# The Effect of Xylitol on the Growth of Three Normal Oral Commensal or Probiotic Bacteria

BY

LAURA WALTERS

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| Philip Whalen | Karen Aguirre |
| Director, Honors Program | Thesis Advisor |
| | Michael Roberts |
| | Honors 499 Professor |

**Abstract**

Xylitol is a pentitol often used as a sweetener in products such as chewing gum. It is recommended to prevent dental caries because of its inhibitory effect on the most common etiological agent of caries, *Streptococcus mutans*. Xylitol inhibits the growth of *S. mutans* by inhibiting its glycolysis, causing a futile cycle. It also inhibits the adhesion of *S. mutans* by reducing the expression of the gene *gtfB*, which causes the secretion of sticky substances. The purpose of this study was to investigate the effect of xylitol on normal oral bacteria, particularly commensal and probiotic strains. The strains tested were *Streptococcus salivarius*, *Lactococcus lactis*, and *Lactobacillus casei*. The strains were tested on agar and in solution at different concentrations of xylitol, .5%, 2.5%, 5%, and 10%. Transmittance of the solutions was recorded to determine differences in the amount of bacteria present. Xylitol was found to have an inhibitory effect on *S. mutans* as reported in addition to an inhibitory effect on *L. lactis*. *S. salivarius* and *L. casei* were only affected by xylitol at the highest concentrations tested, 5% and 10% xylitol.

**Introduction**

Xylitol is a 5-carbon polyol sugar alcohol (Söderling & Hietala-Lenkkeri, 2010). It is a non-cariogenic sweetener that can be found in chewing gums, tablets, dentrifice, and oral rinses (Holgerson, Stecksen-Blicks, Sjostrom, Oberg & Twetman, 2006). It can also be found in fruits and plants (Makinen, Saag, Isotupa, Olak, Nommela, Soderling, & Makinen, 2003). Xylitol is recommended as a preventative measure to reduce dental caries (Milgrom, Roberts, Rothen, Mueller, & Yamaguchi, 2006), an infectious disease which causes cavities (Makinen et al., 2003). Xylitol has been shown to reduce plaque accumulation by decreasing *gtfB* expression,

which causes a decrease in production of sticky substances. It also reduces the production of metabolic acid, lowers the growth and metabolism of Streptococci by generating an energy-consuming futile cycle, and contributes to the remineralization of tooth surfaces by forming a xylitol calcium complex (Lee, Choi, Jeong, Kim, Lee, & Song, 2008). It has also been shown to reduce mother-child transmission of *Streptococcus mutans* in pregnant woman (Lee et al., 2008). When introduced in starch and sucrose diets, xylitol has been shown to cause significant reduction in the area of dentinal carious lesions (Hietala & Larma, 1995).

*Streptococcus mutans* has been found to be the primary etiological agent of dental caries (Söderling & Hietala-Lenkkeri, 2010). Glucosyltransferases (GTF) and glucan-binding proteins contribute to the sucrose-dependant adherence of *S. mutans* (Söderling & Hietala-Lenkkeri, 2010). Xylitol inhibits the growth, acid production, and adherence of *S. mutans* by inhibiting glycolysis and the production of sticky substances in the bacteria (Söderling & Hietala-Lenkkeri, 2010). The inhibition is caused by the intracellular accumulation of xylitol-5-phosphate, which is the result of a futile cycle generated by the presence of xylitol. Xylitol is taken in by *S. mutans* and phosphorylated to xylitol-5-phosphate, then dephosphorylated and expelled as xylitol (Lee et al., 2008). Altered *gtfB* gene expression due to xylitol also causes morphological changes in *S. mutans*. *S. mutans* inhibited with xylitol develops lamellated cytoplasmic membranes and intracellular vacuoles. The colonies are smaller in size and smoother than those grown in the absence of xylitol. Colonies grown in the presence of xylitol produce less sticky substances than those grown without xylitol, another result of the reduced expression of *gtfB* (Lee et al., 2008).

