

## Avirulent Gram-negative Bacteria *E. coli* K-12 or *E. coli* C Compared with Gram-positive Virulent Diplococcic *Streptococcus pneumoniae*

Sunil Palchaudhuri<sup>1\*</sup>, Hansini Mundigala<sup>1</sup>, Eldar Kurtovic<sup>1</sup>, Anubha Palchaudhuri<sup>2</sup> and Archita Biswas<sup>2</sup>

<sup>1</sup>Department of Immunology and Microbiology, Wayne State University School of Medicine, 540 E Canfield St, Detroit, MI 48201, USA

<sup>2</sup>Atlanta Health Centre, Kolkata, India

\*Corresponding author: Sunil Palchaudhuri, Department of Immunology and Microbiology, Wayne State University School of Medicine, 540 E Canfield St. Detroit, MI 48201, USA, Tel: +13135771335; E-mail: spalchau@med.wayne.edu

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### Abstract

Growth curve of *E. coli* C is similar to that of the pathogenic Gram positive strain *Streptococcus pneumoniae* (Sp). In 1928 Dr. Griffith studied Smooth and Rough colonies on blood agar (BA) medium and significance of his observation has been ignored for a long time. In 1944, Dr. Avery et al. have made wrong interpretation of this observation. TCA insoluble precipitation contained only a few nucleotides or at best a fragment of 0.1 Kb. It is unlikely to contain any genetic character. Recently, Palchaudhuri et al. revived and redefined Dr. Griffith's Smooth and Rough colonies. These colonies are actually the growth curve of *S. pneumoniae*. Our objective is to demonstrate a way of combating the pathogen *S. pneumoniae* by utilizing the knowledge of growth pattern.

However the same *S. pneumoniae* appears to be responsible for several diseases like bacterial pneumonia in children and the elderly, paediatric dental caries, otitis media, meningitis and septicaemia (differentiation of the diseases) [3,4]. In recent years investigators have reported that some non-translational small RNAs are responsible for the differentiation [5].

During 1960-1975, three different strains of *E. coli*, *E. coli* K-12, *E. coli* B and *E. coli* C have been used worldwide to develop microbial genetics [6,7]. This includes primarily *E. coli* K-12, bacteriophages (lytic, lysogenic and semi-temperate) and bacterial plasmid DNA (mostly double-stranded covalently closed circle). Recombinant of *E. coli* K-12 and *E. coli* C formed in vivo by genetic cross linkage. *E. coli* HF4714 thus formed shows some interesting characters of parents. Like *E. coli* C, it forms plaques when infected with bacteriophage  $\phi$ X174 and carries genes needed for the utilization of 5-carbon sugar alcohol xylitol. *E. coli* C is very small in size (~1  $\mu$ m), K-12 is long (2.5  $\mu$ m) but the recombinant *E. coli* HF4714 is more like *E. coli* K-12 in size. However such knowledge of genetics has been ignored. Around this time Stanford University School of Medicine faculty and their laboratory technicians begin an in vitro gene cloning experiment (1972-1980) [8-10]. In the formation of such recombinant DNAs, several antibiotic resistant determinants are extensively used although the antibiotic resistance has created a crisis in Medicine. What is the difference between genetic determinants and their transposons? Usually bacterial determinant means genetic character but transposons are mobile DNA elements. Mobile DNA elements are not replicons as defined by Jacob and Monod, but they still multiply [11]. Insertion sequence (IS) and transposons (Tn) are both mobile DNA elements [12,13]. The transposons (Tn) are flanked by IS DNA sequences like IS1 with antibiotic resistance characters and/or genes for exotoxin (stx) production, both stable and unstable [14,15].

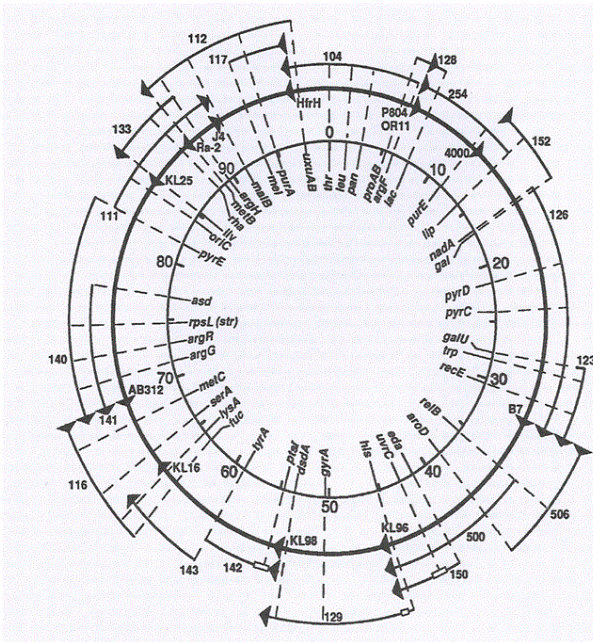
DNA sequence based dissimilarity leads us to conclude that an operon for the metabolism of xylitol (5-carbon sugar alcohol) is absent in *E. coli* K-12 and *E. coli* B but present in *E.*

