**Xylitol Blocks Streptococcal Signal Transduction Pathway**

**and Biological Continuity**

**Short Title: Xylitol blocks signal transduction of S. oralis**

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**ABSTRACT**

*Microbiologically and morphologically, the Mitis group bacteria including S.oralis and S.pneumoniae responsible mostly for diseases in children and elderly are closely related to Gram-positive dental pathogen like S.mutans. Combination therapy that combines fluoride with xylitol has also been recognized by the dentists for the last 25 years as a preventive therapy against the S.mutans but without the information required that all the related diplococcic Gram positive bacteria co-ordinate their biological functions using signal transduction pathway and our data previously published demonstrate that xylitol blocks signal transduction by their genetic inheritance. Signal transduction also co-ordinates the growth cycle of these bacteria that includes bacterial log-phase (smooth colonies ) and stationary –phase (rough colonies). We have observed that the smooth colonies of S.oralis growing in blood agar medium, become rough colonies with their characteristic uneven edges after 24 -48 hours of incubation at 37oC. SEM analysis of* ***rough colonies*** *from solid growth medium or* ***stationary phase cultures*** *(liquid), show that the* ***S.oralis*** *population is clustered around their old parents. The same stationary phase culture if exposed to fluoride (100ppm or less) shows a spontaneous increase of titer even without any fresh growth. Additionally, such clustering of* ***S.oralis****populaton in their stationary-phase , is re-confirmed by subjecting them to a shearing force. The increase in bacterial titer occurs by such rupturing of the cluster has also been confirmed by a standard microbiological assay as well as by SEM. Bacterial population resulted starts re-growing in the fresh growth medium (liquid broth or solid blood agar media). Individual member of the chain also starts growing but again in –chains like a tree with branches. If such a bacterial tree with branches is exposed to fluoride, the entire bacterial population reaches a high titer by their disintegration. This is not desirable because such a high titer will satisfy the ID-50 dose required for the disease pneumonia.*

***Keywords:*** *Streptococcus oralis; xylitol loci ; signal transduction; biological continuity; rough and smooth colonies.*

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**INTRODUCTION**

In 1928, Dr. Fred Griffith made an unusual effort to co-relate Pneumococci colony morphology, "Rough and Smooth" on blood agar medium, to their virulence behavior (1). Many interesting observations have been reported during the period, 1944 - 1990 but assuming chromosomal DNA transfer (transformation) is responsible for Dr Griffith’s colony variations (rough and smooth) on blood agar media (2--5). In fact, the Pneumococci chromosomal- DNA isolated during this period are mostly double stranded DNA fragments, not even segments( DNA isolation and the DNA degradation effect of phenol ,Palchaudhuri, BBRC, 1977)(6) (Palchaudhuri and Palchaudhuri, Chicago Intl meeting on vaccines, 2012)(7). Available data on chromosomal double stranded DNA transfer by transformation involves release of DNA fragments ( not even segments) by the fratricidal effect of competent population on old incompetent parents who are probably prevailing in their chains of inheritance.

Such transformation begins with adherence of DNA fragments (double stranded) to their competent progeny, possibly at their site of cleavage (an index of new growth) initiated by the environmental stress (e.g presence of non-bactericidal concentrations of antibiotics (3). EndA nuclease located in the recipient cleavage site degrades only one strand of their donor DNA, the complementary leading strand with 3-prime OH end enters into an eclipse phase ( 8). Until now the investigators have questions about this eclipse phase, but we think the single stranded donor DNA attaches to the growing membrane of the recipient and integrates into the recipient chromosome by homologous or homeologous recombination. Precise location of the S. pneumoniae chromosome remains to be explored.

