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Xylitol Sensitivity among Oral Streptococci

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Xylitol is a five-carbon sugar alcohol that inhibits the growth of oral streptococci, including *Streptococcus mutans*. In this study, we tested xylitol sensitivity among the oral streptococci. We also compared nucleotide homology of putative fructose phosphotransferase system (PTS) and xylitol sensitivity, since xylitol is transported via the fructose PTS. Among the tested Streptococci, *S. pneumonia* showed the highest resistance to xylitol while *S. gordonii* and *S. sanguinis* showed the most sensitive growth inhibition. These streptococci could be grouped according to their xylitol sensitivity. *S. mutans* and *S. salivarius* showed similar bacterial growth inhibition by xylitol. *S. mitis*, *S. oralis, S. pneumonia, S. intermedius* and *S. anginosus* showed relatively low sensitivity to xylitol. When the genetic homologies of five fructose PTSs were compared among the tested streptococci, closely related streptococci showed similar sensitivity to xylitol. Taken together, fructose PTSs may mediate the sensitivity to xylitol in oral streptococci.

Key words: oral bacteria, *Streptococcus*, xylitol, fructose phosphotransferase system

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Introduction

Oral streptococci constitute the dominant population in supragingival dental plaque and are involved in a number of human diseases, such as dental caries, meningitis, brain abscess, and endocarditis, usually as opportunistic pathogens [1,2]. Oral streptococci can be divided into five distinct groups based on 16S rRNA sequences analysis: Mutans group, Salivarius group, Anginosus group, Sanguinis group and Mitis group [3,4].

Oral streptococci are dependent on sugars as an energy source. Since the free sugar concentration in the oral cavity is often low, the main energy supply for the oral streptococci is carbohydrates in dietary food [5]. In order to compete successfully with the other oral bacteria, oral streptococci must economize on expending energy for acquiring and metabolizing of sugars. They should thus select the most appropriate energy sources and take up the preferred sugars from the external milieu. Studies have demonstrated that the physiological mechanisms involved in controlling carbohydrate utilization and metabolism of bacteria involve multiple regulatory mechanisms [6]. Phosphoenylphyruvate (PEP): phosphotransferase system (PTS) is one of the most well studied systems for the regulation of sugar metabolisms in bacteria [7].

Xylitol is a naturally occurring five-carbon sugar alcohol that have been widely used as sugar substitutes in chewing

gums and confectionary products. Xylitol is non-cariogenic since it is not fermented by most oral microorganisms [8,9]. The mechanism of action was suggested to be an energy-consuming cycle related to the uptake and phosphorylation of xylitol to xylitol 5-phosphate at the expense of phosphoenolpyruvate via the PEP:PTS in *S. mutans* and the subsequent dephosphorylation and expulsion of xylitol [10-12]. However, the inhibitory effects of xylitol on the growth of oral microorganisms have been attributed mainly to its interference with *Streptococcus mutans* [13-15].

In this study, we examined xylitol sensitivity against several oral streptococci and further compared the correlation between the nucleotide homology of predicted fructose PTS system and xylitol sensitivity.

Materials and Methods

Oral streptococci and growth conditions

Representative strains of oral streptococci were selected to study the inhibitory effect of xylitol. For mitis group, *S. oralis* (ATCC 35037), *S. mitis* (KCTC 5638), and *S. pneumoniae* (KCTC 5412) were used. For sanguinis group, *S. sanguinis* (ATCC 10556), and *S. gordonii* (KCTC 3286) were used. For mutans group, *S. mutans* (ATCC 25157) was used. For salivarius group, *S. salivarius* (ATCC 19258) was used. For anginosus group, *S. anginosus* (KCTC 3983) and *S. intermedius* (KCTC 3268) were used. All strains were obtained from the American Type Culture Collection (ATCC) or KCTC (Korean Collection for Type Cultures). Xylitol (Sigma Chemical Co., St. Louis, Mo.) was sterilized by filtration (0.22-μm-pore size, Millipore, Billerica, MA, USA) and added aseptically to the medium to a final concentration of 5%, 10% or 15%. Each strain was cultured in BHI at 37 °C in a 5% CO_2 atmosphere up to the late log phase of growth (optical density [OD], 1). Ten microliters of each strain were diluted into 1 ml of test medium containing various xylitol concentrations. The OD of each tube was measured at a wavelength of 650 nm with a spectrophotometer (Molecular device, Sunnyvale, CA, USA) 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.

