# *In Vitro* Studies of Xylitol and Erythritol Inhibition of *Streptococcus Mutans* and *Streptococcus Sobrinus* Growth and Biofilm Production

Cannon M l */ Merchant M **/ Kabat W ***/ Le Catherine ****/ White K *****/Unruh B ******/ Ramones A *******

*The **aim** of this study was to evaluate synergy and inhibitory effects of xylitol and erythritol on Streptococcus mutans and Streptococcus sobrinus growth and biomass production on a polystyrene plastic surface. **Study design**; S. mutans and sobrinus strains (American Type Culture Collection reference strains 31341, 35668, 25175, sobrinus 33478) were cultivated in media (Todd Hewitt Broth with 1% sucrose or heart-brain infusion broth with 1% sucrose) at differing concentrations of xylitol or erythritol in microtiter assay plates incubated for 48 hours. Bacterial growth was quantified and measured by optical density using a microplate reader. Experiments assessing synergy and biofilm growth were carried out also using microdilution assays. All four strains were inhibited by 30% (w/v) xylitol, and 15% erythritol at 150mg/ml erythritol, 2/4 strains had reduced growth; at 270mg/ml, 4/4 strains were inhibited. Bactericidal effects were not observed at any polyol concentration. Combinations of both polyols in a checker board array were used to determine if there were any benefits of polyol combinations. **Results** The combination studies yielded mixed outcomes with indifference in growth for strains 68 and 78, potential additive effect for strain 75 and possible antagonism for strain 41. Assessment of biomass formation and polyol interference were also performed post MIC assessment. Strains 41, 68 and 75 produced significant biomass in the absence of either polyol. Both polyols inhibited biomass formation in a dose-dependent fashion. Strain 75 is a poor biomass producer and could not be assessed for polyol effects in our assay. **Conclusion:** Our results demonstrate significant polyol influence on the oral Streptococcal strains tested in our laboratory.*

***Keywords:*** *Polyol, erythritol, xylitol, Streptococci.*

From Ann and Robert Lurie Children's Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL USA.
*Cannon ML, DDS MS.
**Merchant M, BS.
***Kabat W, MS.
****Le C, DDS.
*****White K, DMD.
******Unruh B, DDS.
*******Ramones A, DMD.

Send all correspondence to:
Mark L Cannon
E- mail: drmarkcannon@comcast.net

## INTRODUCTION

In 1975, Mäkinen and others first published that xylitol could greatly reduce dental caries by inhibiting the growth of *Streptococcus mutans*. [1] Since then clinical studies have demonstrated that xylitol products decrease the oral microbiome level of *S. mutans*, the amount of plaque, and the incidence of dental caries in children. [2-4] Additionally, other studies have suggested that *S. sanguinis* and *S. salivarius* strains may also be inhibited by xylitol. [5, 6] Total or partial substitution of xylitol for sucrose in the human diet reportedly resulted in more than a 85% reduction in the incidence of dental caries. [7] According to Mäkinen *et al* the majority *of S. mutans* strains transport xylitol into the cell via the phosphotransferase system, phosphorylated to xylitol-5-phosphate and then expelled from the cell. [8, 9] This energy-consuming pathway is thought to be responsible for inhibiting the growth of *S. mutans*. [10] Erythritol has been reported by Mäkinen and others to also be a dental caries preventative, but with fewer studies having been published. [10-12]

This series of studies was designed to expand on previous reports of polyol inhibition of oral Streptococcal strains associated with dental caries. Previous studies reported inhibition with polyols dissolved in Brain Heart Infusion Broth (BHI). Some data was generated in this study using Todd -Hewitt Broth (THB) as we

awaited a supply of BHI. THB is commonly used to propagate other human Streptococcal pathogens such as *Streptococcus pyogenes* (Group A Strep) and *Streptococcus agalactiae* (Group B Strep). The primary goals of this study were to: 1.) determine the effects of increase polyol concentrations over those reported previously (4% at the upper end) and 2.) determine if combinations of xylitol and erythritol displayed any synergy *in vitro*.

## MATERIALS AND METHOD

### Establishing maximal polyol concentrations in broth media

Solubility of xylitol and erythritol in aqueous solutions have been previously established but our goal was to dissolve each polyol directly into broth media which may alter polyol solubility. We empirically determined solubility (near saturation) of xylitol and erythritol in BHI and THB and found xylitol to be soluble at 61 grams/100mL and erythritol at 32 grams/100mL. As a result we prepared our polyol containing media at the highest concentrations of 60% for xylitol and 30% for erythritol. Final concentrations used after addition of equal volume inoculums tested moving forward were 30% and 15% for xylitol and erythritol respectively.

### Preparation of THB and BHI media containing polyols

THB (Cat# 2024417) and BHI (Cat# 4045078) were purchased from Becton Dickinson (BD, Sparks MD) as powders and prepared according to manufacturer's instructions and steam sterilized. 1% sucrose was added to the basal media (THB or BHI) prior to sterilization. Xylitol and erythritol were added directly to test media to achieve the concentrations immediately below saturation of 60 and 30% respectively. The high concentration polyol containing media for all susceptibility studies were filter sterilized through 0.22 micron filter membranes and stored refrigerated until used.