However, one component signal transduction mechanism (previously thought to be two component STKP) is now accepted as a major player (9,10). Briefly, the pheromone (17 amino acid long peptide) is secreted by the recipient under environmental stress via com CDE operon. What is this environmental stress? Presence of antibiotics is definitely an environmental stress and the donor DNA nucleotides (it does not mean any homologous particular DNA donor chromosomal segment) may initiate the transformation process. The end result of such DNA transformation is to bring about an alteration even a single base alteration of the recipient chromosome by a point mutation. Such point mutation in any PBP gene may render the recipient resistant to penicillin and initiate treatment crisis. Accumulation of point mutations in PBP genes and alterations of affinity of PBPs against penicillin drugs is a real crisis in modern medicine. (8- 10.S.pneumoniae penicillin binding protein PBP 2x is an enzyme involved in final stage of peptidoglycan assembly and essential for their growth. PBP2x co-localizes with PASTA at 51 bacterial cleavage sites. Microbiologically and morphologically, the Mitis group bacteria including S.pneumoniae responsible mostly for diseases in children and elderly are closely related to Gram-positive dental pathogens like S.mutans(10;11. They are all diplococcic in shape, Gram-positive, catalase–negative and facultative anaerobes. They all are related by their low DNA base ratio (G/C content approximately 42%).

What is worse, DNA sequence based identification may result in mis-identification of the specific organism belonging to the Mitis group (12). The cariogenic bacteria consisting of acidogenic S. mutans and S.sobrinus reside in the oral cavity with accumulation of proteins and adhesive glucans on the enamel surface of teeth (13). The glucan binding protein (lectin) activity, apparently responsible for aggregation of these bacteria by acidic pH, is significantly reduced in the presence of fluoride (14, 15). We therefore believe that these bacteria if exposed to non-bactericidal doses of fluoride will break their aggregates and result in an instant increase of titer. The population of these diplococcic individuals thus produced may reach a high titer even without any growth or within minimal time of growth. Fluoride (1000 ppm or more) has long been used in toothpastes, mouth wash and more recently in the drinking water supply (low 66 ppm) as a preventive agent for dental caries and tooth decay caused by the dental pathogens. Mehta and Shah have also reported that the fluoride in our drinking water is a major risk factor for dental fluorosis (16). We therefore have made an effort to fully understand how these diplococcic Gram-positive bacteria multiply. We have shown in this work that they grow in-chains, clusters or combination of both to reach stationary phase or latent phase (no metabolism). Usually, these stationary phase bacteria may escape the presence of penicillin (because they are not in growth –phase). In the recent years, **penicillin resistance crisis** has been a serious problem in medicine but an affordable, harmless preventive alternative therapy against the Mitis group streptococci needs to be developed.

Xylitol is a natural low calorie sugar- alcohol. Unlike our commonly usedsix -carbon sugars (glucose ), xylitol has five carbons but can be used even for the diabetics who are prone to bacterial infections. During the last two years we have also developed the Flow Cytometry technique to monitor their in vivo metabolism of xylitol by the diplococcic Mitis group, S.oralis or S. mitis and dental pathogen S.mutans, with or without fluoride. Significantly, genes for xylitol metabolism are expressed even in the presence of other carbon sources resulting in reduction of their cell wall thickness as confirmed by the formation of protoplasts (17-20). These protoplasts are completely trapped in this xylitol-cloud produced by the unfolding of peptidoglycan layers (7).

An alternative but broad spectrum harmless therapy with xylitol, if appropriately developed, should be equally effective against all these closely related diplococcic bacteria, regardless of their resistance to penicillin, penicillin derivatives (alterations even by point mutations)and antigenic variations (21-24). Our work now proves that the simultaneous presence of fluoride and xylitol (2% or higher) is necessary during their growth. Xylitol should provide a useful alternative preventive therapy.

**Materials and Methods**

Bacterial strains and experimental conditions A Gram-positive diplococcic, catalase negative, non-hemolytic (gamma) and bile- esculin negative Streptococcus oralis (ATCC 6249) and a dental pathogen S.mutans (ATCC 25175) were used. Bacteria were always genetically purified as a single colony isolate and then grown in99 the BHI broth containing dextrose 5g/liter (DIFCO, Becton Dickinson) or in solid BHI-agar media (1.5%). Whenever necessary, they were also grown at 37oC as well as at ambient temperature. Bacterial growth was monitored by counting the colony forming units on a BHI agar media after appropriate dilutions as well as by measuring optical density at 580 nm.