Nucleotide sequence analysis

Database searches were performed with BLAST at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). To compare the nucleotide sequence among the bacteria strains, one of each strain whose complete genome sequence are available were used in the analysis.

Results

Effect of Xylitol on Streptococci

Streptococci are classified into several groups including mitis, sanguinis, mutans, salivarius, anginosus and pyogenic group. In this study, we focused on Streptococcus mainly found in oral cavity. Among the tested Streptococcus, *S. pneumonia* showed the highest resistance to the xylitol while *S. gordonii* and *S. sanguinis* showed the most sensitive growth inhibition. Fast growing Streptococci including *S. anginosus, S. mitis, S. mutans* and *S. salivarius* showed delayed bacterial growth in the presence of xylitol. Slow growing Streptococci including *S. intermedius, S. oralis,* and *S. sanguinis* showed sustained growth inhibition in the presence of xylitol (Fig. 1).

Comparison of Bacterial Growth inhibition at Late Log phase

To compare the growth inhibition effect of xylitol at various concentrations, each bacterial growth inhibition was determined at the late log phase (Fig. 2). In the presence of 5 % xylitol, *S. pneumoniae, S. intermedius, S. anginosus, S. mutans* and *S. salivarius* showed less than 50% of growth inhibition while *S. gordonii* and *S. sanguinis* showed more than 75% of growth inhibition. In the presence of 10% xylitol, *S. pneumoniae* and *S. intermedius* showed less than 50% of growth inhibition while *S. mutans, S. salivarius, S gordonii* and *S. sanguinis* showed more than 75% of growth inhibition. Finally, in the presence of 15% xylitol, only *S. pneumoniae* showed less than 50% of growth inhibition while other tested Streptococcus showed more than 75% of growth inhibition.

Comparison of putative fructose PTS systems in various Streptococcus species.

