

## Growth Curve of *S. PNEUMONIAE* -A Serious Pathogen

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### Objective

The *Pneumococcus* is a member of Streptococcus Mitis group and a serious pathogen capable of causing several severe diseases like bacterial pneumonia, meningitis, bronchitis, otitis media (mid-ear infection) and septicemia. In order to stop these diseases caused by the same pathogen, the preventive therapy appears to be now an option because of our antibiotic resistance crisis, born in 1960 in Tokyo (Japan) has not yet been solved. On the contrary the antibiotic resistance crisis has been increasing for the past 60 years by the abuse of antibiotics by our physicians and in the absence of any alternative therapy. These physicians have been increasing the doses of antibiotics with longer duration. How about our vaccines? That could have been an excellent solution provided the antigenic variation by the pathogenic bacteria is clearly understood. Many of these patients are children who are almost lacking body immunity and the elderly also losing their body immunity. Solution for our global population is to develop now an alternative preventive, low-cost home- therapy by inhibiting the growth of this pathogen. In view of such an objective, we must understand the growth curve of such a pathogen.

### Introduction

Dr Fred Griffith's case studies as recorded in his clinical note book of 1928 need to be completely understood [1]. He had observed two kinds of bacterial colonies (Smooth and Rough) when he had streaked the blood samples immediately after collection from the patients with lobar pneumonia on his blood agar medium (solid agar medium with sheep blood) and incubated for two days. These two colonies need to be clearly understood as a growth curve of *S. pneumoniae*. In microbiology text books this bacterium has been defined as diplococci but the diplococci never means the two; but it is merely the morphological appearance while the pathogen is in its post-competent phase or spheroplast. There are two other phases of growth: *pre-competent phase and competent phase*. We the investigators are fully responsible because of our temptation

for fame and fortune after the sudden death of Dr Griffith in the world war II. In 1944 Avery et al misunderstood these colonies of Gram –positive pathogen and mixed up these colonies with the artificial genetic transformation which has recently been widely used by the investigators to learn in-vitro gene cloning techniques but mostly in *E. coli* K-12, a Gram-negative laboratory strain [2,3]. We want to propose that we have not yet recognized the difference between natural transformation and artificial transformation [4]. What is worse, Avery et al made an effort to understand the difference between the Smooth and Rough colonies of Dr Fred but without the required academic preparation. They had isolated the “DNA” as genetic material (TCA insoluble precipitate) but assuming that the DNA means genetic traits. and therefore, their isolation procedure of DNA as genetic material was without the knowledge of DNA double helix as bio-macromolecule [5]. We must not forget that DNA isolated as TCA insoluble precipitate is NOT the same as DNA bio-macromolecule.

### Result and Discussion

DNA is our genetic material, not only in prokaryotes but also in eukaryotes. There are also two kinds of genetic transformation in bacterial genetics—natural transformation and artificial transformation. The artificial transformation has been mostly used in *in-vitro* gene cloning experiments with Gram-negative *E. coli* K-12. Natural transformation has not yet been recognized in an appropriate manner and therefore the growth curve of *Streptococcus pneumoniae* is still hovering in darkness. We are now confirming that the natural transformation is really the growth curve of *S. pneumoniae*.

This article wants to establish that the natural transformation is the growth curve of bacterium, pathogenic or non-pathogenic which obviously grows in three phases (pre-competent, competent and post competent). There is heterogeneity of sizes and shapes even in their pre-competent phase. In the competent-phase (adult but

not that all are carrying progeny). For some years the reputed investigators have recognized the early competent phase, mid – competent phase and late- competent.