***Flouride experiments***

For one experimental set varying concentrations of fluoride: 0 to 300 ppm (NaF, Bio labs) were added to the overnight cultures of *Streptococcus oralis*without diluting the growth media. Afteraddition of fluoride the bacteria were incubated at 22o C for 2- 20 min. In another experimental set the overnight cultures of *Streptococcus oralis*were diluted 100 fold in fresh BHI medium containing xylitol with varying concentrations of xylitol.( Xylitol stocksolution (20% in BHI), pre-warmed to 37 o109 C, was used to have varying concentrations of xylitol without diluting the growth media. The resulting cultures were incubated in a water bath at 37°C with shaking and samples were withdrawn at one hour time intervals with varying concentrationsof fluoride (0 to 300 ppm).

**Xylitol metabolism of S.oralis**

The logarithmic growth -phase of the bacteria was 113 monitored by counting the colonies on rich agar medium as well as by measuring optical density at 580 nm. Gram-stain preparations of *S.oralis*were made after growing similary in BHI medium. These experiments were repeated several times using the standard Gram-staining technique and optical microscopy (final magnification 1000-fold).

***Scanning electron microscopy (SEM)***

Bacterial culture (overnight or diluted 100-fold in BHI broth containing 6.5% sucrose) was fixed on a mica sheet with 2.5% glutaraldehyde. Bacteria on the mica sheet were washed serially with 25% ethanol to absolute ethanol and the fixed bacteria were finally dried and shadowed with gold particles for 10 seconds. They were then seen by a scanning electron microscope (JEOL JSM- 7600F) at 15KV (20). The aliquot of the same bacterial sample was also critically examined by optical microscopy following a standard Gram-staining procedure. For each condition in this study at least 3 replicates were performed.

**Smooth and rough colonies of S.Oralis**

**RESULTS**

Microbiological experiments and analyses by microscopy (optical as well SEM) lead to a conclusion that the S.oralis grows in-chains of diplococcic individuals (growth phase), gradually these chains grow longer and reach to their stationary- phase, approximately within 6-7 hours. Duration of growth phase is determined by the availability of essential nutrients in their growth environment.

We have spread these bacteria on blood agar medium by soft agar overlay technique minimizing the shearing forces, which are responsible for the breakage of their population in –chains and clusters. We have also observed two kinds of colonies in the course of their growth in incubator (37 ° C): smooth or small colonies which appear after 10 hours of growth become rough colonies after 24 hours or beyond. Contour of roughXylitol metabolism of S.oralis colonies with edges fully satisfies the description of Dr Griffith's rough colony (1). If we start fresh cultures transferring these rough colonies again into rich broth and then spread on fresh blood agar medium, we always find smooth colonies become rough with longer incubation (24- 48 hr). By definition, these colony types representing bacterial phenotypes and therefore they are equally capable of producing diseases under physiological conditions of growth. We also want to emphasize that their genotypes (reading frames in- codons as contained in the chromosome) responsible for colony variations are stable for a simple reason that these colony types are reproducibly reversivble. In support of our above observations, we have also critically examined them under both optical and scanning electron microscopes taking precautions so that their physiological states of growth are minimally disturbed.

The smooth colonies are picked up after 10 hours of growth on blood agar plate and suspended in our liquid broth and visualized by both optical microscopy and SEM. The majorities are mostly diplococcic individuals and the minorities are diplococci in pairs and in short chains (19, 20). In our published work we have clearly shown that the diplococcic Gram positive bacteria S.mitis, S.mutans and S.pneumoniae have only two phases of growth, log -phase and stationary- phase (8, 20). Under unfavorable growth conditions, for example antibiotics at non- bactericidal concentrations or environmental stress (regular microbiological dilution techniques or eppendorf pipetting), these diplococcic bacteria dissociate from the chain of their incompetent parents and start re-growing. The heterogenty of stationary -phase S.oralis population is seen by optical microscopy at 1000 fold magnification and by scanning electron microscopy (SEM )at 3000-fold magnification, um time of shadowing with gold particles was 10 to 20 seconds, **Fig 1(a),1(b**).

