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Research Article

Xylitol Stabilizes the *In Vivo* Existence of Streptococci in Heterogeneity

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Abstarct

Based on data published in international journals, it appears that the five carbon sugar alcohol Xylitol has high potential to provide such an alternative preventive therapy since it is equally effective regardless of bacterial antibiotic sensitivity pattern. Xylitol derivative (Xylitol phosphate) produced by the phosphorylation of Xylitol in mitis group Streptococci even in pre-competent phase blocks their two component signal transduction pathway of growth. These diplococcic Gram-positive bacteria in the pre-competent phase, inside mother's womb and or newly born but in - chain with their mothers are also affected by Xylitol phosphate. Thinning of cell wall affects the cleavage that forms at the mid-cell position during development of the competent status. This is also the junction of septal and equatorial biosynthesis of PG (cell wall biosynthesis). Therefore, the progeny still in pre-competent phase needs to be fully understood because Xylitol phosphate formed in this phase affects bacterial maturation starting from the pre-competent phase. The bacterium *S. pneumoniae* responsible for the increasingly high mortality rate of children from the diseases like, bacterial pneumonia, meningitis, otitis media because it has become increasingly resistant to antibiotics like penicillin and their derivatives. Even newly developed polyvalent vaccines are not very effective against this pathogen apparently for their serological variation (?) and therefore an alternative, low - cost preventive therapy is immediately necessary.

Keywords: *penicillin resistance; bio- signaling; S. oralis; growth curve; pneumonia; dental caries*

Introduction

The bacterium *S. Pneumoniae* responsible for the increasingly high mortality rate of children from the disease pneumonia has become resistant to penicillin and their derivatives. Recently developed polyvalent vaccines are also not very effective against the Mitis group streptococci and therefore an alternative preventive therapy is absolutely necessary. In order to achieve this goal we need to unify our total knowledge about Gram-positive diplococcic streptococcus growth curve. Unlike laboratory strain of Gram negative *E. coli* K-12, the Mitis group Gram-positive bacteria multiply in an entirely different pattern. During the period 1944- 1993, we have been dominated by the artificial transformation mostly used in gene cloning experiments with a laboratory strain of *E. coli*, K-12 [1]. We have also extensively used antibiotics resistance Trans positions or mobile DNA elements but without the knowledge how these elements are spread in our environment [2]. Surprisingly, we have ignored the repeated appeals of Nobel Prize winner Dr Alexander Flemming against abuse of penicillin from 1950 onwards.

As an outcome, we find that stool culture of a female patient in Tokyo Hospital establishes that penicillin has failed to cure her infection with Shigella. From 1960 onwards similar reports of antibiotics failure have come from several countries. Based on all these reports we have conclusively confirmed that the transposable mobile DNA elements encoding antibiotic resistance traits are evolved by our unwise use of penicillin. Bugs survive in penicillin and the preventive treatment with vaccines is still questionable. Antigenic variation in modern bacteriology is not any new subject. We must accept the truth that *S. pneumoniae* growth curve is very different than that of laboratory *E. coli* K-12 [3]. And not accepting the truth the progress in *S. pneumoniae* genetics has been delayed. Growth curve of diplococcic *S. pneumoniae* and *S. oralis* should not differ. We have preferred working with S. *oralis* for several good reasons: *S. oralis* is much safer in handling, its complete DNA sequence is available and they are closely genetically related. Mortality rate of children and elderly from *Strept*

pneumoniae is increasing at a very high rate even in the presence of many newer derivatives of penicillin and availability of polyvalent vaccines for the last 22 years (1993-2015). We have recently published articles in reputed journals defining the growth of Mitis group bacteria in three phases: pre-competent, competent and post-competent [3-5]. It is becoming increasingly clear that Griffith's Smooth (S) and Rough (R) colonies as reported in 1928 has laid the foundation of bacterial genetics (6). We have repeated his experiment and fully analyzed the R colony formed when grown to saturation on blood agar solid medium. In fact, his rough colony is equivalent to the stationary phase or latent phase when grown in liquid broth (TSB or BHI). In the latent phase they all silently prevail together in an unusual pattern of existence; genetically their growth in different phases and potential of pathogenesis are not altered except differential expression of pathogenesis [7]. It is high time now that the penicillin resistance has created a crisis in the treatment of infectious diseases. We therefore have developed an idea of alternative preventive therapy with Xylitol, a low calorie, five carbon sugar alcohol, neither alcohol nor sugar.