### Quality Control

Aliquots of each media were plated for sterility and tested for adequate growth support with test Strep strains prior to assay use.

### Preparation of 2% crystal violet suspension for biomass assessments

Crystal violet (Sigma Cat#C-3886, St. Louis MO) was suspended in molecular grade water (Millipore 18ohm) and stirred until dissolved. The final preparation was filtered through thick (~2 mil) filter paper to remove small undissolved and contaminating particulate matter and transferred into a clean sterile container.

### Streptococcal Isolates and growth conditions

Streptococcal isolates used for this study were acquired from the American Type Culture Collection (ATCC Manassas, VA). Three *Streptococcus mutans* isolates, ATCC 35668 (*mutans* type strain), 31341, and 25175 and a *Streptococcus sobrinus* strain ATCC 33478 were used in these studies. All studies Strep isolates were sub-cultured on sheep's blood agar plates (SBAP). On the morning of each experiment 3-5 well isolated colonies of each test strain were inoculated into either 2mL of BHI or THB. Cultures were incubated for 2-4 hours until bacterial turbidity reached approximately 0.5 McFarland units. For culture set up bacterial suspensions were diluted in basal media to a final concentration of ~5 x $10^5$ CFU/mL for inoculation into MIC or Synergy plates. Final colony counts were verified by duplicate plating for quantitative CFU values.

### Minimal inhibitory concentration (MIC)/minimal bactericidal concentration (MBC) and Synergy studies

Microdilution assays were employed to assess polyol inhibitory, bactericidal and combination synergy activity using CLSI M07-A9 (2012) procedures. Briefly, Costar #3596 (Corning Inc., Corning N.Y.) 96 well flat bottom culture plates were used for all studies. MIC/MBC plates were prepared by adding 100 microliters (mcL) of polyol containing media to each well followed by 100 mcL of an appropriate dilution (1-10 x $10^5$ CFU) of Streptococcal test isolate. Cultures were incubated in a $CO_2$ (5%) incubator @36C. Cultures were observed at 24 hours for growth and returned to the incubator for final readings and processing at 48hrs. At 48 hours bacterial turbidity was visually observed, scored for growth and assessed for optical density at 620nm on a Beckman-Coulter (BC) microplate reader. MBC determinations were attempted by sub-culturing 100mcL from wells with no visible bacterial growth. Resulting colony counts were made. A reduction in CFUs of 99.9% or more Bacterial growth was considered as bactericidal. For biomass evaluation bacterial growth supernatants were removed by aspiration, washed 5 times with sterile Dulbecco's – Phosphate Buffered Saline (D-PBS)...

### Biofilm assessment

Uninhibited biofilm formation is readily visible after washing off unbound culture material. In some experiments 100 mcL of sterile water was added to each well and biomass was read directly (without staining) at 620nm on the BC microplate reader. For all experiments biofilm assessment was determined with crystal violet staining of washed plates. For staining, 100mcL of 2% crystal violet was added to each well and incubated at room temperature. After 5 minutes, the crystal violet was aspirated from the wells and the plates were washed 5 times with D-PBS. After the 5$^{th}$ wash, the plates were inverted and tapped to near dryness on paper towels. 100mcL of acetic acid is then added to each well to solubilize bound crystal violet. Plates are gently tapped after addition of acetic acid and then read in the BC microplate reader @ 620nm. Reduction of biofilm is compared to mean positive control OD values using the formula: ($OD_{620}$ sample/ $OD_{620}$ Positive Control) x 100 = % of control biofilm.

## RESULTS

### Polyol Growth Inhibition

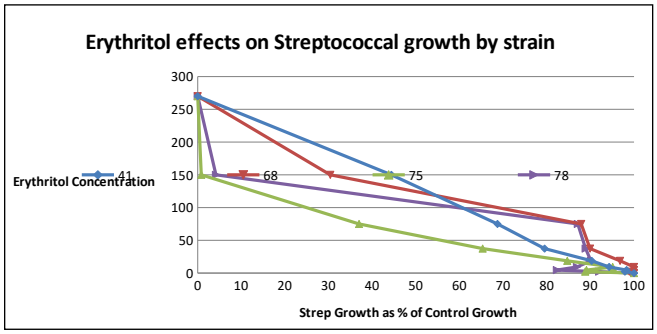
The growth inhibitory effects of polyols were assessed using CLSI standard procedures as described. Multiple MIC studies were performed and are summarized in Table 1. & Figs.1a&b. All four strains tested were inhibited by either xylitol at 300mg/mL with 2/4 strains inhibited by erythritol at 150mg/mL. *S. mutans* ATCC strain 31341 was is inhibited by polyols but to a lesser extent than the other 3 strains tested. We changed the bacterial input method in one experiment by adding a 30 microliter 10 fold bacterial inoculum to 270 microliters of 30% erythritol) so that we could increase the erythritol concentration in our assays to 270mgs/mL (27%). All strains were inhibited by erythritol at 270 mgs/mL demonstrating that complete polyol suppression of growth can be achieved at high concentrations (~27% or greater).

