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# Genetic Transformation in Gram-Positive Streptococcus *pneumoniae*

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## Abstract

There are two kinds of genetic transformation as described in the books of molecular genetics of bacteria: natural transformation and artificial transformation. Many of us are familiar only with artificial transformation because of *in-vitro* gene cloning experiments with recombinant DNA in gram-negative *E. coli* K-12. This is formed by joining a DNA fragment of choice to a multicopy cloning vector pBR322 and its entry into the gram-negative *E. coli* K-12, made competent by its growth in rich broth containing calcium chloride and thermal shock. In natural transformation as studied with gram-positive pathogen *S. pneumoniae*, several investigators have reported the entry of exogenous linear DNA fragments (eclipse phase) but how does such linear DNA fragments originate? However, the established investigators are still not considering our concept of genes, operons and regulons. They have used the word fratricide without the knowledge of growth curve. Now with the knowledge we want to think this pathogen grows in clusters but prevail in chains. Our work on genetic transformation has established that the gram-positive pathogenic bacterium *S. pneumoniae* grows in three phases-pre-competent, competent and post-competent (spheroplast) without the requirement of any exogenous linear DNA fragments.

***Keywords:*** *Genetic Transformation; Spheroplast; E. coli K-12; S. pneumoniae; Natural Transformation; Artificial Transformation; DNA Fragments; pBR322*

In 1928 Dr Fred Griffith made an attempt to develop a growth curve for the gram-positive pathogen *S. pneumoniae* but unfortunately, we lost him in the World War II before his project was done. He left his clinical laboratory note book as his legacy [1]. Briefly, the blood samples as collected from his patients with lobar pneumonia were immediately streaked on blood agar medium and then incubated overnight at 37°C with or without $CO_2$. In the following morning he observed tiny colonies (Smooth) and therefore he decided to go for longer incubation (about 48 hours). These small colonies grew large but with uneven contour (Rough)! Dr Griffith had not examined this serious pathogen *Streptococcus pneumoniae* under optical microscope after gram staining but I (SP) have seen this pathogen and its avirulent derivative morphologically both appear diplococcic under the scanning electron microscope (SEM) (Figure 1). When ruptured a heterogeneity of population is observed (0.1 um to 1 um) but many of them could not produce colony forming unit (cfu). Were they contaminants but our answer is "No". Because they grow in heterogeneity of sizes and shapes (round-oval-diplococcic).

After 16 years of Dr. Griffith's death, Avery., *et al*. of Rockefeller University made an attempt to understand Dr. Griffith's two colonies (Smooth and Rough) of *S. pneumoniae* but mostly on an assumption that the entry of exogenous DNA (TCA insoluble precipitate) changes the Smooth colony to Rough colony (1944). How did they isolate the DNA? Their isolation procedure used an alkaline pH to lyse the bac-

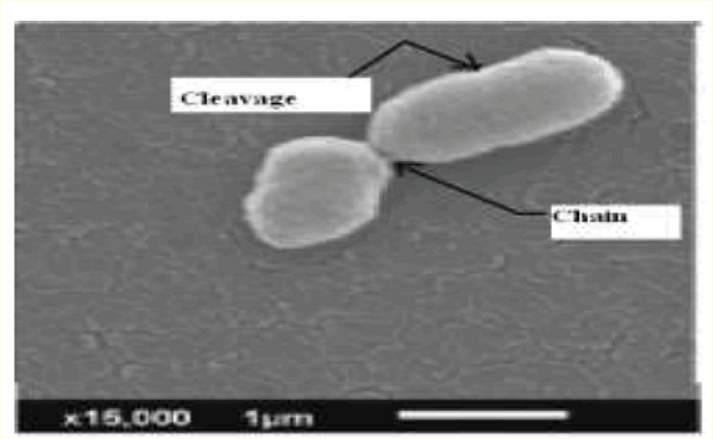


***Figure 1:*** *Scanning electron micrograph of the two diplococcic S. pneumoniae linked in a chain, also recognized as spheroplast, conditionally looks diplococcic but never the two.*