In mit is group Streptococci, penicillin resistance takes place by the point mutations of their chromo *somal* PBP (penicillin binding protein) genes. These genes encode five high molecular weight proteins, essential for peptide glycan bio synthesis. The genes of these five proteins PBP1a, PBP 1b, PBP2a, PBP 2b and PBP2x are all chromosomal and are altered only by point mutations (a single base alteration). We think that the Xylitol phosphate formed by the mitis group bacteria during growth in Xylitol containing nutrient rich broth and inhibits their reproduction. Thinning of cell wall affects the cleavage that forms at the mid-cell position. This is also the junction of septal and equatorial biosynthesis of PG (cell wall biosynthesis). We find that these investigators have not considered the pre-competent phase at all and therefore the competent phase has received all the attention. What is more, the bacterial population even in their pre-competent phase if grown in Xylitol, Xylitol phosphate (or any other intermediate compound) formed has highly derogatory effection their continuation by cell division. Recently, *Morlot et al*. have reported an involvement of PBP2x and ser/there protein kinase in *S. pneumoniae* morphogenesis [8,9]. We have also presented our data in an international meeting in India (abstract published [10]) that we should be able to apply Xylitol in combination with fluoride to prevent pneumonia and TB caused by Gram positive bacteria by a common signaling mechanism even their G/C ratio are significantly different. Morphogenesis or an index of growth in different phases needs to be fully appreciated to think of an effective remedy to stop the increasing mortality rate of our children and the elderly from bacterial pneumonia and morbidity from dental caries.

Materials and Methods

Bacterial strains and experimental protocols have been published in our recent articles [11,12]. Gram-positive diplococcic Streptococcus strains saved in their separate stabs (mother stock): overnight cultures of *S. oralis* are diluted 10,000-fold in a rich broth TSB or BHI by our previously described dilution procedure and grown at 37^0C with or without shaking to saturation. Optical microscopy after Gram-staining technique with crystal violet and scanning electron microscopy has been used. Crystal violet solution is always prepared fresh and filtered through a sterile membrane filter disc (0.2um). Before starting experiments we have streaked our over night cultures on blood agar medium and the single colony transferred with sterile tooth picks onto CNA as well as MacConkey-lactose plates. Then the pure colony which grows only on CNA medium is used in our experiments. After shadowing with gold for 10 to 20 seconds, the sample is visualized by a scanning electron microscope, JEOL JSM -7600F at 15 kV.

Results

Penicillin resistance by point mutations in their PBP genes

Bacterial bio-communication via one or two component signal transduction system appears to be regulating bacterial growth cycle even in the presence of bactericidal penicillin but their details need to be fully analyzed. These diplococcic bacteria live silently in a stationary phase or latent phase in the nasopharynx of children. Silent phase means bacterial metabolism is completely turned off by the bacteria. The conditionally non-growing bacteria in their latent phase are usually resistant to all our beta- lactams (penicillin and its derivatives) but question is how the growing bacteria survive in our beta lactam drugs. Penicilin resistant viridans group streptococci including *pneumococcl* and the dental pathogen *S. mutans* have altered their PBPs by point mutations. The few mutants already present may grow faster by the nutrients as the majority of their population are lysed by the bactericidal effect of penicillin. The beta-lactamases are not known to occur in Streptococcal species [13]. Question remains how are these PBPs mutated Unlike Gram-negative bacteria, an

involvement of any extra-chromosomal DNA elements is a remote possibility but alterations of the progeny chromosome (2000- 2300Kb long) and alterations of PBPs may be induced by an abuse of penicillin has a role not in the alterations of PBPs but in the selection of rare mutants which usually arise during error prone DNA replication.