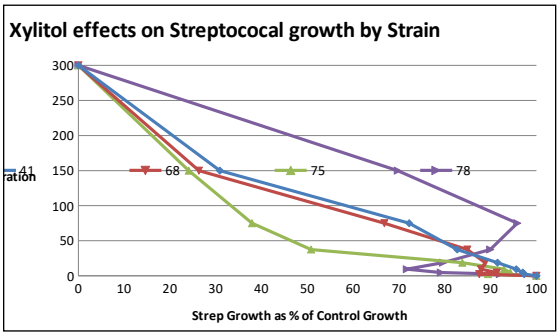
**Table 1: Summary of Streptococcal/Polyol MIC Studies: Average of N=4 replicates**

| Polyol Concentration in mg/ml (%) | | | | | MIC | | Breakpoint** | | | No Polyol |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial Growth: OD 620nm Values | | | | | | | | | | Control* |
| | 300 (30%) | 150 (15%) | 75 (7.5%) | 37.5 (3.7%) | 18.75 (1.9%). | 9.4 (0.9%) | 4.7 (0.47%) | 2.3 (0.23%) | 1.15 (0.15%) | 0 |
| **ATCC 35668 *Strep. mutans* type strain** | | | | | | | | | | |
| Xylitol | 0.001 | 0.122 | 0.311 | 0.394 | 0.412 | 0.409 | 0.425 | 0.407 | ND | **0.464** |
| Erthyritol | ND | 0.142 | 0.409 | 0.418 | 0.449 | 0.464 | 0.464 | 0.459 | 0.478 | |
| **ATCC 25175 *Strep. mutans* type strain** | | | | | | | | | | |
| Xylitol | 0. | 0.107 | 0.169 | 0.226 | 0.373 | 0.414 | 0.421 | 0.398 | ND | **0.4485** |
| Erthyritol | ND | 0.037 | 0.165 | 0.291 | 0.3775 | 0.424 | 0.997 | 0.3955 | 0..404 | |
| **ATCC 31341 *Strep. mutans* type strain** | | | | | | | | | | |
| Xylitol | 0 | 0.176 | 0.411 | 0.47 | 0.52 | 0.544 | 0.551 | 0.553 | ND | **0.5685** |
| Erthyritol | ND | 0.252 | 0.391 | 0.452 | 0.513 | 0.536 | 0.559 | 0.5565 | 0.572 | |
| **ATCC 33478 *Strep. sobrinus* type strain** | | | | | | | | | | |
| Xylitol | 0 | 0.3505 | 0.482 | 0.452 | 0.4005 | 0.3605 | 0.398 | 0.413 | ND | **0.503** |
| Erthyritol | ND | 0.054 | 0.438 | 0.447 | 0.453 | 0.437 | 0.414 | 0.462 | 0.463 | |

*Value corrected for background **Defined as sustained 15% decrease in OD or more

Note: Growth levels of both S. mutans and S. sobrinus in the absence of polyols were similar throughout all studies.




**Figures 1a and 1b: Changes in Streptococcal growth in the presence of Erythritol and Xylitol**

MBC determinations were attempted for all four strains that were inhibited by either polyol. A bactericidal drug concentration is defined as a concentration that reduces viable bacteria by 99.9%. At no time did we observe any significant reduction in viable bacteria and in most cases the post 48 hour culture viable count was at or near the initial input concentration of $10^5$ bacteria /ml (Data not shown).

## Polyol reduction in Biomass Formation

A summary of biomass formation data associated with the MIC assays is shown in Table 2 and Figures 2a and 2b. Comparison of polyol effects on growth and biomass formation are seen in Figures 3a-d. The data demonstrates a direct correlation between growth and biomass formation with each polyol for 3 of the 4 strains tested. *S. mutans* ATCC 25175growth is impacted by polyol but biomass is cannot be assessed as this strain makes little too little to no biomass relative to other the other 3 strains tested in this study. Note that there is considerably more biomass produced by strain 41 which requires proportionally more polyol to affect biomass formation in this group of stains.