teria and then neutralized by using TCA [2]. The TCA insoluble precipitate thus prepared is merely the collection of deoxyribonucleotides but never any bio macromolecule [3]. In 1971, in the New York University laboratory of Prof. WK Maas, when I (SP) repeated the isolation procedure of Avery., *et al*. to prepare the fertility factor or F plasmid from *E. coli* K-12 male but in vain. Then Dr. Sunil Palchaudhuri developed a new isolation procedure and measured the size of *E. coli* sex factor which is 31 um or 100 Kb [3]. In order to make it clear an electron micrograph of a bio-macromolecule (F plasmid of *E. coli* K-12) containing several genes or operons as shown in figure 2. The same isolation procedure confirms that the TCA insoluble precipitate of Avery., *et al*. was really polynucleotides but never a bio-macromolecule (Palchaudhuri S., unpublished data).

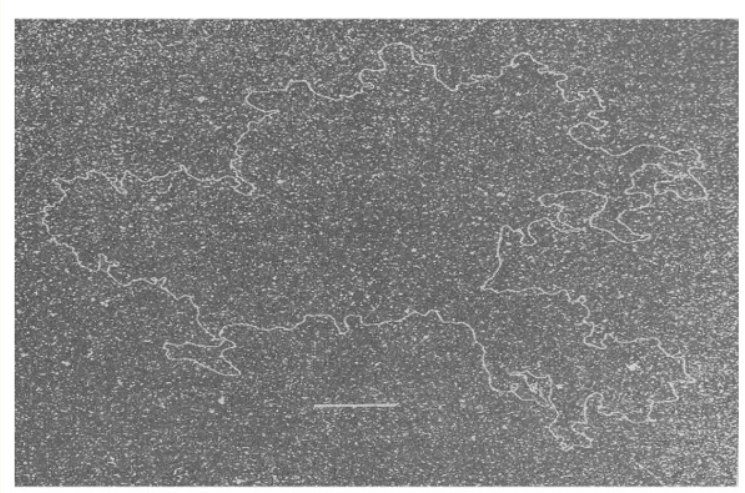

***Figure 2:*** *Electron micrograph of E. coli K-12 sex factor or F plasmid (100 Kb) prevails as CCC DNA bio-macromolecule (supercoiled) but exposed to X-irradiation to convert it into open circular bio-macromolecule of length 31 um [3].*

In fact, double helix DNA was discovered by Watson and Crick in 1953 [4]. In 1944 Avery., *et al*. used the word DNA but it seems his ignorance of DNA as bio-macromolecule and their TCA insoluble precipitate is nucleotides long but never any genetic characters [3]. The TCA precipitate of Avery., *et al*. was never DNA bio-macromolecule nor even DNA fragments [2,3]. Ignorance of our investigators has delayed the progress of gram-positive bacterial genetics because they were biased by the *in-vitro* recombinant DNA as formed in *E. coli* K-12 [4,5]. After the entry of radio labelled DNA nucleotides but never bio-macromolecule, the radiolabelled $P_{32}$ DNA nucleotides formed a complex with a protein (eclipse complex) [6,7]. Unfortunately, Morrison observed an eclipse phase because of his bias as if the TCA insoluble precipitate of Avery., *et al*. does carry genetic characters! [6].

*Streptococcus pneumoniae* grows in three phases- pre competent, competent and post competent. The post-competent phase is also known as spheroplast and looks diplococcic because their progeny is growing in heterogeneity of growth phases [8]. Previously our text-book has defined *S. pneumoniae* as diplococcic but without the reason why it looks diplococcic, now we want to give a reason why a single bacterium looks diplococcic-because mother bacterium (post-competent phase) is carrying the progeny. In order to study its growth curve-the overnight culture was diluted in fresh rich broth by pipetting (10-fold, 100-fold, 1000-fold and 10000-fold), spread on blood agar medium, and observed after overnight incubation at 37°C. We observed equal number of cfu (colony forming unit) at higher dilutions but the optical density increased after 90 minutes of growth and continues but not the colony forming unit (cfu). How is it possible? Professor Palchaudhuri has made it clear because the shearing force induced by pipetting the progeny is released, many of these progeny can't form cfu [8]. Previously some investigators have also observed two kinds of colonies-transparent and opaque instead of smooth and rough. Now we know that this pathogen also grows in clusters but still all clusters may not prevail in the same chain if not grown in the presence of Xylitol (2% or higher) (Palchaudhuri, Ms in preparation).