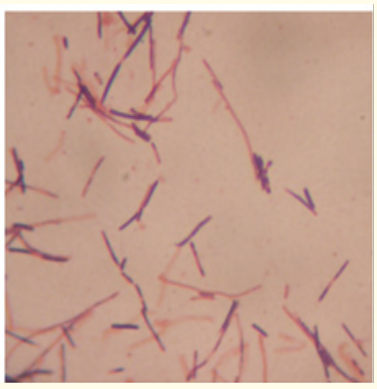
The role of *StkP* inc ell division and its modulation by an Interaction with penicillin binding protein PBP2x has been reported in a recent article of Morlot et al, 2013 (8). Their published article has stated "*StkP* and PBP2X interaction is mediated by their extracellular regions and that the complex thus formed is inhibited *in vitro* in the presence of cell wall fragments". What do they mean by extracellular regions? The *StkP* and PBP2x proteins are located in the membrane but at the junction where peripheral and septal bio-synthesis takes place. In this article we call this junction a cleavage site. Growth in Xylitol causes a displacement of the *stkP* and PBP2X by the thinning of cell wall thickness apparently ending in spheroplasts [10,11]. Such dislodgement of PBP2X, *StkP* affects their ability to bio-communicate (Figure 1, Saha Institute presentation). Xylitol derivative, or probably Xylitol phosphate is formed by the phosphorylation of Xylitol during their growth even in pre-competent phase. Disturbance in the orientation of *StkP* and PBP2x is an obvious outcome by the thinning of cell wall thickness (probably cell wall cross-links are collapsed) and the unfolding of peptidoglycan layers occurs in those who are in the competent phase; but we should not forget that these bacteria grow in heterogeneity of their population in different growth phases, pre-competent, competent and post-competent [3,4].

The pre-competent phase affected by Xylitol phosphate formed during their import via fructose

PTS transporter

In our experiments we have strictly followed pneumococcal growth in both solid blood agar media and liquid broth (BHI/TSB). We agree with Trahan *et al.* that Xylitol uptake and accumulation is mediated via a constitutive fructose PTS transporter while *S. oralis* is growing in nutrient broth [5,6]. Presence of Xylitol in normal growth media causes thinning of cell wall until protoplasts are formed and the entire population prevails in heterogeneity. When these protoplasts prevailing in the bacterial growth chain are ruptured, the population falls apart resulting in the release of entire population and the heterogeneity is seen even by optical microscopy after staining with crystal violet as used by century old Gram-staining technique [14]. But these protoplasts may not be visualized by standard Gram- staining which depends on an interaction between crystal violet and the bacterial cell wall. Thus the latent phase (Silent existence) of bacteria is affected and they initiate a new growth (Figure 2). Figure 2a shows the heterogeneity of the population usually prevails in their latent phase and Figure 2b shows the individual members in the chain after pipetting commonly used in microbiological labs. The members in the competent phase as well as in pre-competent phase are affected by their growth in Xylitol (*StkP* and PBP2x displaced), unfolding of the cell wall peptide glycan layers occurs around their protoplasts. We should also think about those in pre-competent phase with limited numbers of peptidoglycan layers are also affected, dimension reduces. Heterogeneity of sizes and shapes can be stabilized in chain by their growth in Xylitol, 2% or higher. The old has already lost their cell wall and has been ignored by many investigators because crystal violet dye used in our century old Gram staining technique fails to make them visible. Dislodgement of the PBP2X, *StkP* will interfere in their ability to bio-communicate (Figure 1a, 1b). Until now complete growth curve of *S. pneumoniae* is not available except Dr. Griffith has observed two types of bacterial colonies on blood agar medium: Smooth and Rough colonies.

Significantly, the population in the pre-competent phase either growing in mother's body or just born but both are affected in the presence of Xylitol. The entire population has been affected by the growth in Xylitol (2%): They all are stabilized but sizes are much smaller than the normal ones and appear spherical or spheroplsts. A few competent ones appear as diplococcic but most of the population have been affected in sizes from 1.8um ± 1um to approximately 0.1um but not shapes. They are diminished in sizes (spheroplasts) but still in chains, larger than 100nm, 100nm and smaller (Figure 1 with 100nm scale). A few diplococcic bacteria are also seen attached to their children. Comparison between the two populations of the same mitis group bacteria grown in rich broth, with and without Xylitol, helps us to appreciate their growths in chains and clusters.