Increased exposure to xylitol decreases *S. mutans* concentration in plaque, with the ideal dose being between 6.88 grams and 10.32 grams. Salivary levels of *S. mutans* can be reduced to a lesser extent with a similar dose (Milgrom et al., 2006). Use of xylitol containing products

such as chewing gum, sucking tablets, candy, toothpaste, and oral rinses has been shown to cause significant elevations in xylitol concentration for up to 16 minutes. Xylitol rinses containing 6.0 grams of xylitol can cause an increase in xylitol concentrations in plaque for up to 30 minutes (Holgerson et al., 2006). Concentrations of about 10 milligrams per milliliter are needed for xylitol to have an antibiotic effect (Holgerson et al., 2006). Xylitol concentrations as low as .1% have been shown to significantly inhibit most strains of *S. mutans*. (Soderling, Ekman & Taipale, 2008).

Xylitol reduces dental plaque accumulation on teeth, reducing the accumulation of *S. mutans* on tooth surfaces. It can reduce plaque up to 50% (Iwata, Nakagaki, Morita, Sekiya, Goshima, Abe, Isogai, Hanaki, Kuwahara, Tatematsu, & Robinson, 2003). Xylitol also increases salivary flow, which helps to remove acid producing bacteria from teeth, resulting in a higher pH. This causes an increase in the amount of soluble calcium in dental plaque, which increases enamel remineralization (Iwata et al., 2003). Streptococci can adapt so that they still produce acid in the presence of xylitol, but because of xylitol's ability to inhibit adhesion, the amount of plaque and *S. mutans* will still be decreased (Söderling & Hietala-Lenkkeri, 2010).

Other sugar alcohols can also effectively inhibit oral bacteria. Concentrations of 4% erythritol have been shown to reduce adhesion of oral polysaccharide-forming streptococci and inhibit growth by more than 2% (Söderling & Hietala-Lenkkeri, 2010). Erythritol has been shown to have an inhibitory effect even after 4 and 5 hour cultivation. Erythritol, a tetritol or sugar alcohol with 4 carbons, and xylitol, a pentitol or sugar alcohol with 5 carbons, are both effective in reducing plaque and presence of *S. mutans* in plaque and saliva (Makinen et al., 2003).

Xylitol can also have an inhibitory effect on other oral and nasopharyngeal bacteria in addition to *S. mutans*. Lactobacillus has been shown to be inhibited by xylitol, and also to adapt and produce acid when xylitol is present (Richard, Castaing-Debat, de Flaujac, & Dorignac, 2004). Xylitol also inhibits *Streptococcus pneumoniae, Haemophilus influenza*e, and *Moraxella catarrhalis*. The inhibition of these bacteria makes xylitol effective in preventing acute otitis media, or chronic ear infection (Uhari, Tapiainen, & Kontiokari, 2001).

There are over 700 bacterial species found in the oral cavity, more than in any other part of the human body. This includes many commensal species and species that are used as probiotics (Kolenbrander, Jakubovics, & Bachrach, 2009). Commensal bacteria are bacteria that have evolved with the host and are beneficial to the host. *Streptococcus salivarius* and *Lactococcus lactis* are both commensal bacteria found in breast milk. Both are also common oral bacteria. They have been found to inhibit *Staphylococcus aureus* infections and *L. lactis* has been shown to produce nicin. Nicin is a bacteriocin, or a toxin with inhibits similar bacteria. Nicin is used to prevent bacterial pathogens in the food industry (Heikkilä & Saris, 2003). *S. salivarius*, along with other oral commensals, plays an important role in the suppression of pathogenic yeast like *Candida albicans* in human buccal epithelial cells (Nair & Samaranayake, 1996).