We are now presenting the growth curve of *Streptococcus pneumoniae* but accepting the truth that the pre-competent phase is the progeny released by the lysis of spheroplasts. Spheroplast is really the mother morphologically appears diplococci because of bearing the progeny. These diplococci population should be handled with care and the use of pipettes or Eppendorff to dilute has mostly ruptured the spheroplasts releasing the population of progeny prevailing in mother's womb but in heterogeneity. Denial of such truth delayed the discovery of growth curve of Gram-positive mitis group diplococci which are serious pathogens. In 1928 Dr. Fred Griffith observed two colonies, Smooth and Rough colonies, the truth is this is a single colony gradually becomes rough but our textbooks describe as if these colonies are two separate colony but the truth is the same smooth colony becomes rough (uneven contour) in the course of growth in blood agar medium for about two-days [4,5]. Moreover, Avery et al have added additional confusion thinking involvement of DNA even they did not know the difference between the TCA insoluble DNA precipitation (nucleotide fragments) and the DNA isolation of bio – macromolecules (F and F-prime plasmids, Palchadhuri S,1971 unpublhed data). Until the discovery of double helix DNA by Watson and Crick in 1953 and the concept of bio-macromolecule as gene or genome, several reputed investigators had accepted Avery's TCA insoluble precipitate as if carrying genetic character [6]. TCA insoluble precipitate was free from RNA but collapsed deoxy-ribonucleotides originated by the denaturation at alkaline pH but re-naturation gives rise to insoluble precipitate [7]. *S. pneumoniae* grows in three phases- pre-competent, competent and post-competent. In 1964 Tomasz et al. have published an article entitled "The fine structure of Diplococcus Pneumoniae", they observed the ultrathin sections of *S. pneumoniae* under electron microscope at 2,00,000 times magnification [8]. Their data clearly shows the presence of progeny in the spheroplasts of *S pneumoniae*. Unfortunately, their mesosome or chondroid was not properly recognized as a temporary structure of mother *S. pneumoniae* bearing the heterogenous population of progeny. In this article I have described the progeny (pre-competent phase), which is originated by the lysis of spheroplasts. What causes such lysis? Answer is the undesirable shearing force induced by the use of pipettes or Eppendorff during the dilution of overnight culture.

Usually, we diluted our overnight cultures of *S pneumoniae* (spheroplasts) in fresh medium by pipetting or Eppendorff and the population of spheroplasts are lysed by such shearing force releasing the progeny in the growth medium. The progeny consists of individual members in heterogeneity of shapes and sizes. This is the population in pre-competent phase. Figure 1 shows this population in heterogeneity.

What is worse, the younger investigators have published an excellent article in 1964 entitled "The fine structure of diplococcus pneumoniae" but their interpretation suffered for

their bias towards Avery et al. without the correct interpretation of their own experimental data. What is worse, these investigators have also ignored Watson and Crick's double helix DNA as bio-macromolecules [6]. In 1972 our article published in the proceedings National Academy Sciences USA, after the recommendation of 1960 Nobel prize winner Professor Severo Ochoa, we have made it very clear that the TCA insoluble precipitate of DNA as reported by Avery et al in 1944 is really collapsed nucleotides of DNA fragments resulted by the alkaline denaturation of chromosomal DNA of *S. pneumoniae*. This is also true in the isolation of Gram-negative *E. coli* K-12 chromosomal DNA (Palchadhuri Sunil, unpublished data). Therefore, I had to modify the existing DNA-isolation procedures to visualize the chromosome or extra-chromosome (F-plasmid in super-coiled covalently closed circular DNA) [7].



**Figure 1:** Mitis group Streptococcus progeny when overnight culture diluted by using pipettes. Progeny sizes and shapes confirm their growth in heterogeneity. Gram-staining technique needs to be modified to re-confirm the heterogeneity of such a population in pre-competent phase, competent phase and post-competent phase (spheroplast).

Because of our modification of existing Gram –staining technique to visualize the entire population in the pre-competent phase the morphological look of spheroplasts in diplococci shape (mother carrying progeny) was sacrificed to some extent. The crystal violet solution was not completely washed with acetone –alcohol mixture. However, the progeny in heterogeneity of growth become barely visible but looked pink to pinkish. The spheroplasts, fully lysed by the complete rupture looked as pink- bush adjacent to another spheroplast not lysed but they still prevail in chain.

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