

## RESEARCH ARTICLE

# Low Xylitol Concentration induces Ultrastructural Cellular Changes in *Streptococcus mutans* and *Streptococcus sobrinus*

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## ABSTRACT

**Background:** Xylitol is a sugar alcohol frequently used in the prevention of dental caries. However, the mechanism of action of xylitol on *mutans streptococci* is still unclear. This study evaluated the ultrastructural cellular changes in *Streptococcus mutans* and *Streptococcus sobrinus* after xylitol treatment.

**Materials and methods:** *S. mutans* ATCC 25175 and *S. sobrinus* ATCC 33478 were grown separately in BHI medium plus 0.2% sucrose at 37°C under microaerophilic conditions overnight. Then, planktonic cells were harvested by centrifugation, resuspended in a fresh medium and incubated with either 0.2% glucose or 0.2% xylitol solutions for 2 hours. To prepare bacterial cells for the evaluation of morphology of cellular walls and polysaccharide capsules, cultures were subsequently fixed with 2.5% glutaraldehyde, stained with ruthenium red, post-fixed with 1% osmium tetroxide, dehydrated in acetone, embedded in Epon LX112 and analyzed by a transmission electron microscopy.

**Results:** Bacterial cells incubated with glucose showed well-defined cellular walls and well-stained cytoplasmic membrane. Unlike, xylitol yielded diffuse cellular wall and irregular polysaccharide capsules in both microorganisms.

**Conclusion:** Low xylitol concentrations also can induce cellular damage to both *S. mutans* and *S. sobrinus*. Further studies are needed to provide more evidence on the anticariogenic effect of xylitol.

**Keywords:** Xylitol, *Streptococcus mutans*, *Streptococcus sobrinus*, Microscopy, Transmission.

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## INTRODUCTION

*Streptococcus mutans* and *Streptococcus sobrinus* have been strongly implicated as the main etiological agents in human dental caries. These microorganisms are able to form biofilm and produce weak organic acids, leading to mineral loss and progression of dental caries lesions.<sup>1</sup>

Mutans streptococci can incorporate but not metabolize xylitol, a five-carbon sugar alcohol frequently found in chewing gums and oral healthcare products. The consumption of xylitol-containing products might decrease the growth of mutans streptococci and its acid production;<sup>2</sup> however, the mechanism of action of xylitol on pathogenic bacteria is still unclear. Theoretically, xylitol is phosphorylated, dephosphorylated and expelled from the cells without producing energy.<sup>3</sup> This 'futile cycle' promotes the depletion of intracellular metabolic activity and a lower production of adherent extracellular polysaccharides and lipoteichoic acids, resulting in the formation of loosely attached biofilms to tooth surfaces.<sup>3</sup>

Tapiainen et al<sup>4</sup> and Tuompo et al<sup>5</sup> have reported respectively more diffuse and damaged *Streptococcus pneumoniae* cells and autolysis of *S. mutans* after treatment with xylitol solutions. The effectiveness of xylitol against microorganisms depends on its concentration. To our knowledge, however, the effects of <0.38% xylitol on the ultrastructure of mutans streptococci have not yet been investigated. Besides, there is no evidence on the effect of xylitol on the cellular morphology of *S. sobrinus*. This study aimed to evaluate the effect of 0.2% xylitol on the ultrastructure of *S. mutans* and *S. sobrinus*.

## MATERIALS AND METHODS

Laboratory of Microbiology of Bauru School of Dentistry, Bauru, Brazil. Pure stock solutions of *S. mutans* ATCC 25175 and *S. sobrinus* ATCC 33478 were grown

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separately in Brain Heart Infusion (BHI) Medium (Difco Laboratories, Detroit, MI, USA) plus 0.2% sucrose at 37°C under microaerophilic conditions overnight (OD $\approx$ 0.3-0.4 at 650 nm, Spectrophotometer Ultrospec 2000) (Pharmacia Biotech, Cambridge, England). Cultures were harvested by centrifugation and re-suspended in a fresh medium. Each cell suspension (1 ml) was incubated with a final concentration of 0.2% glucose (control) or xylitol (test). Planktonic cells were incubated in 5% CO<sub>2</sub> at 37°C for additional 2 hours. Then, bacterial cells were harvested by centrifugation (3,615 xg, 10 min) (Eppendorf, Hamburg, Germany) from a volume of 1.5 ml and directly prepared to ultrastructural analysis by transmission electron microscopy.