Probiotics are living microbes used as a diet supplement in order to improve the microbial balance in the host (Aryana & McGrew, 2007). *S. salivarius* strain K12 is used as an oral probiotic. It produces bacteriocins that make it useful in inhibiting *Streptococcus pyogenes* and other bacteria thought to cause halitosis (Burton, Wescombe, Moore, Chilcott, & Tagg, 2006). It also expresses urease in low pH conditions in the mouth, resulting in the hydrolysis of urea into carbon dioxide and ammonia and ultimately a raised pH. Elevation of the pH creates

an environment that is not conducive for acidogenic bacteria like *S. mutans* (Chen, Hall, & Burne 1998). Studies show that *L. lactis* has the potential to be an effective oral and gastrointestinal probiotic because of its ability to adhere to cells and survive in the intestine (Kimoto, Kurisaki, Tsuji, Ohmomo & Okamoto, 1999). *Lactobacillus casei* is another common oral microbe used as a probiotc. It has been shown to support healthy cellular function and immune system response while promoting healthy bacteria and modifying harmful bacterial activities (Aryana & McGrew, 2007). *L. casei* has potential as a treatment to maintain remission in Crohn's disease patients because it has been shown to inhibit the adhesion and invasion of adherent invasive *Escherichia coli*, which is usually present in abnormally large amounts in Crohn's disease patients (Ingrassia, Leplingard, & Darfeuille-Michaud, 2004).

The purpose of this study was to investigate the effect of xylitol on *Streptococcus salivarius*, *Lactococcus lactis*, and *Lactobacillus casei*, three beneficial bacterial strains, in addition to *Streptococcus mutans*, a pathogenic bacterium, in order to determine whether or not xylitol has a detrimental effect on normal oral flora. Inhibition of commensal and probiotic bacterial strains could have an adverse effect on oral health overall. These strains are important in inhibiting pathogenic strains of yeast and bacteria and in maintaining a healthy microbial balance. If xylitol was found to have an inhibitory effect on normal commensal oral microbes then usage of xylitol for its benefits, inhibition of *S. mutans* growth and adhesion, could eventually result in reduced oral health. The absence of commensal bacteria would eventually result in less competition for pathogens like *S. mutans*, allowing them to flourish.

**Methods**

Freeze dried *S. mutans*, *L. lactis*¸*S. salivarius*, and *L. casei* were purchased from Ward's Natural Science with transfer and growth media. The pellets were resuspended with .5 milliliters of the liquid transfer medium provided with each, Brain-Heart Infusion broth for *S. mutans*, Tryptic Soy broth for *L. lactis* and *S. salivarius* and tomato juice broth for *L. casei*. The bacteria were subcultured onto the appropriate agar slants provided using sterile swabs. The subcultures were incubated at 37$^{o}$C for one week. A solution of 1% xylitol was prepared using 300 milliliters of distilled water and 3 grams of xylitol. The solution was sterile filtered. Cells from the subcultures were removed and resuspended in 250 microliters of distilled water. One hundred microliters of this solution were added to a solution containing 150 microliters of 1% xylitol and 150 microliters of distilled water and to 300 microliters of distilled water as a control. Fifty microliters of the xylitol-containing solution were added to 3 wells in a 6 well culture plate and 50 microliters of the non-xylitol control solution were added to the other 3 wells. *S. mutans* and *L. casei* were plated on Brain-Heart Infusion agar while *S. salivarius* and *L. lactis* were plated on tryptic soy agar. Each plate was placed in a Bio Bag Type A from BD Diagnostic Systems with one anaerobic indicator, one anaerobic generator, and one catalyst container. The Bio Bag was placed inside a sealed Ziploc bag. The indicator and generator were crushed to create an anaerobic environment and the sealed bags were stored in a 37$^{o}$C incubator for one week. This procedure was repeated with new *S. mutans* and *L. lactis* cultures.