Since xylitol is taken up by fructose PTS, putative

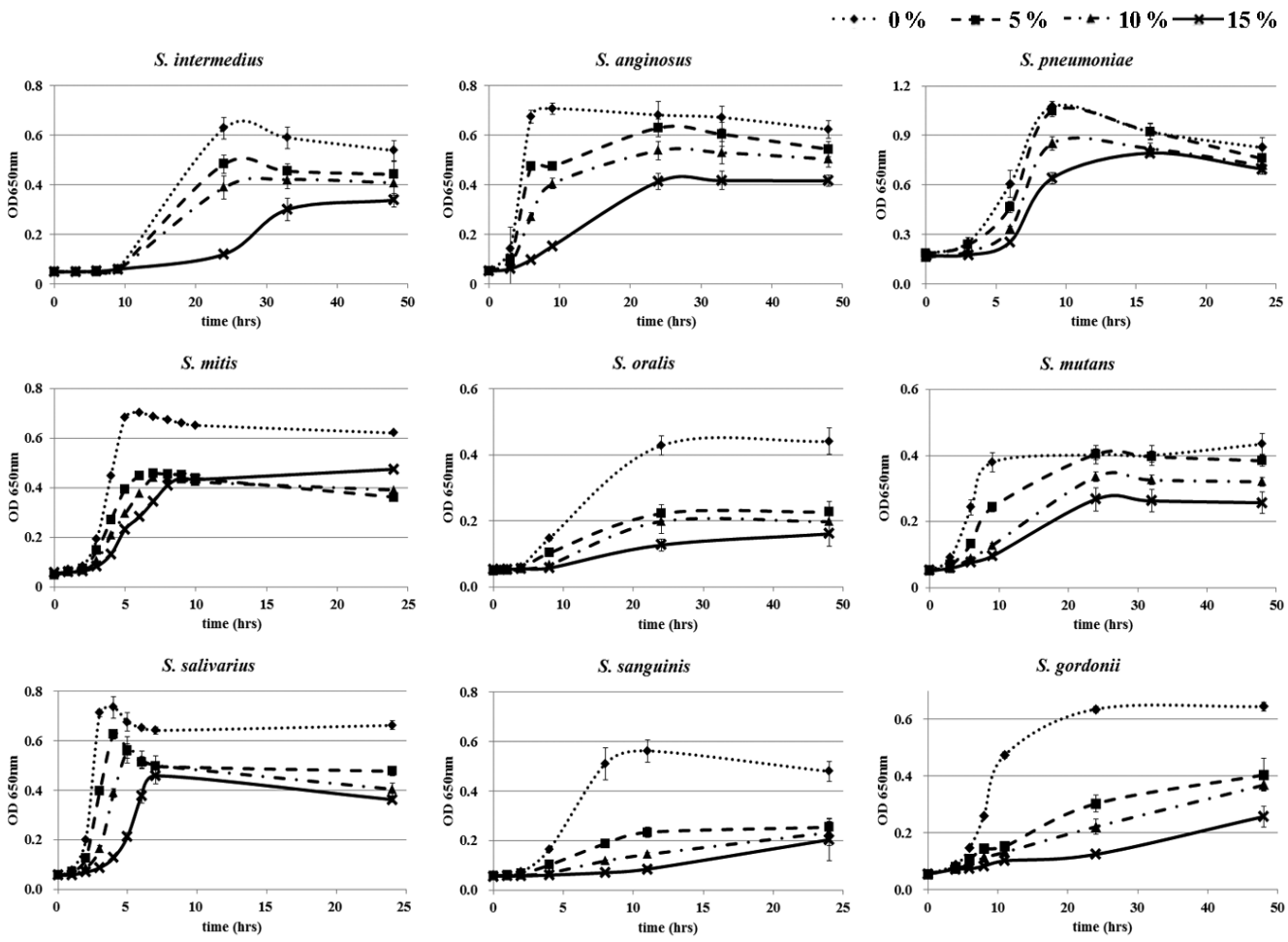

Figure 1. Growth of Streptococci measured in terms of OD counts, in BHI and BHI containing 5% xylitol, 10% xylitol, and 15% xylitol. The OD of each tube was measured at a wavelength of 650 nm with a spectrophotometer against the standard medium, with the measurements being performed during the bacterial growth. The OD results were calculated as the means of at least three measurements.

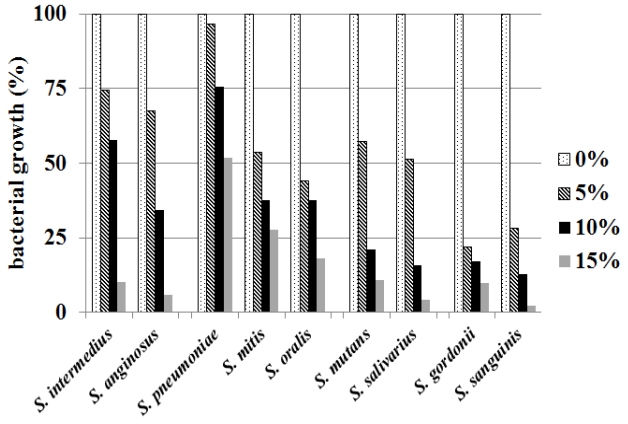

Figure 2. Inhibition of bacterial growth was determined at the late log phase. Bacterial growth was calculated by [(OD 650 nm read in each xylitol concentration)x100 / OD 650 nm read in BHI].

fructose PTS of the tested Streptococci were searched with BLAST at the National Center for Biotechnology Information. Among various putative fructose PTSs, five clusters were commonly found and were selected for the comparison. All five clusters were found in *S. intermedius* and it was selected to compare the homology with other Streptococci.