## Polyol Synergy Studies

Sample synergy results (composite of 2 assays for each synergy) are shown in Figures 4a-h. Synergy studies were initially done with the ATCC 35668 (68) which is the Strep mutans type strain. Synergy results consistently showed that the polyols were not synergistic but possibly additive. We proceed to check our other 4 Strep stains in the synergy assays. We found similar results with the Strep sobrinus strain (78), Strain 75 growth synergy results with this strain but biomass reduction was inconclusive as this strain is a poor biomass producer. The *S. mutans* 41 strain showed growth inhibition with the polyols but surprisingly and consistently behaved differently in terms of biomass formation. The Biomass assessment in the synergy assay repeatedly suggested that there was an antagonistic effect of the combined polyols in a dose dependent manner. Figures 4a and 4b show the phenomena as described. This antagonism issue combined with the lack of true synergy suggests that combination of polyols is not likely to be useful in a therapeutic setting

**Table 2: Assessment of Biomass formation in the presence of polyols.**

| Polyol Concentration in mg/ml (%) | | | | | MIC | | Breakpoint** | | | No Polyol |
|---|---|---|---|---|---|---|---|---|---|---|
| BIOMASS | | | | | OD 620nm Values | | | | | Control* |
| | 300 (30%) | 150 (15%) | 75 (7.5%) | 37.5 (3.7%) | 18.75 (1.9%). | 9.4 (0.9%) | 4.7 (0.47%) | 2.3 (0.23%) | 1.15 (0.15%) | 0 |
| | E@270 | | | ATCC 35668 *Strep. mutans* type strain | | | | | | |
| Xylitol | 0.001 | 1.655 | 0.898 | 0.809 | 0.844 | 0.84 | 0.864 | 1.02 | ND | **2.1775** |
| Erthyritol | 0 | 1.52 | 0.944 | 0.898 | 1.519 | 1.749 | 2.048 | 2.499 | 2.011 | |
| | | | | ATCC 25175 *Strep. mutans* type strain | | | | | | |
| Xylitol | 0.011 | 0.018 | **0.025** | 0.063 | 0.035 | 0.077 | 0.049 | 0.041 | ND | **0.081** |
| Erthyritol | 0 | 0.032 | 0.036 | 0.1185 | 0.1125 | 0.07 | 0.06 | 0.053 | 0..076 | |
| | | | | ATC**C 31341** *Strep. mutans* type strain | | | | | | |
| Xylitol | 0.044 | 0.284 | 0.719 | 1.115 | 1.308 | 1.29 | 1.29 | 1.274 | ND | **1.365** |
| Erthyritol | 0.027 | 0.32 | 1.137 | 1.214 | 1.201 | 1.444 | 1.407 | 1.359 | 0.392 | |
| | | | | ATCC 33478 *Strep. sobrinus* type strain | | | | | | |
| Xylitol | 0.0805 | 0.472 | 0.284 | 0.308 | 0.286 | 0.351 | 0.398 | 0.489 | ND | **0.523** |
| Erthyritol | 0 | 0.231 | 0.391 | 0.249 | 0.285 | 0.248 | 0.496 | 0.483 | 0.392 | |

*Value corrected for background **Defined as sustained 15% decrease in OD or more