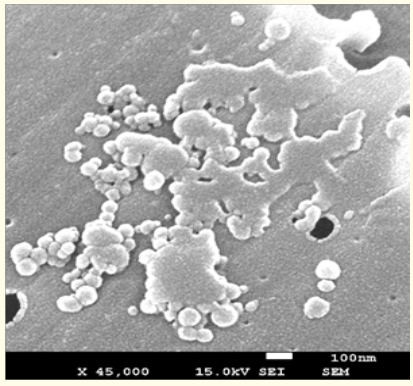


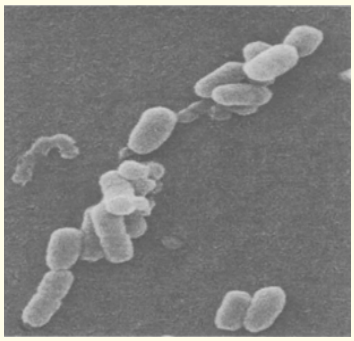
Figure 1a*: Population grown overnight in TSB plus 2% xylitol and visualized by SEM at high magnification (x45,000). The xylitol phosphate or xylitol derivative is formed which apparently affects the biosynthesis of cell wall peptidoglycan layers. Multiplication of these Gram-positive Streptococcus is very different than that of Gram negative laboratory strain E.coli K-12*

Figure 1b: *Optical microscopy magnified 1000X. The S.oralis population after 7 hours growth (stationary phase) is standard Gram staining with crystal violet6. It confirms that stationary phase population represents the mixture of purple band pink combined in the same chain. We think that the old ones are pink (like Gram negative bacteria) due to thinning of S.oraliscell wall thickness, and the young ones appers stronger than the pink, because of their cell wall's peptidoglycan layers difference. They are also showing theirt growth in branches*

Latent phase of growth in liquid broth and opaque or rough colonies in solid blood agar

The growth pattern of *S. oralis* as presented in this work provides an excellent explanation about the origin of rough colonies as recorded by Griffith in his 1928 laboratory note book [6]. The irregular contour of his rough colony on solid blood agar medium after 24 hours or longer period of growth supports streptococcal population growing in heterogeneity: baby, adult and the old in a single chain. We have established that they all do prevail in -chains but differ considerably from the Gram negative *E. coli* K-12. The old Gram-positive Streptococcus appears pinkish but still differs from the pink Gram-negative *E.coli* K-12 (As shown in Figure 1). Thinning of cell wall thickness renders them in competent to interact with crystal violet. Such a difference is not due to any bacterial contamination but appears to be thinning of their cell wall peptide glycan layers.

Because these bacteria grow in a long chain but in heterogeneity of sizes, it is not possible to accurately measure their dimension but their prevailing in heterogeneity has been confirmed by our SEM analysis of the stationary phase bacteria (Figure 2). The post-competent phase contains their total population with heterogeneity of morphology and sizes. The post- component phase culture, equivalent to R colony grown on blood agar medium shows their prevailing in heterogeneity. One such chain of *S. oralis* is presented in Figure 2(a). In the same field of electron micrograph, the diplococcic individuals in a pair and the old in a short chain are seen probably dissociated from the main chain during our sample preparation. The old incompetent have lost the cell wall thickness by the loss of peptidoglycan layers and therefore they look like spheroplasts with diminishing sizes. Size heterogeneity of the entire population has been clearly visualized by SEM when the same sample is subjected to shearing force as shown in Figure 2(b). It is a possibility that such shearing force may rupture the mothers who hide their progeny in their body. Figure 3 also supports this conclusion.



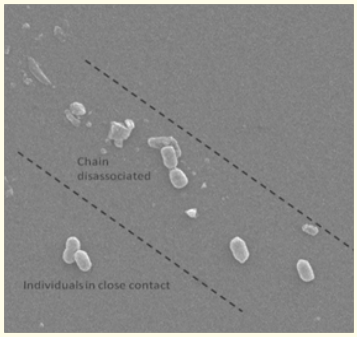


Figure 2(a): *(Left): rough colony of the S.oralis as visualized by scanning electron microscopy(SEM) at a magnification of 3000X. The members of the rough colony prevail in a long chain with heterogeneity. Individual diplococcus in a pair (below this long chain) and the old population in a short chain (above the long chain) appear due to breakage at random during sample preparation.*

Figure 2b: *(Right): the size heterogeneity of the S.oralis individuals when rough colony is suspended in a broth and grown at 37oC with vigorous shaking, with varying sizes from 0.2um to 1.8 um.The small ones are almost buried by the gold particles because of their dimensional differencewithparentswho are much larger in size (1.8um +0.1um).*