Three 50 milliliter xylitol solutions were prepared at concentrations of 1%, 5% and 10% xylitol. They were prepared with 50 milliliters of distilled water and .5 grams, 2.5 grams, or 5 grams of xylitol, respectively. Each solution was sterile filtered. Cells were obtained from the *S. mutans* subculture and resuspended in 1.4 milliliters of distilled water. Three hundred and fifty

microliters of the solution were added to microcentrifuge tubes containing 350 microliters of either distilled water, 1% xylitol solution, 5% xylitol solution, or 10% xylitol solution. Fifty microliters of each solution were added to 3 wells in a 96 well plate containing BHI agar. The process was repeated with each bacterial strain, then repeated in another BHI plate and two plates containing Tryptic Soy agar. One BHI plate and one TSA plate were placed in Bio Bags to create an anaerobic environment. The other two plates were incubated aerobically. The four plates were incubated at 37$^{o}$C for one week. The plates were observed and the solution was extracted from each well to observe growth on the agar under a microscope.

Three 50 milliliter xylitol solutions were prepared at concentrations of 1%, 5% and 10% xylitol. They were prepared with 50 milliliters of distilled water and .5 grams, 2.5 grams, or 5 grams of xylitol, respectively. Each solution was sterile filtered. Cultures of each of the four bacterial strains, *S. mutans, S. salivarius, L. lactis,* and *L. casei* were prepared in approximately .5 milliliters of Brain-Heart Infusion broth. 20 sterile cuvettes were labeled and 2.5 milliliters of water or xylitol solution at each concentration were added to 5 cuvettes each. One hundred microliters of the *S. mutans* culture was added to one cuvette of each concentration. This process was repeated with the *L. lactis, S. salivarius,* and *L. casei* cultures, leaving one cuvettee of each concentration with no bacteria as a control. 2.5 milliliters of BHI broth were added to each of the 20 samples, making the final concentrations 0% xylitol, .5% xylitol, 2.5% xylitol, and 5% xylitol. Table 1 shows the contents of each cuvette. Initial transmittance readings at 900 nanometers were recorded using a spectrophotometer with the control sample containing only water and BHI broth set at 100% T. The samples were stored at 37$^{o}$C with shaking. Final readings of transmittance at 900 nanometers were recorded at 72 hours. The control sample containing only water and BHI broth was set at 100% T.

Table 1. Contents of each sample in experiment 3, trial 1.

| **Sample Number** | **Bacterium** | **% Xylitol** | **Sample Number** | **Bacterium** | **% Xylitol** |
|---|---|---|---|---|---|
| 1 | *S. mutans* | 0 | 11 | *S. salivarius* | 2.5 |
| 2 | *S. mutans* | .5 | 12 | *S. salivarius* | 5 |
| 3 | *S. mutans* | 2.5 | 13 | *L. casei* | 0 |
| 4 | *S. mutans* | 5 | 14 | *L. casei* | .5 |
| 5 | *L. lactis* | 0 | 15 | *L. casei* | 2.5 |
| 6 | *L. lactis* | .5 | 16 | *L. casei* | 5 |
| 7 | *L. lactis* | 2.5 | 17 | Control | 0 |
| 8 | *L. lactis* | 5 | 18 | Control | .5 |
| 9 | *S. salivarius* | 0 | 19 | Control | 2.5 |
| 10 | *S. salivarius* | .5 | 20 | Control | 5 |

The second trial was an expanded version of the first. Xylitol solutions of 1%, 5%, 10% and 20% xylitol were prepared. 25 sterile cuvettes were set up with 2.5 milliliters of water, 1% xylitol, 5% xylitol, 10% xylitol, or 20% xylitol and .1 milliliter of each bacterial culture was added one cuvette of each concentration, leaving 5 for control samples. 2.5 milliliters of BHI broth were added to each sample, making the final xylitol concentrations .5%, 2.5%, 5%, and 10%. Table 2 shows the contents of each sample. Initial transmittance readings were taken at 900 nanometers. The control sample containing only water and BHI broth was set at 100% T. The samples were stored at 37$^{o}$C with shaking. Final readings of %T were taken at 900 nm with the water and BHI broth control sample set at 100%T.