Note that Erthyritol is at 270mg/ml for this dataset

Note: No breakpoint for strain 25175 because biomass is not concisely measurable

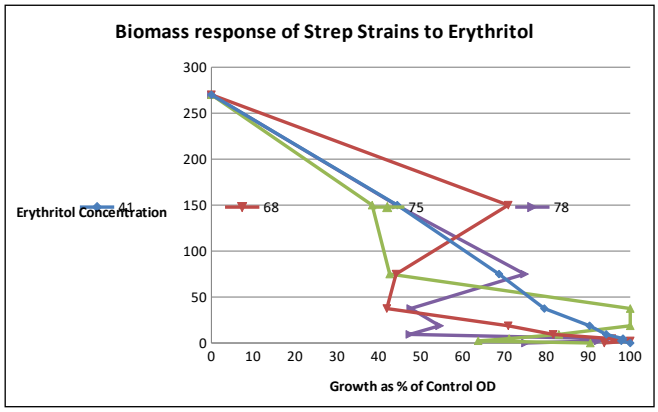



**Figure 2a**

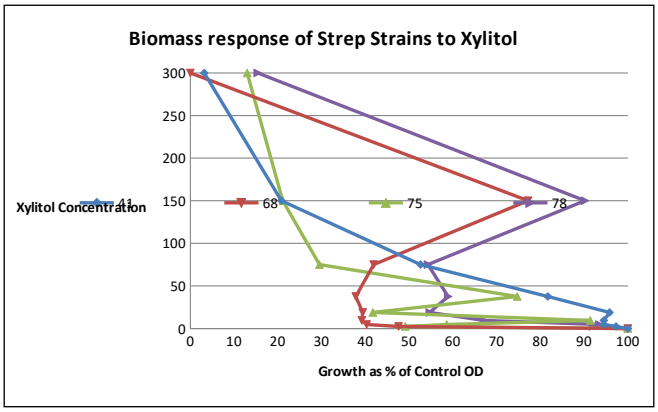



**Figure 2b**

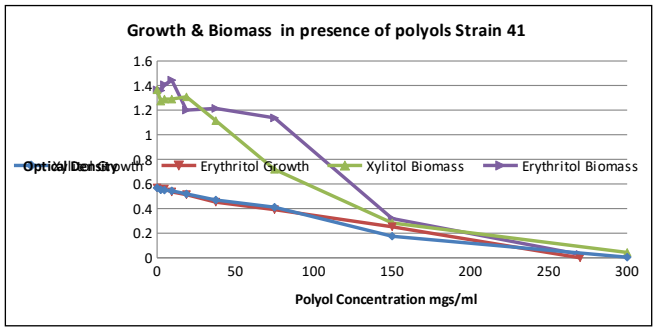



**Figure 3a**

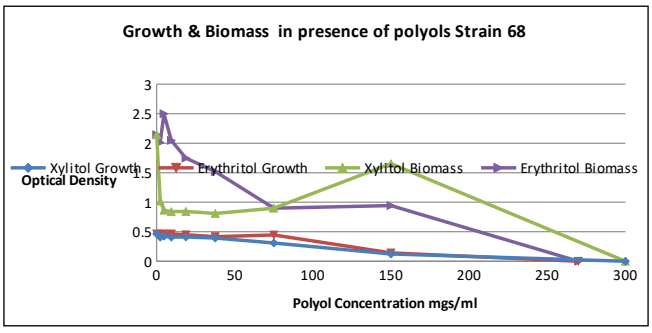



**Figure 3b**

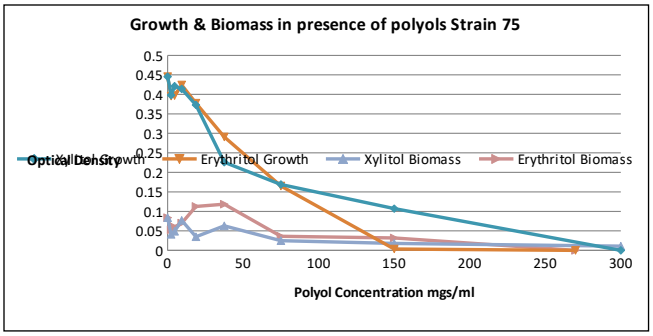



Figure 3c

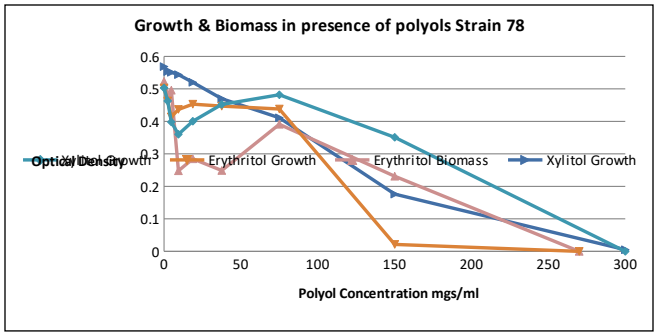



Figure 3d

| | | ATCC 41 | Growth as OD 620 | Yellow values indicate visually inhibited wells | | |
|---|---|---|---|---|---|---|
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0.0255 | 0.1665 | 0.268 | 0.356 | 0.356 |
| " | 37.5 | 0.063 | 0.251 | 0.3855 | 0.414 | 0.4365 |
| " | 19.75 | 0.137 | 0.332 | 0.4 | 0.452 | 0.4855 |
| " | 9..375 | 0.1555 | 0.374 | 0.483 | 0.491 | 0.516 |
| " | 4.69 | 0.1325 | 0.4 | 0.462 | 0.502 | 0.544 |
| | | | Control OD = 0.559 | | | |

**Figure 4a: ATCC 41 growth pattern in synergy array (OD 620)**

| | | Red values suggest antagonism of the polyols | | | | |
|---|---|---|---|---|---|---|
| | | ATCC 41 | Biomass | | | |
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0.288 | 1.1125 | 0.977 | 0.6975 | 0.573 |
| " | 37.5 | 0.953 | 1.986 | 0.581 | 0.29 | 0.354 |
| " | 19.75 | 1.988 | 1.2125 | 0.3665 | 0.3285 | 0.331 |
| " | 9..375 | 2.24 | 0.668 | 0.2685 | 0.21 | 0.306 |
| " | 4.69 | 2.452 | 0.7275 | 0.3205 | 0.259 | 0.239 |
| | | | Control OD = 0.3885 | | | |

**Figure 4b ATCC 41 biomass pattern in synergy array (OD620)**

| | | ATCC 68 | Growth | | | |
|---|---|---|---|---|---|---|
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0 | 0.008 | 0.805 | 0.215 | 0.1905 |
| " | 37.5 | 0.004 | 0.0695 | 0.239 | 0.255 | 0.283 |
| " | 19.75 | 0.006 | 0.1595 | 0.3565 | 0.434 | 0.483 |
| " | 9..375 | 0.02 | 0.2525 | 0.439 | 0.534 | 0.5485 |
| " | 4.69 | 0.027 | 0.304 | 0.4765 | 0.5868 | 0.5485 |
| | | | Control OD = 0.465 | | | |