Pre-competent and competent phases

Natural transformation of diplococcic Gram-positive bacteria including *S. pneumoniae* is really their growth curve. Based on our most recent data we claim that the life cycle of diplococcic Streptococcus is their gradual physiological changes: baby (small, round) grows to young (oval, pre-competent), then the young becomes competent (diplococcic) with an ability to produce pheromone and finally reach their latent or stationary phase. However, donor DNA in an eclipse phase has not been isolated and therefore we think there is no evidence of homologous recombination between the donor DNA of any length and the recipient chromosome. The ultimate question remains who is the donor in natural transformation? This is not artificial transformation which has been widely used in gene cloning experiments with Gram negative *E. coli* K-12. Gram staining technique identifies these bacteria in heterogeneity of sizes and colour (Figure 3). The large Gram-positive, sizes 0.05-1.8um are purple but we may describe the heterogeneity of the total population in varying in sizes: baby (small, round) grows to young (oval, pre-competent), young becomes competent (diplococcic). However, a fraction of the mother may even get ruptured by the thinning or unfolding of their cell wall but the baby released should prevail in the environment (Figure 3). In conclusion we accept the truth that the heterogeneity of population if properly analyzed or observed should account for the total population but the diplococcic or elliptical purple ones (purple) are the fraction of the total population.

Significantly, overnight culture, diluted 10,000-fold by the standard laboratory protocol shows the purple colored diplococcic population as a minority and the remaining population with their heterogeneity of shapes and sizes (Figure 3). The small, round shaped bacterial baby (0.2um) grows gradually to oval shape and then the size of the oval increases from 0.2um to its full size about 1.8um. Cleavage appears at the mid-cell position of the oval shaped bacterium as an index of their competence for reproduction. The diplococcic bacterium is just the shape during reproduction. Pre-competent (spherical to oval), competent (oval to diplococcic) and the incompetent (spheroplasts or protoplasts with thin cell wall) representing their different phases of growth are clearly labeled in the micrograph (Figure 3). Some of them are stained pink, many of them may even remain invisible because of their inability to adsorb crystal violet, an essential dye used in Gram staining technique [13]. When our child is in mother's womb, we have not counted her as two!!

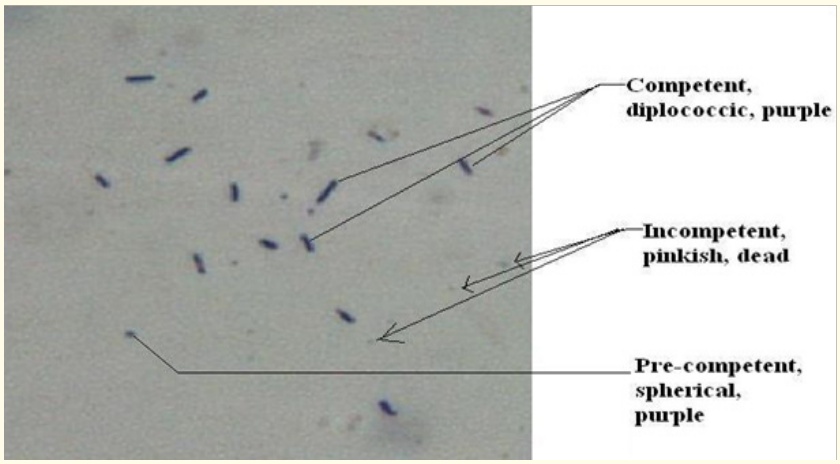


Figure 3: *The sample prepared after 10000 –fold dilution of stationary phase cultures to a lower titer (approximately105/ml) and Gram-stained by the Gram staining technique (modified). Such heterogeneity of S.oralis (a close kin of Pneumococcus) population is visualized by optical microscopy at 1000X magnification.*