Table 2. Contents of each sample in experiment 3, trial 2.

| Sample Number | Bacterium | % Xylitol | Sample Number | Bacterium | % Xylitol |
|---|---|---|---|---|---|
| 1 | *S. mutans* | 0 | 14 | *S. salivarius* | 5 |
| 2 | *S. mutans* | .5 | 15 | *S. salivarius* | 10 |
| 3 | *S. mutans* | 2.5 | 16 | *L. casei* | 0 |
| 4 | *S. mutans* | 5 | 17 | *L. casei* | .5 |
| 5 | *S. mutans* | 10 | 18 | *L. casei* | 2.5 |
| 6 | *L. lactis* | 0 | 19 | *L. casei* | 5 |
| 7 | *L. lactis* | .5 | 20 | *L. casei* | 10 |
| 8 | *L. lactis* | 2.5 | 21 | Control | 0 |
| 9 | *L. lactis* | 5 | 22 | Control | .5 |
| 10 | *L. lactis* | 10 | 23 | Control | 2.5 |
| 11 | *S. salivarius* | 0 | 24 | Control | 5 |
| 12 | *S. salivarius* | .5 | 25 | Control | 10 |
| 13 | *S. salivarius* | 2.5 | | | |

**Results**

After the first experiment, the *L. casei* and *S. salivarius* plates showed some growth. Neither bacterium appeared to be inhibited by the 1% xylitol solution. After lengthy incubation, the *L. lactis* plate showed some growth but the *S. mutans* was not successfully cultured in this experiment. The results were not analyzed because the *S. mutans*, the control organism, did not grow.

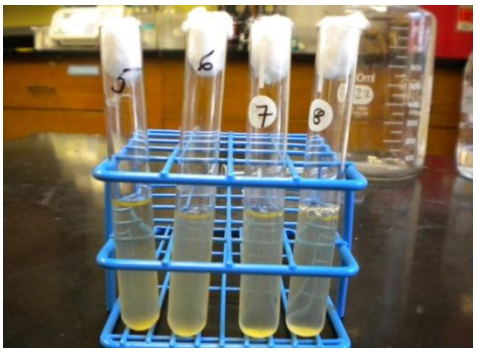
The bacteria grew successfully in the second experiment on each plate. However, we were unable to analyze the results because of possible contamination. There was growth in wells that no bacteria had been added to. The majority of the growth was in solution, so once the solution was extracted it was difficult to determine if there was growth inhibition based on growth on the agar.

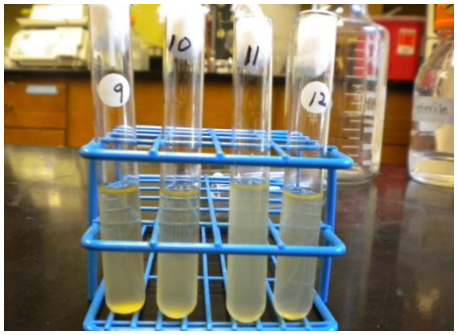
The third experiment yielded usable results. Figure 1 shows the samples after 72 hours. The *S. mutans* samples containing 2.5% and 5% xylitol contained visibly less bacteria than the *S. mutans* control and .5% xylitol samples. There was also a visible decrease in turbidity in the *L. lactis* samples corresponding with increased xylitol concentration. There was no visible decrease in the *S. salivarius* and *L. casei* samples. Figure 2 shows the change in transmittance with increased xylitol concentration for each bacterium. Transmittance increased greatly between 0% and .5% xylitol and between .5% and 2.5% xylitol for *S. mutans*. The increase was present but smaller between 2.5% and 5% xylitol. *L. lactis* showed a slight decrease in transmittance between 0 and .5% xylitol followed by a sharp increase from .5% to 2.5% xylitol. The inhibition seemed to reach a peak between 2.5% and 5% xylitol. *S. salivarius* showed a very slight increase from 0 to 2.5% xylitol with a greater increase between 2.5% and 5% xylitol. *L. casei* showed a decrease in transmittance between 0 and .5% xylitol with very slight increases from .5% to 5% xylitol. The transmittance at time zero and 72 hours are shown in table 3.