**Keywords:** Growth curve; Comparison; Xylitol operon; *E. coli* C; *Streptococcus pneumoniae*

### Introduction

In 1928 Dr. Fred Griffith has introduced the concept of bacterial genetics in his famous pathology notebook. Gram-positive growth curve includes two colonies grown on blood agar media [1]. He has seen two types of *S. pneumoniae* colonies: SMOOTH and ROUGH. Smooth colonies as observed on blood agar media represent the log phase (early, mid and late log) and rough colonies represent the stationary phase (equivalent to the stationary and decline phase of *E. coli* K-12). Smooth colony is more severe than the Rough colony. We have proven earlier that the Smooth colony represents bacteria in log phase and Rough represents stationary phase [2]. These two colonies represent the bacterial physiological changes. While they are in log phase, we think these bacteria are responsible for the degree of severity of diseases [1,2].

*coli* C chromosome at 47.5 min location of linkage map (**Figure 1**), proximal to Met G loci [16,17]. It is well established now that the 10% of *E. coli* C isolates contain xylitol operon but the origin of xylitol operon remains unknown.



**Figure 1:** Linkage map of F-prime factors. Two red stars show the position of oriC (*E. coli* C or *E. coli* K-12 origin of replication). F-prime argECBH and metB are located proximal to the oriC are highly unstable. Therefore, it dissociates into complete F plasmid and chromosomal segment carrying these two operons. This chromosomal segment is flanked by mobile DNA element gamma-delta or Tn1000.

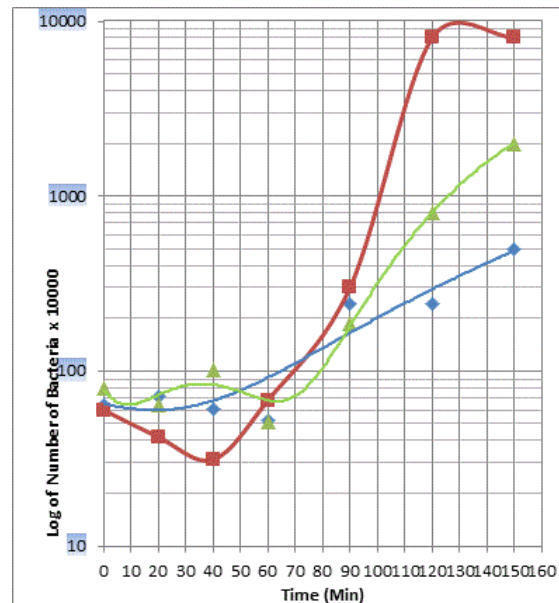
## Experimental Procedures

All these experiments and bacterial strains have been described in our previous articles [2,14].

## Results

### Comparison of growth curves *E. coli* K-12, *E. coli* C and *E. coli* HF4714

Growth curves of Gram positive Streptococci and Gram negative *E. coli* K-12.

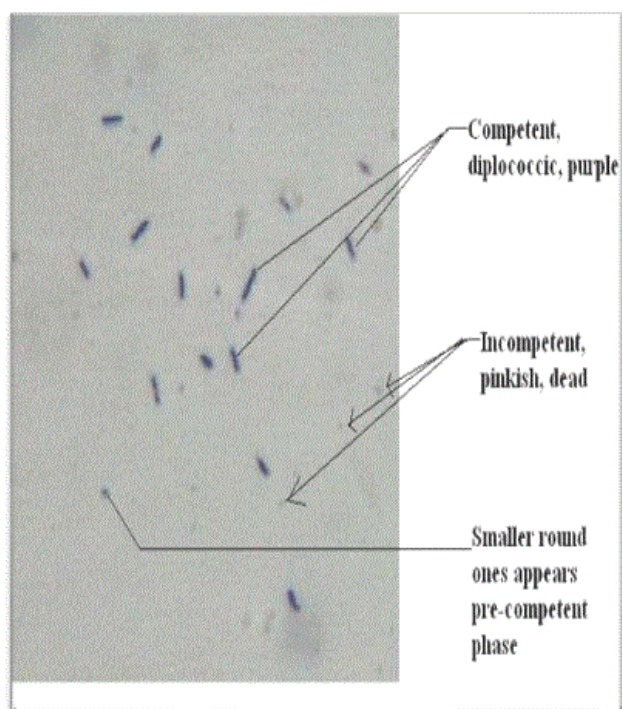


**Figure 2:** Growth curve of *E. coli* C, *E. coli* K-12 and their recombinant *E. coli* HF. Blue curve represents growth curve of *E. coli* C. Red represent that of *E. coli* K-12. Green represents growth curve of the recombinant *E. coli* HF4714.