Our data presented in this work clearly demonstrates that the diplococcic Strep to *coccus* grows in- chains with heterogeneity of their shapes, sizes and colour (purple, pink and colour less) after standard gram-staining technique, although all are originated from the genetically pure diplo *cocci*c Gram-positive bacterium. Obviously, the population in-chain gets ruptured in the course of sample preparation for microscopy and thus the truth has so far been overlooked and/or ignored by the investigators. We conclude the following: a) the diplococcic Strep to cocci grow in a long chain with heterogeneity of colour (pink, purple and pink - purple in combination) and a percentage probably remains colour less, b) the heterogeneity of sizes varies from 0.2um to 1.8um, c) the pink ones have lost peptidoglycan layers either by aging or by growth in 2% Xylitol (irreversibly old) looks extremely fragile, d) thinning of cell wall thickness produces protoplasts via spheroplasts, e) the adult population (competent) that appears deep purple appears to be them in ority of the total population and f) lysis of the population (autolysis) is likely to occur by the too much thinning of cell wall thickness under adverse condition like starvation when grown for 24 hours or longer on blood agar growth medium (Palchaudhuri S, data not included). Similar situation arises if they are grown in the presence of Xylitol (2% or higher concentration). Un folding of the peptide glycan layers (or autolysis) occurs and the peptide glycan layers spread which looks like a mesh around the protoplasts formed. This is not for the pre-competent who still affected. The competent ones are not all equally affected there is a gradient. The percentage of population who are already at the late phase of reproduction, the new born still prevailing in the mother or attached to the mother but shearing force will dissociate them. In fact we see this new population when the overnight cultures are diluted 10000 fold in nutrient broth by pipetting in the laboratory experiment population who has already reached their maturity (Figure 3). In this micrograph some ghosts or spheroplsats are barely seen which indicates the weak interaction of crystal violet and therefore identification may suffer (our unpublished data). Because of their heterogeneity of growth, the population in pre-competent phase may not show any autolysis but may still be affected by phosphorylation but without rupturing presence in the chain of clusters.

Discussion

Because antibiotic resistance has created a crisis in medicine, we have already developed an alternative but preventive therapy because in many cases the patients die before the correct diagnosis of pneumonia caused by the bacterium *S. pneumoniae. For the loss of peptidoglycan layers by thinning (spheroplasts) affects their cleavage formed at mid cell position which constitutes an essential site for the*

PG layers to diverge or bifurcate into septa land peripheral synthesis during bacterial multiplication. Formation of cleavage is initiated at the junction of peripheral and septal synthesis of PG; we think this is an index of the competent phase or adulthood. At this phase they start excreting pheromone under the regulation of two- component signal transduction or/and com genes [8,15]. It appears that *StkP* and PBP2X are both located in the cleavage and both are playing essential roles in the reproduction of bacteria. Growth in the presence of Xylitol affects bacterial cell wall cross- linkage and the spheroplasts are formed. The nutrients in the growth media are also slowly depleted and therefore they face starvation. Lysis of the population with thin cell wall may release some nutrients and feed the young for a short period. Exposure to fluoride at non- bactericidal doses will break them apart and the young will start their growth a fresh but if simultaneously Xylitol is present then the population in growth phase is affected. Obviously, we have to accept that they can't grow synchronously. However, presence of Xylitol is absolutely necessary. How does Xylitol work? The intermediate compound Xylitol phosphate is formed, affects the cell- wall thickness and the cleavage. The orientation of PBP2X and *StkP* is affected and signaling is fully blocked. *Fleurie et al.* have recently identified a protein Map Z at the mid cell position [16]. In agreement with these authors we also believe that the target of ser/thr kinase (*StkP*) which plays a central role in cytokines is and morphogenesis of the *S. pneumoniae.* Even the competent induced by using synthetic competent stimulating peptide 1 (CSP 1). They have shown that both phosphorylated and the non-phosphorylated forms of Map Z are required for proper positioning of Fts Z -ring formation. However, these authors remain silent a b out bacterial competence and the role of PBP2X but speculates about an interaction with the PG structure at mid cell position (cleavage, or equatorial mark) and becomes visible at the bacterial surface. Phosphorylation of bacterial kinase *StkP* affects bacterial cell division and their regulation via two component signal transduction. Obviously, the functioning of PBP2X and *Stkp* will be affected if they are displaced from their normal location. We have previously shown that the growth of viridians group Streptococci in Xylitol (2%) stops their multiplication in 2-3 hours by stabilizing irreversibly the entire population which grows in heterogeneity. However, we have recently defined their growth curve starting with a pre-competent phase and followed their other phases by measuring OD at 580mu, their colony forming unit (CFU) and their morphology and shapes by both optical and scanning electron microscopy. Many of us are not yet aware of the difference between strains of *E. coli*, K-12 and C in their growth patterns and sensitivity to a single stranded DNA containing phage phiX174.