**Xylitol metabolism of S.oralis**

If any chain is completely broken, we should 159 see their individual members. This helps us for a direct analysis of their clusters formed around their incompetent parents who also grow in chains, **Fig 2(a) and 2(b**).

Figure 2(a) clearly demonstrates that the heterogenity of the population in chain, comprising of both the young (diplococcic) and the old (some of them are incompetent). The incompetent have lost their cell wall thickness (size) and therefore their morphology (shapes) differ. Fig 2(b) confirms that if the chain is broken during sample preparation, then all the members of the chain are released with their heterogeneity of sizes (the bacterial population is shown within two parallel dotted lines). Previously, we have established how the presence of xylitol in their growth media contains them in- chains, regardless of their difference of their physiological states of growth. We establish that initiation of their growth in the absence of xylitol these chains may fall apart, also proving their existence in heterogeneity (19,20).

**The competent and incompetent population in stationary –phase**

Previously, it has been shown that the fratricide of the incompetent members occurs during stationary phase of their growth but without affecting the viability of competent ones (3). Similarly, we like to propose that the rough colonies of S.oralis incompetent population are partially lysed (fratricide!) releasing their nutrients and probably for crosse-feeding their younger generations who may multiply in-chains but slowly until they are transferred to fresh growth media for normal multiplication. Rough colonies on blood agar media show occasionally spontaneous lysis in their centre area. This is the basis of the uneven shape of rough colonies as observed by Dr. Griffith after 24 -48 hours incubation on blood agar (1). (Data not shown). Their growth –in chains and clusters are broken by the standard dilution techniques commonly used for measuring biological titer and the Gram staining technique for visualization by optical

Xylitol metabolism of S.oralismicroscopy. In order to minimize the breakage of chains, we 182 have added sucrose to a final concentration of 6% before these bacteria are fixed in glutaraldehyde (2.5%) prepared in buffer. Such existence in –chains and in-clusters have been visualized by microscopy both optical and SEM after growth either in broths TSB, BHI or solid blood agar media. Xylitol protocol to be defined.

***Stationary-phase S. oralis increases in titer in the presence of fluoride (0-100 ppm)***

The increase of *S.oralis*colony forming units (cfu) takes place when the stationary –phase189 culture is exposed to different concentrations of fluoride for 2min or 20 min at room temperature(22o190 C). The cfu gradually increases with the increasing concentrations of fluoride: (a) after treating with 0 ppm fluoride, control (530 cfu), (b) after treating with 50 ppm fluoride (980 cfu) and (c) after treating with 100 ppm fluoride (1320 cfu). However, such increase of titer does not require any fresh growth but their incubation with fluoride for 2- 20min at room temp. However, such increase is not observed at 200 ppm of fluoride or higher but to the contrary the titer starts decreasing. This is a bactericidal effect of fluoride. Under similar conditions but without exposure to fluoride, the stationary –phase bacteria show no increase in titer at room temperature and therefore, we conclude that stationary-phase bacteria in clusters are dissociated by the action of fluoride.

When similar experiments are carried out at 37 ° C we have observed the similar dissociation of *S.oralis*chains by fluoride. Interestingly some round shaped members of these bacterial population but highly diminished in size have also been seen by optical microscopy. These smaller colonies are not able to produce colony forming units when transferred to another fresh TSA agar media apparently they cannot multiply to form visible colonies. This appears to be the percentage of the incompetent population present in the same chain (old).