**Figure 4c: ATCC 68 growth pattern in synergy array (OD 620) Indifference noted.**

| | | ATCC 68 | Biomass | | | |
|---|---|---|---|---|---|---|
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0.006 | 0.0865 | 1.036 | 2.675 | 2.3255 |
| " | 37.5 | 0.0145 | 1.187 | 2.715 | 3.0055 | 2.1805 |
| " | 19.75 | 0.0535 | 1.3735 | 1.412 | 0.803 | 0.8265 |
| " | 9..375 | 0.573 | 1.677 | 0.6875 | 1.1505 | 0.7925 |
| " | 4.69 | 0.7765 | 1.4935 | 0.735 | 1.26 | 0.7405 |
| | | | Control OD = 2.205 | | | |

**Figure 4d: ATCC 68 biomass pattern in synergy array (OD620)**

| | | ATCC 75 | Growth | | | |
|---|---|---|---|---|---|---|
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0.001 | 0.004 | 0.0185 | 0.104 | 0.15 |
| " | 37.5 | 0.002 | 0.0395 | 0.185 | 0.3095 | 0.3095 |
| " | 19.75 | 0.0025 | 0.1365 | 0.2265 | 0.3385 | 0.406 |
| " | 9..375 | 0.004 | 0.186 | 0.3185 | 0.3895 | 0.429 |
| " | 4.69 | 0.004 | 0.212 | 0.2945 | 0.42 | 0.4385 |
| | | | Control OD = 0.449 | | | |

**Figure 4 e: ATCC 75 growth pattern in synergy array (OD 620)**

| | | This strain does not produce a biomass | | | | |
|---|---|---|---|---|---|---|
| | | ATCC 75 | Biomass | | | |
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0 | 0 | 0.057 | 0.174 | 0 |
| " | 37.5 | 0.0085 | 0 | 0.056 | 0.1305 | 0.122 |
| " | 19.75 | 0.0155 | 0.002 | 0 | 0.0085 | 0.0305 |
| " | 9..375 | 0 | 0.043 | 0.001 | 0.102 | 0 |
| " | 4.69 | 0.3 | 0.53 | 0.4 | 0.1755 | 0.074 |
| | | | Control OD = 0.0395 | | | |

**Figure 4 f: ATCC 75 biomass pattern in synergy array. (OD 620)**

| | | ATCC 78 | Growth | | | |
|---|---|---|---|---|---|---|
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0 | 0.2565 | 0.4195 | 0.4015 | 0.442 |
| " | 37.5 | 0.0865 | 0.423 | 0.465 | 0.4655 | 0.4805 |
| " | 19.75 | 0.279 | 0.44 | 0.496 | 0.524 | 0.4635 |
| " | 9..375 | 0.3385 | 0.4805 | 0.508 | 0.515 | 0.418 |
| " | 4.69 | 0.371 | 0.515 | 0.541 | 0.4815 | 0.5265 |
| | | | Control OD = 0.480 | | | |

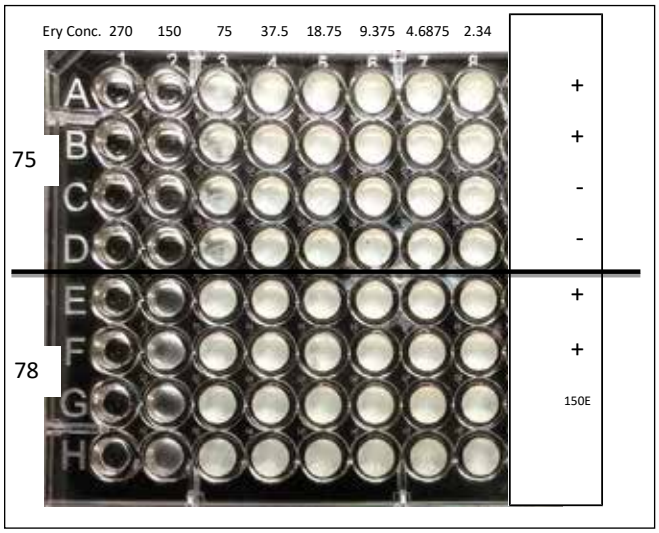
**Figure 4g: ATCC 78 growth pattern in synergy array (OD 620)**

| | | ATCC 788 | Biomass | | | |
|---|---|---|---|---|---|---|
| | | Concentration values in mg/mL | | | | |
| [Xyl] | | 150 | 75 | 37.5 | 18.75 | 9.38 |
| [Eryth] | 75 | 0.16 | 0.4345 | 0.2855 | 0.7325 | 0.962 |
| " | 37.5 | 0.203 | 0.266 | 0.344 | 0.606 | 0.846 |
| " | 19.75 | 0.194 | 0.677 | 0.578 | 0.9165 | 1.052 |
| " | 9..375 | 0.161 | 0.593 | 0.6095 | 1.086 | 1.078 |
| " | 4.69 | 0.208 | 0.4035 | 0.64 | 1.0185 | 1.236 |
| | | | Control OD = 1.274 | | | |