In a very recent article, *Fleurie* et al. have produced their competent state of the same bacterial population by treating the pre-competent cells with synthetic competence stimulating peptide [16]. We have observed the similar situation under *in vivo* growth condition and it leads to a truth that there is no involvement of any exogeneous donor DNA! Such assumption has delayed the subject area and what is worse; many of our investigators like to accept that artificial transformation of *E. coli* K-12 with recombinant DNA is true for natural transformation in Gram positive Streptococci.

Conclusion

The progress of the natural transformation has been delayed because we are biased by the recombinant DNA technology for the period 1970-90 [17]. Many of these investigators are gene cloners who have introduced recombinant DNA (antibiotic resistant plasmids) into the recipient *E. coli* K-12, a gram negative bacterium by the presence of divalent ions and thermal shock. Please note that the natural transformation in Gram positive Streptococci doesn't have any similarity with such artificial transformation. of *E. coli* K-12. Based on our data presented in this article, we like to conclude that *E. coli* K-12 is a strain used in the laboratory of Professor Lederberg used extensively in gene cloning experiments. This is one reason why the growth of *S. pneumoniae* genetics has been delayed. In the absence of this knowledge alternative preventive therapy has not grown even in the presence of antibiotic resistance crisis. In fact, we have recently shown the natural transformation is the growth curve of mitis group of streptococci. They grow in heterogeneity of their growth phases and the entire population can be stabilized if grown in the presence of Xylitol (2% or higher) [4,5,11].

Bibliography

1. Lacks SA. Transformation In: "The Pneumococcus". Tuomanen Elaine I., et al. ASM press, Washington D (2004): 89-115.

2. Lodish H., *et al*. "Molecular Cell Biology". *4th edition. New York: W. H. Freeman* (2000).

3. S Palchaudhuri S., *et al*. "Blocking the two component signal transduction pathway of diplococcic Gram-positive pathogens by xylitol cloud as visualized by SEM". *International conference in India* (2013): 43-44

4. Palchaudhuri S., *et al*. "Xylitol blocks Streptococcal Signal transduction pathway to reduce children mortality rate from pneumonia". *International Journal of research* 1 (2015): 2394-2397.

5. Palchaudhuri S., *et al*. "Growth curve of Streptococcus oralis". *European Journal of Biological Research* 6.1 (2016): 36-41.

6. Griffith FJ. "The significance of pneumococcal types". 27 (1928): 113-159.

7. Marx P., *et al*. "Identification of genes for small non-coding RNAs that belonging to the regulation of the two component regulatory system Cia RH in Streptococcus". *BMC Genomics* 11 (2010): 661-667.

8. Morlot C., *et al*. "Interaction of Penicillin-Binding Protein 2x and Ser/Thrprotein kinaseStkP, the two key players in Streptococcus pneumoniae R6 morphogesis". *Molecular Microbiology* 90.1 (2013): 88-102.

9. Morlot C., *et al*. "Growth and division of S.pneumoniae: localization of high molecular weight penicillin-binding proteins during the cell cycle". *Molecular Microbiology* 50 (2003): 845-855.

10. Palchaudhuri S., *et al*. "International Meeting Abstract". Kolkata SINP, India (2013).

11. Czarnecki G., *et al*. "Gram positive S.mitis become sensitive to colistin and nalidixic acid when grown in Xylitol". *Journal of Molecular and Genetic Medicine* 7 (2013): 4172.

12. Palchaudhuri S., *et al*. "Raman spectroscopy of xylitol uptake and metabolism in Gram-positive and Gram-negative bacteria". *Applied and Environmental Microbiology* 77 (2011): 131-137.

13. Tuomanen Elaine I., *et al*. "The Pneumococcus". *ASM press, Washington DC* (2004).

14. Ton T. "MS thesis. Wayne State University under the guidance of Professor S. Palchaudhuri (2006).

15. Håvarstein LS., *et al*. "An unmodified heptadecapeptide pheromone induces competence for genetic transformation in Streptococcus pneumoniae". *PNAS USA* 92.24 (1995): 11140-11144.

16. Fleurie A., *et al*. "MapZ marks the division sites and positions FtsZ rings in Streptococcus pneumoniae". *Nature* 516.7530 (2014): 259-262.

17. Cohen SN. "DNA cloning: a personal view after 40 years". *PNAS USA* 39 (2013): 15521-15529.

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