Among five clusters, cluster II, III and IV were found in all the tested Streptococci. Comparing the distance tree, several groups were found, even though they were closely related. *S. salivarius* and *S. mutans* were relatively far from other Streptococci. Cluster III which includes *ptsI* and *ptsH* showed higher nucleotide homology compared to other clusters suggesting that *ptsI* and *ptsH* are highly conserved within Streptococci and these proteins may serve

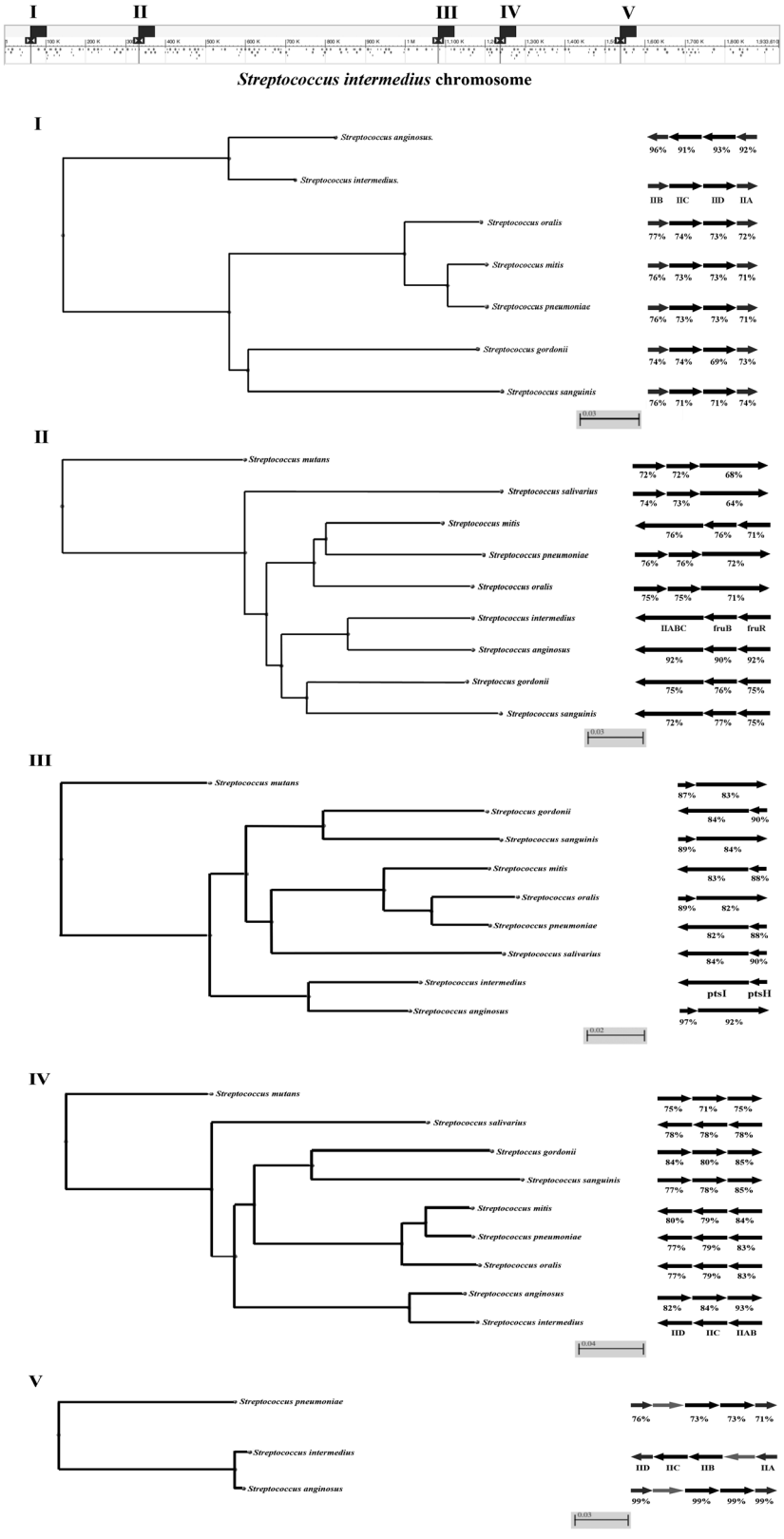

Figure 3. Comparison of putative fructose PTSs in various oral Streptococci. Related proteins are linked indicating the different levels of nucleotide identity, expressed as percentages.

as a common pathway for metabolizing the substrate.

Cluster I was not found in *S. mutans* and *S. salivarius*. The distance within cluster I was similar to cluster II, III and IV. Cluster V was only found in *S. intermedius, S. anginosus* and *S. pneumoniae*, which showed relatively high resistance to xylitol suggesting that this cluster may mediate the selectivity for fructose and xylitol. Taken together, there might be close correlation between the nucleotide homology of putative fructose PTS and sensitivity to the xylitol.