**Xylitol metabolism of S.oralis**

***Growth-phase S. oralis increases in*** 205 ***titer when exposed to fluoride (NaF, 0-100 ppm)***

We have seen that the addition of fluoride breaks the chains of diplococcic *S. orali s*and initiates their growth phase, i.e. they become metabolically active, **Fig 3(a**). **Fig 3(a)** shows the *S.oralis* growth curve for 3 hours under normal conditions by diluting overnight *S. oralis*cultures 1000 fold in fresh BHI broth. Addition of fluoride (100 ppm) to a fixed volume of growing culture at different time intervals shows an increase of bacterial titer. The increase of the bacterial titer due to the fluoride exposure is highest at 3 hours relative to 0 min, 1h or 2h increments. Our interpretation is that these bacteria grow in chains with branches of varying lengths. Therefore, when these bacteria are grown for longer time, their branches also keep growing longer. Once these longer chains are dissociated by fluoride, bacterial titer also increases.

***Xylitol grown S.oralis becomes resistant to fluoride (within 3 hours of growth)***

If grown in xylitol and then exposed to fluoride at different time intervals (0-3 hours), the increase of titer plateaus after 3 hours of growth, **Table 1**, and **Fig 3(b)**. We conclude the growth in xylitol makes them resistant to fluoride within 2-3 hours of growth. Previously, we have shown that the chains of *S.oralis*, are stabilized upon growth in xylitol (17). In continuation, we are now reconfirming by the analysis of our data obtained by the SEM of these bacterial population, **Fig 4(a),** grown to stationary phase in the presence of 2% xylitol. The bacterial sample is over-shadowed to illustrate the dimensional differences among their heterogenous members which are all present in the same chain. The arrowhead points out the location of a member which is considerably reduced in dimension and therefore they are buried under the gold particles, as scattered in shadowing technique for the visualization by SEM. The presence of size heterogeneity including their protoplast-like shape, considerably diminished in sizes; but interestingly all are still present in the same chain with decreasing contrast, **Fig 4(a)**.

**Xylitol metabolism of S.oralis**

It grows well in our CNA medium (colistin and nalidixic 228 acid containing agar) which is not buffered by adding sodium or potassium phosphates (20).

**Blocking the One-component Signal Transduction Pathway of Diplococci Gram-positiveBacteria by Xylitol Cloud as Visualized by SEM:**

Bacterial receptors for signal transduction are usually located in their membranes (possibly S.oralis cleavage site). In our experiments, a five-carbon sugar, when added to the growth medium, their xylitol operon is expressed. An irreversible xylitol compound is formed with a serious toxic effect on bacterial reproduction (17). Bacterial chains consisting of their progeny, mostly protoplasts which are irreversibly stabilized to prevent their run away from clusters. However, this population of protoplasts is enveloped in a xylitol cloud which is apparently originated by the systematic unfolding of bacterial peptidoglycan layers (starting from the outermost layer) and around these bacteria growing in- chains with branches (cluster). Bacterial bio-communications via StkP which requires their cleavage site for signaling are thus affected. In support of these statements, scanning electron-micrographs (SEM) are systematically presented here in Figures 4(a), 4(b), 5, 6(a), and 6(b) with expanded legends.

**DISCUSSION**

During a period, 1960- 1990, many laboratories have been mostly interested in E.coli K-12 genetics and the report on smooth and rough colonies of S.pneumoniae which represents the multiplication pattern of diplococcic Gram-positive Mitis group bacteria remains in obscurity for a long time. The Mitis phylogenetic group of streptococci includes S.oralis, S.pneumoniaeand S.mitis. In order to understand the difference between these rough and smooth colonies of S.pneumoniae as reported by Dr Griffith in 1928, we have used S.oralis which is closely related S.pneumoniae also by their GC content ( ~42%) (25). We have clearly seen by microscopy, both optical and SEM that the rough colonies (R) which are equivalent to our stationary phase culture which is grown in rich broth. They are consisting of both old incompetent and competent population in –chains, but reduced in size by the thinning of their cell wall thickness ( stained pink ) and their diplococcic live population(deep purple) remains clustered around them. If stationary cultures are ruptured by vigorous pipetting or by vigorous shaking they show their heterogeneity of sizes (approx., 1.8um-0.2); but the majority of repopulation thus released are stained purple by Gram staining (17-20).