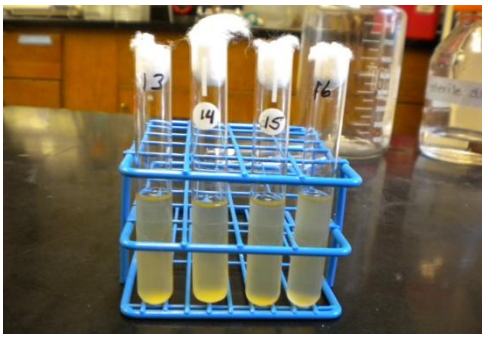
Table 3. Transmittance (%T) values before and after trial 1.

| **Tube Number** | **Time Zero** | **72 Hours** | **Tube Number** | **Time Zero** | **72 Hours** |
|---|---|---|---|---|---|
| 1 | 101.0 | 10.0 | 11 | 98.6 | 16.0 |
| 2 | 99.4 | 46.00 | 12 | 101.8 | 36.0 |
| 3 | 99.6 | 83.6 | 13 | 99.2 | -6.4 |
| 4 | 100.2 | 97.4 | 14 | 100.6 | -8.8 |
| 5 | 99.4 | 17.4 | 15 | 99.6 | 4.6 |
| 6 | 99.8 | 11.0 | 16 | 99.6 | 8.6 |
| 7 | 101.0 | 59.8 | 17 | 100.0 | 100.0 |
| 8 | 98.4 | 60.0 | 18 | 99.4 | 98.2 |
| 9 | 99.8 | 14.4 | 19 | 100.2 | 99.6 |
| 10 | 99.2 | 14.2 | 20 | 101.6 | 99.4 |

A. 


B. 


C. 


D. 


E. 


Figure 1. Samples after 72 hours. (A) *S. mutans*, (B) *L. lactis*, (C) *S. salivarius*, (D) *L. casei*, (E) Control samples.

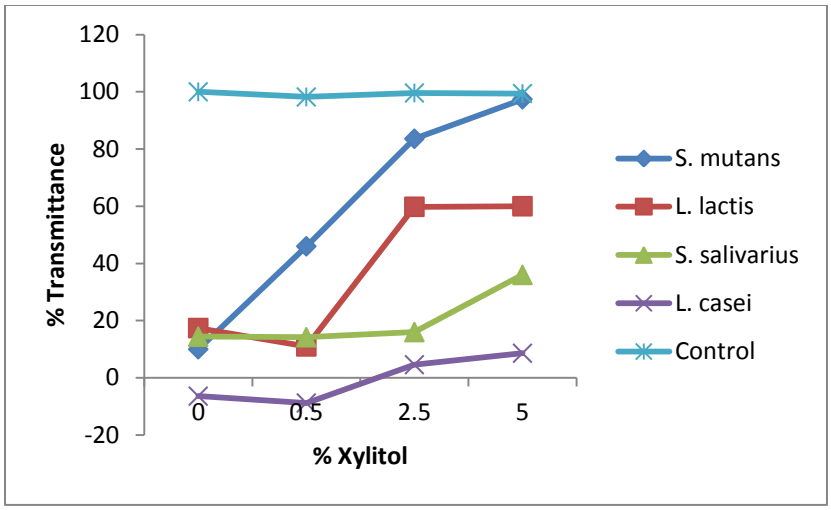



Figure 2. Transmittance readings of each sample from Trial 1 at 72 hours.