To ultrastructural analysis, the bacterial pellets were fixed overnight at 78°C with 1 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer containing 100 ml of ruthenium red (Sigma Chemical Co, St Louis, USA) for staining. It was then washed three times in phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon LX112. Thin sections were cut with the ultramicrotome Leica Ultracut—UC2 (Leica Microsystems, Wetzlar, Germany) and examined with the transmission electron microscope Zeiss EM900 (A = 30,000) (Zeiss, Munich, Germany) for a blinded examiner. One hundred cells of each microorganism were evaluated to describe the cellular morphology changes. All experiments were performed in triplicate.

## RESULTS

Qualitative ultrastructural analysis of *S. mutans* and *S. sobrinus* after 0.2% xylitol and 0.2% glucose treatments are shown in Figures 1 and 2. Glucose solution did not yield morphological changes in both streptococci. Right

side of Figures 1 and 2 show well-defined cell walls and well-stained cytoplasmic membrane of *S. mutans* and *S. sobrinus* respectively.

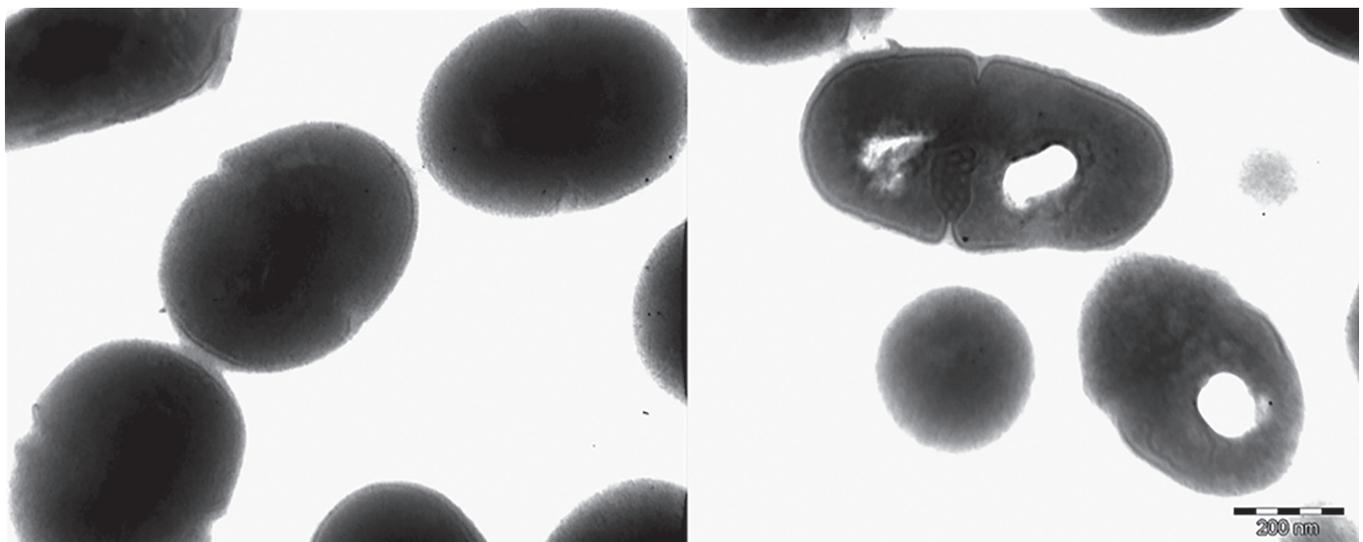
General morphology of bacterial cells was damaged for xylitol solutions. *Streptococcus mutans* and *S. sobrinus* treated with 0.2% xylitol showed more dispersed and unequal polysaccharide capsules, sloughing, intracellular vacuoles, and autolysis (left side of Figs 1 and 2). Xylitol made the cell walls of both microorganisms more diffuse and less defined when compared to control groups.

