Fig. 3). At 8 to 24 h of incubation, the difference between the control and 5% xylitol groups remained statistically significant ($P = 0.001$ to 0.02).

Xylitol did not affect the growth of either *H. influenzae* or *M. catarrhalis*, even at a concentration of 5% ($P = 0.14$ to 0.94) (Fig. 4 and 5).

In both 1 and 5% xylitol solutions, the *S. mutans* positive

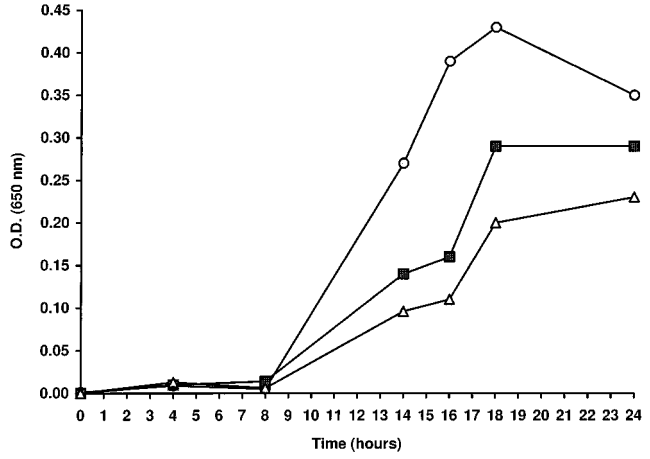

FIG. 2. In vitro growth of *S. mitis*. Addition of 1 and 5% xylitol resulted in 59 and 72% growth inhibition, respectively, at 16 h of incubation and 32 and 53% inhibition at 18 h. The difference between the groups treated with xylitol and the control group was statistically significant throughout the exponential growth phase ($P = <0.001$ to 0.04). The symbols are the same as in Fig. 1.

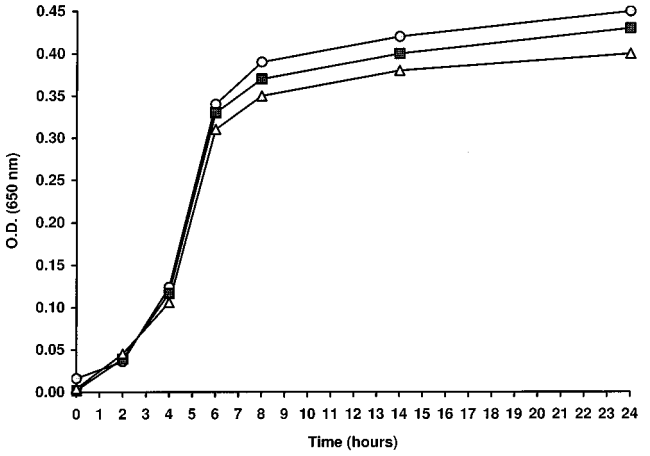

FIG. 3. In vitro growth of beta-hemolytic streptococci, four strains of group G streptococci, and five strains of group A streptococci. In the presence of 5% xylitol, slight growth inhibition was observed after the exponential phase ($P = 0.001$ to 0.02). The symbols are the same as in Fig. 1.

control showed clear inhibition of growth, with maxima of 60 and 78%, respectively (Fig. 6). Differences remained at this level for at least 12 h after the logarithmic phase of growth and seemed to be dose dependent.

O.D. values and numbers of CFU per milliliter showed a good correlation: O.D. values of 0.4 and 0.8 U were equal to 2×10^5 and 2×10^9 CFU of *S. pneumoniae* per ml, respectively; O.D. values of 0.2 and 0.4 U were equal to 1×10^4 and 5×10^6 CFU of *S. mitis* per ml, respectively; O.D. values of 0.2 and 0.4 U were equal to 2×10^4 and 2×10^6 CFU of beta-hemolytic streptococci per ml, respectively; O.D. values of 0.3 and 0.6 U were equal to 5×10^6 and 1×10^8 CFU of *H. influenzae* per ml, respectively; and O.D. values of 0.3 and 0.6 U were equal to 1×10^5 and 6×10^7 CFU of *M. catarrhalis* per ml, respectively.

DISCUSSION

Our results indicate that xylitol markedly reduced the growth of *S. pneumoniae* and *S. mitis* during the logarithmic phase and that this effect increased with increasing concentra-

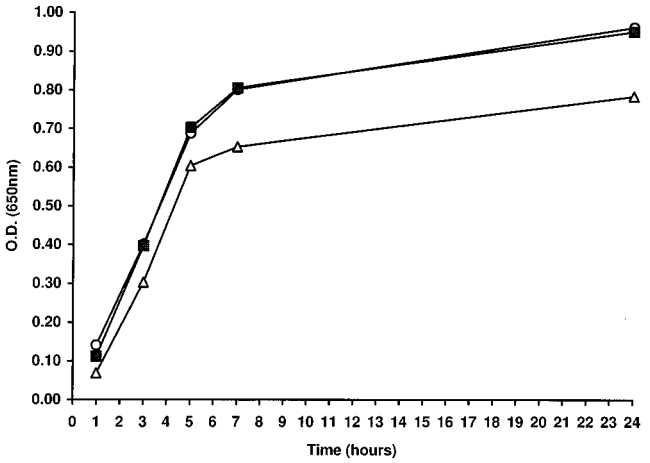

FIG. 4. In vitro growth of *H. influenzae*. One or 5% xylitol was added to the basic medium. No statistically significant growth inhibition was observed during the logarithmic phase or during 24 h of incubation ($P = 0.15$ to 0.64). The symbols are the same as in Fig. 1.