Previously we have published the growth curve of Gram-positive pathogen *S. pneumoniae*. This belongs to Mitis group Streptococci. All members of this group grow in three different phases: pre-competent, competent and post-competent [2]. In many articles investigators have mentioned early, mid and late competent. Linkage map of *E. coli* C does not differ from *E. coli* K-12 except for xylitol operon (partial or complete) and therefore the *E. coli* C is sensitive to single stranded circular bacteriophage  $\phi$ X174. However, in *E. coli* C we have defined log phase in three phases - early, mid and late. Gram-positive *S. pneumoniae* is also carrying Xylitol operon, but it cannot metabolize except for xylitol phosphate which is highly toxic to their continuity. We have already published how this Gram-positive organism is affected by the growth in xylitol (2% or more) [18,19].

Smooth colony of Dr. Fred Griffith represents its growth in log phase (early, mid or late) [1]. Dr. Griffith's ROUGH colony is the same as stationary phase of *E. coli* C or *E. coli* K-12. At the same time, we should not forget that exogenous DNA, single or double, does not have any role in the physiological states (smooth or rough colonies). Meanwhile several distinguished investigators have mixed up the microbiological meaning of a single bacterium with its colony and the bacterial strain (reference not cited). In order to appreciate the difference of colony morphologies, we should go back to the growth curve of *E. coli* K-12.





**Figure 3:** Stationary phase viridans group *streptococci*, diluted 10,000-fold in rich broth, allowed to grow at 37°C and then visualized by optical microscopy after Gram staining (magnification 1000X). Bacterial population maintains heterogeneity of sizes including the pre-competent, competent and the post-competent. Bacterial population in the post-competent phase is diminished in size because of the thinning of cell wall thickness (spheroplasts, protoplasts and peptidoglycan cell wall fragments) [18].

Unlike *E. coli* K-12, *E. coli* C does not have any Lag phase. This reminds us, the growth pattern of Gram positive bacterium *Streptococcus pneumoniae* (Sp). It is well known that Professor J Lederberg has extensively used in his laboratory experiments this strain of *E. coli* K-12 without any adverse effect, so we think that is an avirulent strain. Question still remains that this *E. coli* K-12 becomes male by the presence of fertility factor F. Besides, a single chromosome of *E. coli* K-12, F plasmid continues as an extra chromosome, stringently controlled being a single copy of length 100 Kb [13]. In the Microbiology text book F prevails not always as extra-chromosome but integrates into the chromosome, which is substantiated by Dr, William Hayes as HfrH strain [20]. What is more, an aberrant excision of F forms F-primes like F-prime lac<sup>+</sup> or F-prime trp<sup>+</sup> and retains maleness of the host *E. coli* K-12. *S. pneumoniae* is a pathogenic strain and produces several severe diseases (Bacterial pneumonia in children and the elderly, paediatric dental caries, otitis media, meningitis). However there is a degree of difference in pathogenesis. *E. coli* HF4714 growth pattern is somewhat similar to that of *E. coli* C but not like *E. coli* K-12 (Figures 2 and 3).