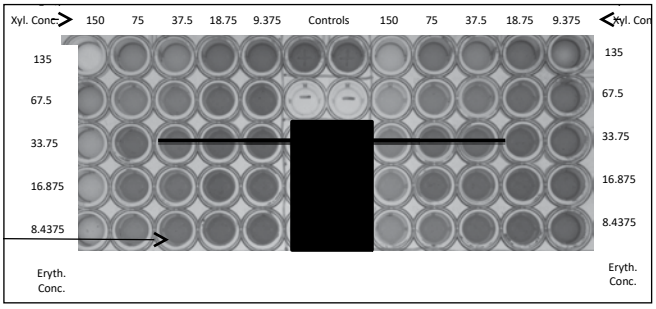
**Figure 4h: ATCC 78 biomass pattern in synergy array. (OD620)**

## Supplemental Images

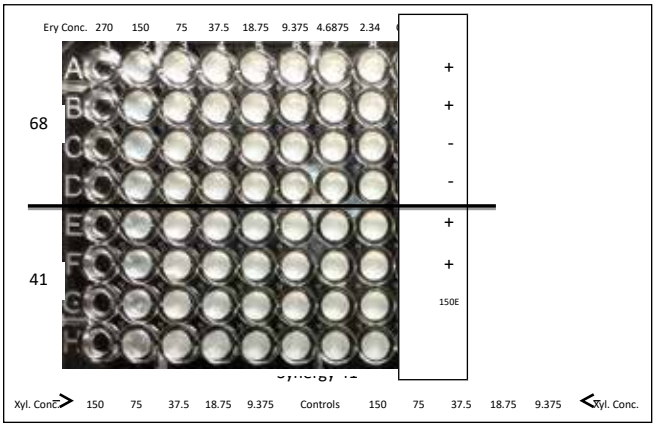
The images below represent a supplemental study using an inoculum schema that permitted using higher polyol concentrations. This was of particular importance as far as erythritol was concerned. Note that both polyols are equivalent in terms of inhibiting bacterial growth. The proceeding synergy images illustrate the biomass anomaly with the Strep mutans 41 strain. While in grey-scale, one can still see the antagonistic pattern of biomass formation which was seen in 2 experiments and a total of 4 replicates.



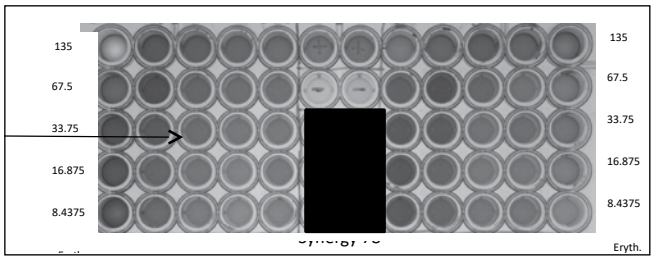

**MIC 75-78**




**Synergy 78**




**MIC 68-41**




**Synergy 41**

Control groups showed positive growth of all bacterial strains. At 300mg/ml xylitol, all four strains showed inhibited growth; at 150mg/ml erythritol, 2/4 strains had reduced growth; at 270mg/ml, 4/4 strains were inhibited. Bactericidal effects were not observed. Antagonistic effects were noted with combinations of xylitol and erythritol for strain 41. Synergy effects were seen w/ strain 75, however reduction in biomass is inconclusive as this strain is a poor biomass producer. For strains 68, and 78, no significant synergy effects were noted.

## DISCUSSION

Previous research by Ghezelbash *et al* reported that in the presence of 4% xylitol and 4% erythritol, the growth of *S. mutans* was decreased by 68% and 71%, respectively. Biofilm formation by *S. mutans* was reduced to 31.32% in the presence of 4% erythritol. [13] Additional studies have demonstrated that erythritol may have a more inhibitory effect on certain pathogens, such as, *S. mutans* and *sobrinus*. [14] Erythritol did not serve as a substrate for cellular aggregation of *S. mutans* and was not utilized for water-insoluble glucan synthesis and cellular adherence by glucosyltransferase from *S. mutans* or *S. sobrinus.* [15] This mechanism is similar to xylitol's reported inhibitory function. [16] But how do they function together, and is there a competitive or synergistic effect? Previously reported studies often compared erythritol to xylitol or to sorbitol, but never as combinations, although many commercial products contain both xylitol and sorbitol. Previous studies reported have not looked at the synergistic potentials of either polyol, or the effect of the respective concentration.