## DISCUSSION

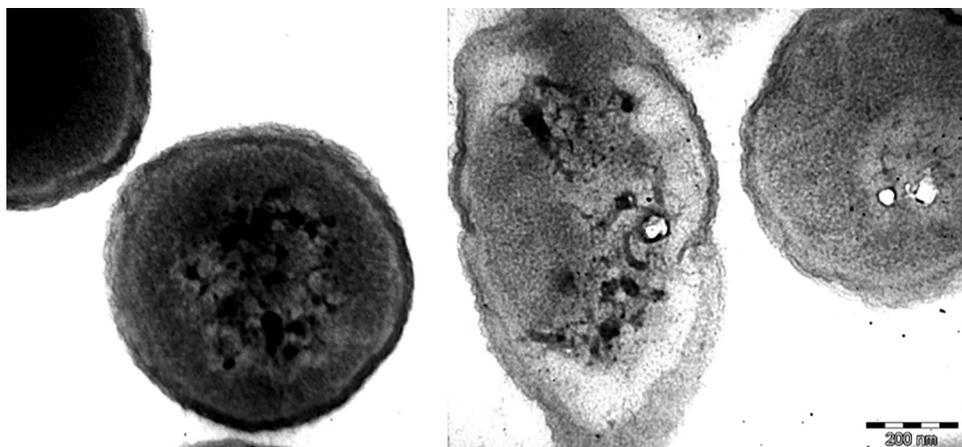
This study demonstrated that 0.2% xylitol yields more dispersed and unequal polysaccharide capsules, sloughing, intracellular vacuoles and autolysis in planktonic cells of *S. mutans* and *S. sobrinus*. Tuompo et al<sup>5</sup> also reported cellular damages in *S. mutans* after incubation in 50 mg/ml xylitol solution. Tapiainen et al<sup>4</sup> described similar effects of 0.5% xylitol on the ultrastructure of *S. pneumoniae*.

To compare the cellular morphology of bacteria from control and test groups, strains were respectively incubated in 0.2% glucose or 0.2% xylitol for 2 hours. This methodological approach were in accordance with Tapiainen et al<sup>4</sup> microorganisms from control group were exposed to glucose based on previous results of Tuompo et al<sup>5</sup> that demonstrated the absence of alterations in the ultrastructure of *S. mutans* after glucose and sucrose treatments. In addition, the authors described autolysis and intracellular vacuoles as common findings among bacterial cells treated with xylitol, which also can be observed in this study.

The intracellular accumulation of xylitol phosphate is responsible for inhibiting many intermediaries of



**Fig. 1:** Ultrastructural cellular morphology of *S. sobrinus* ATCC 33478 after 0.2% glucose (left side) and 0.2% xylitol (right side) treatments



**Fig. 2:** Ultrastructural cellular morphology of *S. mutans* ATCC 25175 after 0.2% glucose (left side) and 0.2% xylitol (right side) treatments

glycolytic pathway, such as glucose-6-phosphate, fructose-6-phosphate and glyceraldehyde-3-phosphate.<sup>3</sup> These intermediaries are essential to enzymatic activation and, therefore, their inhibition prevents the metabolism of xylitol and energy production. Morphological cellular changes lead to reduced survival, virulence and adherence of microorganisms,<sup>6</sup> which might explain the clinical benefits of xylitol on the prevention of dental caries. Söderling et al<sup>7</sup> demonstrated a dose-dependent growth inhibition of *S. mutans* after exposure to xylitol.

Currently, the clinical efficacy of xylitol in the prevention of dental caries is related to the maintenance of minimum salivary levels >1%. However, this concentration can be detected in saliva for approximately 15 minutes after consumption of xylitol-containing products.<sup>8</sup> The evidence that lower xylitol concentrations can affect ultrastructure of cariogenic microorganisms might promote the development of slow-release vehicles, which are able to maintain therapeutic xylitol concentrations in microenvironments, such as polymeric matrix and fluid of dental biofilm, for longer.

In summary, xylitol demonstrated its potential as a caries-preventive treatment by promoting ultrastructural cellular changes in planktonic cells of *S. mutans* and *S. sobrinus*. Further studies are needed to elucidate the effects of xylitol on the ultrastructure of bacterial cells from biofilms and possible mechanisms of antimicrobial action of xylitol on oral microorganisms.

## CONCLUSION

However, dental caries is a multifactorial disease that starts with microbiological shifts within the complex biofilm (dental plaque) and this may be one of the important variables for dental caries risk assessment.<sup>9</sup> Dentistry is

varying with induction of modern science to practice dentistry.<sup>10</sup>

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