We have also demonstrated in this work that the difference between the opaque and transparent variants is their phenotype variation, because opaque colony is equivalent to our stationary phase culture and the transparent is the growth phase, independent of their encapsulation status. Belanger et al have also clearly specified in their recent article about the lineage of S.pneumoniae laboratory strains derived from D39 and their associated phenotypes and genotypes. Based on numerous publications on DNA transfer by transformation and our data included in this work we conclude that the rough colonies are representing S.pneumoniae stationary phase . The R36 with rough colony morphology (R) has been used by Avery et al (1944) to establish DNA mediated character transfer but we must not forget that the smooth and rough colonies represent their phenotype variations (physiological states of growth) and not the transformant which is initiated by the peptide pheromone excreted by the live recipient before their uptake of exogenous DNA fragment (26, 27). In 1964, Tomasz et al have published the fine structure of diplococcusPneumoniae (strains R6 or R1 derived from R36A), after growing them to stationary phase and analyzing by transmission electron microscopy (28). Significantly, their “spheroplasts" of S.pneumoniae are similar to our incompetent parents of S.oralis (pink,biological non-living) who are clustered together with their competent progeny. In combination, it is their stationary- phase.. What is more, their published micrographs show a population of Pneumococci which are also clustered in –chains but apparently ignored by authors (28, Fig 1). We have taken affordable precautions to avoid breakage of these chains during our sample preparation. Dissociation of diplococcic bacteria -in chains (stationary –phase population) into diplococcic individuals is required to start de novo growth and expression of genes required. Under environmental stress induced by pipetting, presence of fluoride or antimicrobials at sub-lethal doses (abuse of antibiotics), these pathogens in long –chains, (latent –phase) may express their disease producing ability by the rupture of their chains into individual diplococcic units to initiate growth phase. It is already known that these diplococcic Gram-positive bacteria may live silently in children's throat (latent phase) or in their dental environment but we must not forget that their pathogenic potential has remained the same. This is supported by the fact that the chromosomal DNA sequence (coding regions) is not much altered. Based on our results with Streptococcus Mitis group, S. oralis, we conclude that when fluoride at non-bactericidal concentration (<100ppm) together with xylitol (2% or higher), the diplococci individuals are clustered around their incompetent parents [Fig 2(a)] will be dissociated and instantly produce high titer. This in turn may satisfy the requirements for their pathogenesis, even in the absence of any new growth. As antibiotic resistance crisis is a major concern globally for the treatment of many diseases, especially for life threatening bacterial pneumonia, meningitis, otitis media, and dental caries of children, a preventive treatment with xylitol should provide a useful alternative but low-cost therapy if combined with fluoride. Our data now distinctly shows that fluoride should not be used in dentistry without the concomitant presence of xylitol (2% or more) since mortality rate of children from bacterial pneumonia has increased enormously during the past 24 years(1990 onwards). Use of antibiotics (especially, at sub-lethal doses) in poultry, pigs and cattle farms appears to have contributed to our antibiotics crisis in medicine during this period. Unlike penicillin resistance crisis, xylitol resistance should NOT be a problem for two good reasons: xylitol acts as bacterial birth control pill (production inhibited), and the population xylitol resistant ones grow at a slower rate than the xylitol sensitive ones ( 29, Palchaudhuri S, unpublished data) . Due to the genetic relatedness (G-C content, ~42%), all three diplococcic pathogens S.oralis, S.pneumoniae and S.mutans are expected to behave in a similar manner but the diseases caused by them are not the same. Until recently we did not think that there are also RNA transcripts of varying lengths, starting with few nucleotides which are not translated but play important role in the regulation of bacterial gene expression and their diversity of pathogenesis (30).The same S.pneumoniae causes diseases like pneumonia, meningitis, otitis media and bacteremia. Marx et al. have already have identified genes for small non-coding RNAs that belong to the regulon of the two-component or one component regulatory system (10,31). The signal transduction mediated bacterial communication within their community regulates many of their biological functions beginning with their genetic ability to reproduce and mutate PBPs to avoid modern medicines, antibiotics and polyvalent vaccines. PBP2X and serine threonine protein kinase (StkP) of S.pneuomoniae interact directly and are located in the same membrane -associated complex (10).