This study looked at the synergistic and concentration effects of both polyols on *S. mutans* and *sobinus.* The resultant inhibition did not demonstrate a synergistic inhibition but there was an additive effect. Of interest is that different pathogenic bacteria strains have different sensitivities to polyols, and this would make the concept of combination therapy even more attractive. In addition, more than one pathogen is involved in dental disease. Indeed, oral disease is due to dysbiosis, with a significant shift in the oral microbiome being foremost the cause of pathology. [17] To rebalance the microbiome, multiple polyol therapy and probiotics should be suggested. [18]

Dental prevention strategies need to adapt to the mounting evidence that dysbiosis is treatable and preventable. [19] Polyols are effective in reducing the level of pathogens and combination therapies may be more efficacious in dental prevention. The periodontal and dental pathogenic strains should be treated as a group, with the different inhibition strengths of the polyols, combined into singular products, such as, toothpastes and mouth rinses. Adjunctive products, candies, gums and other items, should also combine the different polyols to enhance the total benefit to the patient.

## CONCLUSION

*Streptococcus mutans* and *sobrinus* growth was reduced in the presence of xylitol and erythritol in a concentration dependent fashion. Complete growth inhibition occurred at 30% xylitol and 15% erythritol. In combination, additive effects were noted but no synergistic inhibition occurred. Streptococci strains associated with dental caries may respond differently and separately from each other in the presence of polyols.

## REFERENCES


1. Knuuttila MLE, Makinen KK; Effect of xylitol on the growth and metabolism of Streptococcus mutans. Caries Res., 9: 177-189. 1975.
2. Ly KA, Milgrom P, Rothen M; Xylitol, sweeteners, and dental caries. Pediatr. Dent; 28: 154-16. 2006.
3. Bradshaw DJ, Marsh PD; Effect of sugar alcohols on the composition and metabolism of a mixed culture of oral bacteria grown in a chemostat. Caries Res.;28: 251-256. 1994.
4. Soderling EM;Xylitol, mutans Streptococci, and dental plaque. Adv. Dent. Res., 21: 74-78. 2009.
5. Isotupa KP, Gunn S, Chen CY, Lopatin D, Makinen KK). Effect of polyol gums on dental plaque in orthodontic patients. Am. J. Orthod. Dentofac. Orthop; 107: 497-50.1995.
6. Kontiokari T, Uhari M, Koskela M Effect of xylitol on growth of nasopharyngeal bacteria in vitro. Antimicrob. Agents; Chemother.39: 1820-1823. 1995.
7. Scheinin A Caries control through the use of sugar substitutes. Int. Dent. J; 26: 4-13. 1976.
8. Makinen KK, Isotupa KP, Kivilompolo T, Makinen PL, Toivanen J, Soderling E. Comparison of erythritol and xylitol saliva stimulants in the control of dental plaque and mutans Streptococci. Caries Res., 35: 129-135. 2001.
9. Soderling EM. Xylitol, mutans Streptococci, and dental plaque. Adv. Dent. Res., 21: 74-78. 2009.
10. Burt BA. The use of sorbitol- and xylitol-sweetened chewing gum in caries control. J. Am. Dent. Assoc., 137: 190-6.2006.
11. Makinen KK, Isotupa KP, Kivilompolo T, Makinen PL, Murtomaa S, Petaja J, Toivanen J, Soderling E. The effect of polyolcombinant saliva stimulants on S. mutans levels in plaque and saliva of patients with mental retardation. Spec. Care Dentist., 22: 187-193. 2002.
12. Makinen KK, Saag M, Isotupa KP, Olak J, Nommela R, Soderling E, Makinen PL. Similarity of the effects of erythritol and xylitol on some risk factors of dental caries. Caries Res., 39: 207-215. 2005.
13. Ghezelbash G. R., Nahvi I., Rabbani M. Comparative inhibitory effect of xylitol and erythritol on the growth and biofilm formation of oral Streptococci . African J Microbiol Res;6(20):4404–4408. 2012.
14. de Cock, P., Mäkinen, K., Honkala, E., Saag, M., Kennepohl, E., & Eapen, A. (2016). Erythritol Is More Effective Than Xylitol and Sorbitol in Managing Oral Health Endpoints. Int J Dent, 2016, 9868421. https://doi.org/10.1155/2016/9868421
15. Kawanabe J., Hirasawa M., Takeuchi T., Oda T., Ikeda T. Noncariogenicity of erythritol as a substrate. Caries Res. 1992; 26(5):358–362. doi: 10.1159/000261468.
16. Shemesh, M., Tam, A., Feldman, M., & Steinberg, D. (2006). Differential expression profiles of Streptococcus mutans ftf, gtf and vicR genes in the presence of dietary carbohydrates at early and late exponential growth phases. Carbohydrate research, 341(12), 2090–2097. https://doi.org/10.1016/j.carres.2006.05.010
17. Cannon M. A Review of Probiotic Therapy in Preventive Dental Practice, Probiotics and Anti-microbial Proteins, Vol 3, Num 2, July 2011.
18. Cannon ML and Peldyak JN. The prevention and treatment of neural arterial gingival simplex Dental Res Manag; 3: 32-37. 2019.
19. Cannon, Mark. (2019). Pediatric Oral Systemic Health: From Fetus to Adolescence. Interventions in Pediatric Dentistry Open Access Journal. 3. 10.32474/IPDOAJ.2019.03.000158.