Discussion

In this study, we examined xylitol sensitivity against several oral streptococci. Among the tested Streptococci, sanguinis group including *S. gordonii* and *S. sanguinis* showed high sensitivity to 5% xylitol. At 10% xylitol, *S. mutans* and *S. salivarius* showed more than 75% of bacterial growth inhibition. *S. mitis*, *S. oralis* and *S. anginosus* showed similar xylitol sensitivity at the tested xylitol concentration. *S. intermedius*, which showed relatively slow bacterial growth compared to *S. anginosus*, were more resistant to 10% xylitol. However, at 15% xylitol, anginosus group were highly sensitive to xylitol. At 15% xylitol, mitis group showed the highest resistance to xylitol among the tested groups. *S. pneumoniae* showed only 50% of bacterial growth inhibition at 15% xylitol (Fig. 1 and Fig. 2). This result suggests that there is close correlation between xylitol sensitivity and Streptococcus group. To rule out the effect of osmotic pressre, we have grown the oral streptococci in broth containing various concentration of fructose. Even 15% fructose did not inhibit the growth of the tested oral bacteria (data not shown). Thus, we further compared nucleotide homology of genes suggested to be included in xylitol metabolism among the tested Streptococci.

Through the nucleotide search provided by the NCBI, we searched for genes associated with xylitol metabolism including fructose PTS and mannose PTS.

The PEP:PTS is the primary sugar transport system in oral streptococci, especially under carbohydrate-limiting conditions, and plays important roles in global control of gene expression [7,16,17]. The PTS consists of two proteins that are common to all PTS substrates, enzyme I (EI) and the heat-stable phosphocarrier protein HPr, as well as a variety of sugar-specific permeases, known as EII complexes, which catalyze the transport and concomitant phosphorylation of the substrate [18]. The EII complexes usually consist of three domains, A, B, and C, but sometimes a fourth domain, D, is required [16,17]. The A and B domains participate in phosphorylation of the cognate substrates, whereas the C and D domains comprise the membrane permeases [19]. Total of 5 gene clusters from cluster I to cluster V, all found in *S. intermedius*, were selected for homology search. Among 5 clusters, cluster II, III and IV were found in all the tested streptococci.

Cluster II and IV include genes related with fructose transport and concomitant phosphorylation. Cluster II include IIABC, fruB and fruR. FruR is well known as a pleiotropic transcriptional regulatory protein controlling the expression of numerous operons concerned with carbon metabolism. FruR binds to operators within the regulatory region preceding the structural genes of the fructose operon, including fruB [20]. FruB is also known as fructose-1-phosphate kinase which transfers phosphoryl group from ATP to fructose-6-phosphate, yielding fructose 1,6-bisphosphate [21]. The nucleotide homology of IIABC in cluster II ranged from 64% to 92% in our analyses. FruR and fruB showed similar homology compared to the following IIABC gene. Cluster IV includes IIAB, IIC, and IID. The homology of IIAB, C, and D in cluster IV ranged from 71% to 93%. Cluster IV showed higher nucleotide similarity compared to cluster II.

Cluster III includes ptsI and ptsH which forms two general energy-coupling proteins, enzyme I and HPr of PTS. These catalyzes the concomitant transport and phosphorylation of its sugar substrates in a process termed group translocation [22-24]. Compared to other clusters, the nucleotide homology of *ptsI* (82~92%) and *ptsH* (88~97%) among the tested Streptococci was higher, suggesting that this proteins may serve as a common pathway for metabolizing the substrate.

The overall nucleotide homology comparison among the tested Strepotococci indicates that there is close correlation between the nucleotide homology of fructose PTS system and sensitivity to the xylitol. The selectivity of II complex for fructose and xylitol may have played the central roll. Nucleotide seqences of the related PTS genes that differ

among the Streptococci may result in different peptide sequence and protein structure. Thus, further structural comparison of PTSs proteins among the Streptococci may explain xylitol sensitivity difference.

Acknowledgements

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Conflict of interest

The authors declare that they have no competing interest.

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