The second trial showed similar results. None of the samples showed a visible decrease in turbidity, but the transmittance values increased with each xylitol concentration. Figure 3 shows the change in transmittance with increased xylitol concentration for each bacterium. *S. mutans* showed a slight increase in transmittance between 0 and .5% xylitol with a greater increase between .5% and 2.5% xylitol. Between 2.5% and 5% xylitol transmittance seemed to reach a peak, and it decreased slightly at 10%. For *L. lactis* there was a slight increase in transmittance between 0 and .5% xylitol, and a slight decrease from .5% to 2.5% xylitol. There was a very small increase in transmittance from 2.5% to 5% xylitol followed by a very small decrease from 5% to 10%. *S. salivarius* showed a slight decrease from 0 to .5% followed by a very sharp decrease from .5% to 2.5%, the only outlier in the experiment. From 2.5% to 5% there was a sharp increase in transmittance, followed by a smaller increase from 5% to 10%. *L. casei* showed a slight decrease from 0 to .5% xylitol followed by slight increases between .5% and 5% xylitol. At 5% xylitol transmittance seemed to reach a peak and decreased slightly at 10% xylitol. Table 4 shows the transmittance values before and after incubation.

Table 4. Transmittance (%T) readings before and after trial 2. Sample 17 was the only outlier in the experiment.

| Tube Number | Time Zero | 48 Hours | Tube Number | Time Zero | 48 Hours |
|---|---|---|---|---|---|
| 1 | 94.4 | 14.2 | 14 | 90.8 | 48.8 |
| 2 | 97.6 | 17.2 | 15 | 93.4 | 51.0 |
| 3 | 96.8 | 29.8 | 16 | 78.4 | 13.8 |
| 4 | 95.2 | 31.8 | 17 | 77.4 | 9.8 |
| 5 | 97.2 | 28.2 | 18 | 77.0 | 14.2 |
| 6 | 95.4 | 38.2 | 19 | 74.8 | 20.2 |
| 7 | 93.2 | 47.2 | 20 | 78.2 | 18.8 |
| 8 | 94.4 | 38.0 | 21 | 100.0 | 100.0 |
| 9 | 85.2 | 41.4 | 22 | 99.0 | 100.0 |
| 10 | 94.0 | 37.5 | 23 | 96.8 | 97.6 |
| 11 | 92.4 | 50.8 | 24 | 95.6 | 97.6 |
| 12 | 91.6 | 38.2 | 25 | 97.2 | 97.8 |
| 13 | 80.6 | 9.2 | | | |

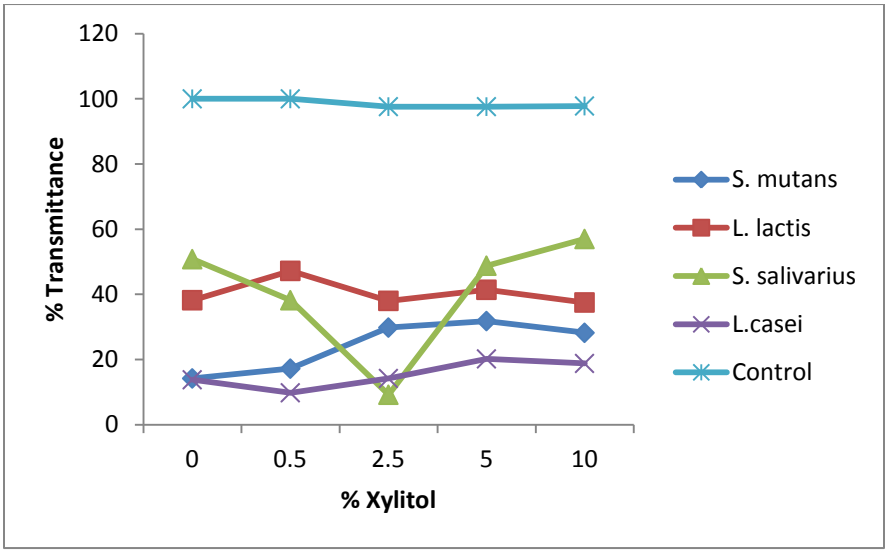



Figure 3. Transmittance readings of each sample from Trial 2 at 24 hours.

