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XYLITOL BLOCKS STREPTOCOCCAL SIGNAL TRANSDUCTION PATHWAY AND BIOLOGICAL CONTINUITY

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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.

ABSTRACT

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.

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).

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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 site. 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).

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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 used six -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).

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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.