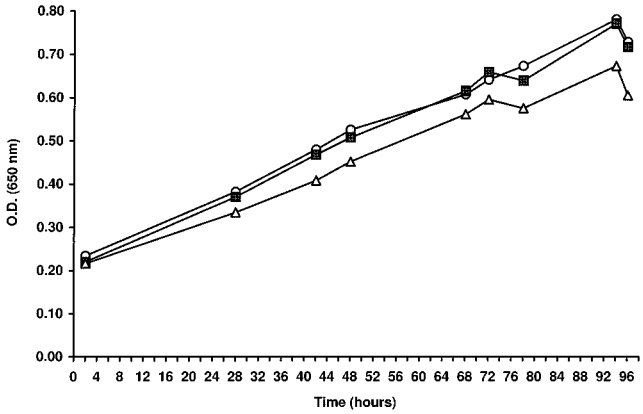

FIG. 5. In vitro growth of *M. catarrhalis*. One or 5% xylitol was added to the basic medium. No statistically significant growth inhibition was observed during 96 h of incubation ($P = 0.14$ to 0.94). The symbols are the same as in Fig. 1.

tions and was statistically significant throughout the exponential growth phase with both 1 and 5% solutions. Slight postexponential inhibition of growth of beta-hemolytic streptococci by 5% xylitol was observed. No statistically significant inhibition of *H. influenzae* or *M. catarrhalis* growth was seen. The growth pattern of *S. mutans* was found to resemble that reported previously (13, 16, 18).

The advantage of xylitol for caries prevention is well documented and is, for the most part, connected with a reduction in the growth of *S. mutans*. This is, in turn, caused by the energy-demanding xylitol transport system (16). It has been shown that the anticariogenic effect of xylitol lasts for several years after the practice of consuming xylitol daily has been discontinued and is associated partly with the reduction in the total levels of *S. mutans* and partly with the decrease in the amount and adhesiveness of dental plaque (8). Some studies have failed to document any long-term decrease in the amount of *S. mutans* bacteria, however (13, 19), and this has been explained by the selection of bacterial strains: habitual use of xylitol may select *S. mutans* strains which are up to 87% nonexpressive of the fructose phosphotransferase enzyme (17) and are thought to have lower adhesiveness to enamel and, therefore, lower virulence (7, 12).

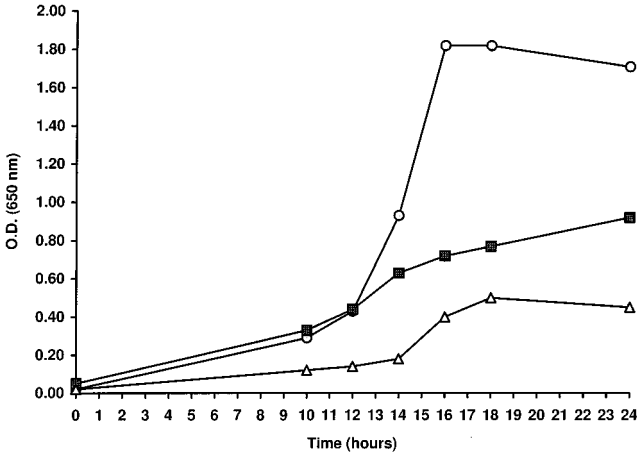

FIG. 6. In vitro growth of *S. mutans* NCTC 10449. One or 5% xylitol was added to the basic medium, resulting in growth inhibition similar to that reported previously (13, 16, 18). The symbols are the same as in Fig. 1.

The mechanisms of inhibition of other alpha-hemolytic streptococci are unclear, although similarity in growth curves indicates the possibility of a similar futile xylitol cycle. The growth-inhibitory effect of xylitol on beta-hemolytic streptococci differs from that on alpha-hemolytic strains and seems to be of minor importance.

Xylitol is widely used in Scandinavia as a sweetener, especially in the candy industry and in dentistry products. When attempting to provide a long-lasting effect in the oral cavity, chewing gum and hard candy offer ideal vehicles. Since one piece of chewing gum contains about 0.5 g of xylitol and there is less than 10 ml of saliva in the mouth at a time, the xylitol concentrations of 1 and 5% used in this study can be easily achieved, at least temporarily, in the saliva and on the mucous membranes. Xylitol is usually well tolerated as a sweetener, although absorption from the gut is slow and therefore osmotic diarrhea is possible. Adults can tolerate doses of up to 200 g per day (11) without symptoms, and children can tolerate 45 g per day (1). Most experiments have used xylitol concentrations of 0.5 to 6% (13, 16, 18). Caries prevention is achieved with doses of 4 to 20 g per day (6, 14, 15).

S. pneumoniae is the most common bacterium causing middle ear infections or sinusitis, and nasopharyngeal carriage of this bacterium has been shown to be a predisposing factor (4). Pneumococcal infections have been easy to treat with penicillin, but the appearance of multiresistant *S. pneumoniae* strains, as carried and spread by children in day care centers in particular (5), has altered this situation. If xylitol reduces the growth of *S. pneumoniae* in the nasopharynx, it could also reduce carriage of the bacteria and thus be of clinical significance in preventing pneumococcal diseases.

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