**Discussion**

The results of the first two experiments did not yield analyzable information. The effect of the 1% xylitol solution on *L. casei*, *S. salivarius*, and *L. lactis* could not be determined

because *S. mutans*, the control organism, could not be successfully cultured, so we were unable to determine whether the solution would have inhibited *S. mutans*. The *L. lactis* plate was incubated for three weeks before visible growth was present. At that point the growth was likely due to fungal contamination. The plates were too large for the Bio Bags to be heat sealed according to the instructions. The manufacturers informed us that the anaerobic environment could still be achieved if the Bio Bags were each placed inside a Ziploc bag. The bacteria did not respond well to the conditions in this experiment, with only *L. casei* and *S. salivarius* presenting any visible growth after the first incubation period of one week. *S. mutans* and *L. lactis* were not growing on the incubated plates or the slants provided to subculture the bacteria. The plates and slants were reincubated and new freeze dried cultures of *S. mutans* and *L. lactis* were purchased along with a culture of *S. mutans* on a slant to ensure that we had a viable culture of the control organism. The experiment was repeated with the newly obtained bacteria but they still did not grow well enough to determine if there was inhibition as a result of the xylitol.

The second experiment was revised to include multiple xylitol concentrations. Each bacterium was also grown on Brain-Heart Infusion agar and Tryptic Soy agar in both aerobic and anaerobic conditions to determine the best growth medium and environment for each. Each bacterium seemed to grow on each plate. However, the results could not be used because of suspected contamination. There was growth in many wells that had had no bacteria added to them. We suspected that the plates had been moved or tipped during incubation, causing over flow into other wells. We also suspected some fungal contamination, but after microscopic examination of a sample from each bacterial strain no fungus was identified. The solutions in each well were quite turbid, so they were extracted in order to observe the growth on the agar. The plates were observed under a microscope. The *S. mutans* colonies did appear to shrink with

increasing xylitol concentration, but there were no measurable results from the other bacteria. The experiment was once again revised.

We chose to grow the bacteria in solution for the third experiment because small changes in growth could be determined using a spectrophotometer. We chose BHI broth because each bacterium grew well on the BHI plates in the previous experiment. Transmittance was recorded for each sample before and after incubation to determine if the growth was inhibited. Increased transmittance, or light passing through the sample, indicated inhibition and decreased growth of the bacterium. The first trial showed that *S. mutans* was greatly inhibited by xylitol at 2.5% and 5% xylitol. The second trial showed less inhibition than the first but followed the same pattern, reaching a peak at 5% xylitol. Each bacterium followed this pattern, showing similar results to a lesser extent in the second trial. This could have been due to a shorter incubation time. The results show that xylitol inhibits both *S. mutans* and *L. lactis*. *S. salivarius* and *L. casei* were only inhibited at the highest concentrations of xylitol, 5% and 10%. However, these concentrations are too high to be achieved and maintained *in vivo* for long periods of time. There was an increase in growth in *L. casei* and *S. salivarius* with .5% xylitol.

Based on these results, use of xylitol could cause inhibition of *L. lactis* over time but does not have a detrimental effect on *S. salivarius* and *L. casei*. Further studies should be performed to determine whether use over long periods of time could inhibit *S. salivarius* and *L. casei* and other normal oral microbes. This study should be extended in order to further validate the results. Further plans include performing trials in which *S. mutans* and one of the commensal or probiotic organisms are tested in solution together to observe their effects on one another. We also plan to test the effect of extended continuous use of xylitol to determine if xylitol resistant strains can be developed quickly enough to hinder xylitol's effectiveness.

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