We have previously established that growth of these diplococcic Streptococci in the presence of xylitol reduces their cell wall thickness and in turn the dislodgment of PBP2x and StkP kinase should take place. We conclude in Fig 7 how xylitol metabolism by S.oralis, their closely related Mitis group members and dental pathogen S.mutans forms a xylitol cloud by the unfolding of peptidoglycan layers and in turn blocks their 320 signaling pathways (Palchaudhuri S, Dissanayake P, and Palchaudhuri A, 2013, Intl Meeting Abstract, Kolkata SINP, India). We now support our published data how xylitol metabolism affects the biological continuity of these diplococcic Gram-positive pathogens, regardless of their antibiotic resistance patterns and high potential as an alternative therapy to minimize the mortality rate of children and elderly. Our conclusion is depicted in Fig 7 by a schematic diagram. **Figure 7** shows schematically how xylitol metabolism blocks the formation of cleavage by the thinning of cell wall which is essential for the action of PBP2X and StkP mediated bio-signaling. The bacterial population are seen as protoplasts (size considerably reduced) which are mostly covered by a xylitol cloud.

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**Table 1 Xylitol grown *S.oralis*titer upon exposure to fluoride**

Time of growth in xylitol (h) Control (0 ppm fluoride) 104 /ml 100 ppm fluoride 104/ml increase of *S.oralis*

Titer 104/ml 0 110 210 100 1 570 1100 530 2 ~1200 ~1200 No increase Xylitol metabolism of S.oralis 26

3 ~2900 ~2900 No increase 483

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Figure 2(a) : Click here to download high resolution image

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Figure 3(a) : Click here to download high resolution image

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Figure 4(a) : Click here to download high resolution image

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Figure 5 : Click here to download high resolution image

Figure 6(a) : Click here to download high resolution image

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**Table 1 Xylitol grown *S.oralis*titer upon exposure to fluoride**

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If xylitol is present with fluoride during this growth period, the bacterial titer reaches a plateau within 2-3 hours being contained in their clusters. Bacterial utilization of xylitol is responsible for stalling the bacterial growth and rendering them attain plateau. There is no lysis of bacterial population as confirmed by the increase of CFU and the OD. During such growth in the presence of xylitol, the thinning of bacterial cell walls occurs and peptidoglycan layers of the cell wall apparently spreads but remains fused together. This xylitol cloud thus formed by the unfolding of peptidoglycan layers still contains all the bacterial protoplasts . The cross wall peptides are collapsed by the displacement of the PBP proteins. These major PBPs of high molecular weight are usually located by the cross wall peptides but between the peptidoglycan layers. Based on our data, we must accept the bio-conversation of bacteria (one component signaling) but blocking such signals with XYLITOL stop these pathogens from causing the dreadful disease of our children for our annihilation. Simultaneous presence of fluoride together with xylitol will not allow these pathogens to hide in their stationary phase or latent phase. We think this cloud is originated by the unfolding of their peptidoglycan layers from the outermost to inward. As a result of breakage of cross- walls, bacterial biological structures and functions indispensable for continuity are affected. In this work we are strictly limiting us in Mitis group diplococcic Gram-positive bacteria. Signal transduction via two component system initiates the formation of physiological cleavage in the middle of cell cytoplasmic membrane the one-component serine/threonine kinase (StkP) to co-ordinate reproduction of S.pneumoniae, distribution of chromosomes into daughter cells during multiplication and diversity, if necessary via DNA transformation.

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