## Discussion and Conclusion

For many years gram positive bacterial genetics was not grown because of our bias towards an *in-vitro* gene cloning technique [5]. During the period 1980 - 2005 many of our reputed investigators have claimed that the entry of linear DNA fragments is required to attain a competent phase [6,9]. We should not forget that there is a distinguishable difference between the cell wall thickness (many layers of peptidoglycan) of the gram-positive bacteria *S. pneumoniae* but the gram negative *E. coli* K-12 which has just one peptidoglycan layer, sandwiched between its two membranes (inner and outer). In the course of growth (optical density increases but not the colony forming unit (cfu), the *S. pneumoniae* grows in clusters but these clusters prevail in chain [8]. Nutrients of growth media are exhausted and the mother(spheroplasts) are lysed from starvation even by minimal shearing force. This is not fratricide. Unfortunately, Haverstein., *et al*. have used the word fratricide as if the competent *S. pneumoniae* is killing the old parents for their nutrients [10]. Natural transformation is really a growth curve of *Streptococcus pneumoniae* but without the requirement of any exogenous DNA fragments, single or double. Until recently the growth curve of this pathogen was not available but the book entitled The *Pneumococcus* published by the ASM Press (USA) in 2006 has included a review article by Sanford Lacks [7]. The first three pages of his review article do not mention the difference between DNA bio-macromolecules and the DNA polynucleotides of Avery., *et al*. (Figure 3). What is worse, he thinks Avery 's TCA insoluble precipitate is very important but not the effort of Dr Fred Griffith who was thinking about growth curve [1,2]. His diagram shown in my figure 3 illustrates how the group force leads us to astray. Figure 3 shows oligonucleotides but dreams the entry of SS DNA! His oligonucleotides may enter and form an eclipse phase complex by binding protein [9,10].

Lacks has supported Avery., *et al*. (1944) for his TCA insoluble precipitate without the knowledge of bio-macromolecule but criticised Dr Fred Griffith who made an honest approach towards developing a growth curve on the basis of his two colonies -smooth and rough on blood agar medium [1,2].

All these publications have delayed the progress of gram-positive genetics, specifically treatment of patients infected by the antibiotic resistant pathogen, *S. pneumoniae* [4-7]. Recently we have defined the growth curve of *S. pneumoniae* in three phases [8]. Without the

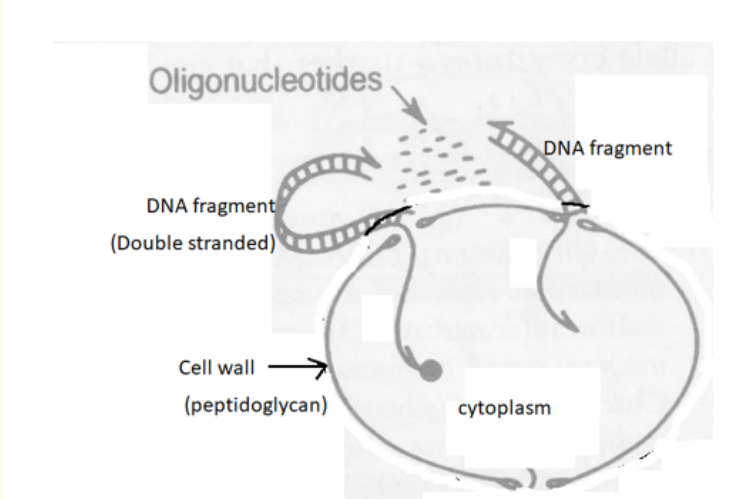


***Figure 3:*** *Model for DNA uptake in transformation of S. pneumoniae showing the attachment of the DNA fragments (linear) to the surface of the competent S. pneumoniae [6,7].*

growth curve of this pathogen and the knowledge of its antigenic variation, vaccines may not remain effective for many years but the xylitol should give us a preventive treatment for children and the elderly (primary victims of bacterial pneumonia [9-11].

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