### *E. coli* C and multiplication of bacteriophage $\phi$ X174

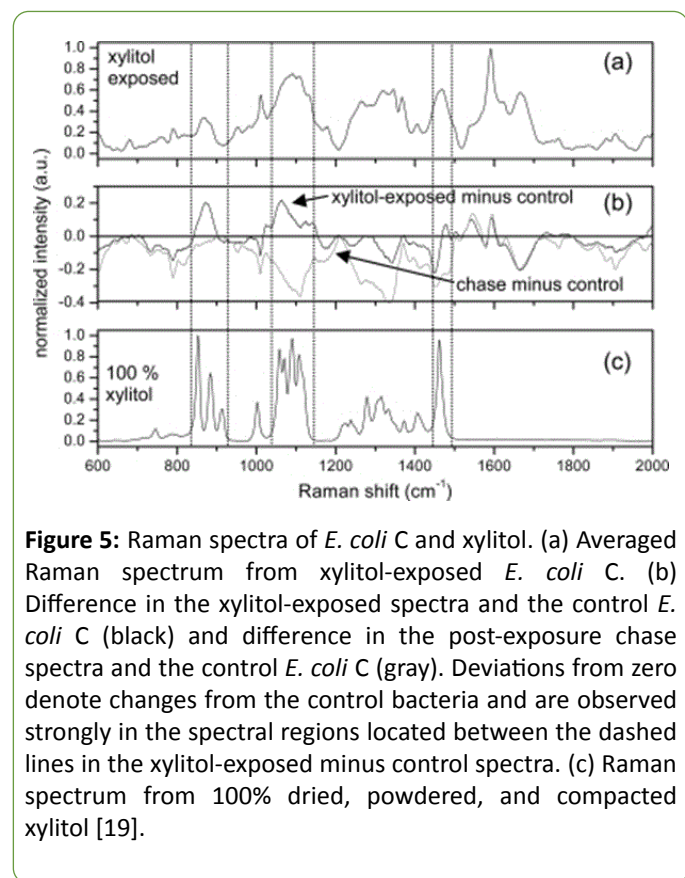
*E. coli* C acts a host for the single stranded DNA containing bacteriophage  $\phi$ X174. But *E. coli* K-12 is resistant to the same phage. We have recently shown by Raman spectroscopy that the *E. coli* C which carries a complete xylitol operon apparently expresses at a low level or remains silent [19].



**Figure 4:** Effect of 2% xylitol on viridians group streptococcus ultrastructure as revealed by differential Gram-staining imaged with the same magnification. (a) Planktonic viridans group streptococcus cells cultured in BHI medium and 0% xylitol anaerobically for 24 h prior to growth in xylitol. The titer has been reduced 10,000-fold to ensure that all bacteria attain log-phase growth in the presence of the xylitol [19].

In this work, we have compared the growth curves of Gram negative *E. coli* C (xylitol positive) and Dr, Lederberg's laboratory strain of *E. coli*, *E. coli* K-12 (xylitol negative). Difference between these two strains is that *E. coli* C is capable of utilizing 5-carbon sugar alcohol xylitol as a carbon source and producing plaques when it absorbs the bacteriophage  $\phi$ X174. *E. coli* K-12, on the contrary, is apparently resistant to the xylitol and  $\phi$ X174. However, *E. coli* HF4714 generated by the homologous recombination between *E. coli* K-12 and *E. coli* C, is capable of metabolizing xylitol constitutively. In short, the recombinant *E. coli* HF4714 grows even in minimal media containing xylitol alone. *E. coli* HF4714 can utilize xylitol, and is resistant to bacteriophage  $\phi$ X174. Genome of bacteriophage  $\phi$ X174 is single stranded, covalently closed circular (CCC) DNA. The phage adheres to the outer membrane of *E. coli* C

using its spikes. Subsequently phage multiplies and releases thousands of mature particles by the lysis of *E. coli* C (Figures 4 and 5) [21,22].



**Figure 5:** Raman spectra of *E. coli* C and xylitol. (a) Averaged Raman spectrum from xylitol-exposed *E. coli* C. (b) Difference in the xylitol-exposed spectra and the control *E. coli* C (black) and difference in the post-exposure chase spectra and the control *E. coli* C (gray). Deviations from zero denote changes from the control bacteria and are observed strongly in the spectral regions located between the dashed lines in the xylitol-exposed minus control spectra. (c) Raman spectrum from 100% dried, powdered, and compacted xylitol [19].

## Discussion

Avirulent Gram negative *E. coli* C is completely lysed by the semi-temperate phage  $\phi$ X174. Based on the similarity of growth patterns, we like to postulate that the phage  $\phi$ X174 should also affect the virulent Gram positive *S. pneumoniae*. At the same time in our recent publications we have demonstrated that there is no such lysis. In this context we like to recall that there is a big difference between cell-wall of Gram positive *S. pneumoniae* and the outer membrane of Gram negative *E. coli* C. Below this membrane there is a single layer of peptidoglycan layer and then the inner membrane encircling the cytoplasm. There is a single chromosome of approximately 4734 Kb and at the same time the cell wall of Gram-positive *S. pneumoniae* consists of several peptidoglycan layers and then the inner membrane. The cell wall is formed via cross-bridges of pentapeptides [23]. Once the unfolding is initiated by their growth in presence of xylitol (2% or more), their spheroplast is formed and followed by protoplast, Cell wall is fragmented and these peptidoglycan fragments inhibit any further growth of bacteria [24].

During the reproduction of *S. pneumoniae*, a cleavage is formed at the midpoint of the diplococcus and it appears as diplococcus. But it is never two bacteria, but one bacterium. Therefore the word diplococcic is misleading. However the cleavage is an index of reproduction which is very similar to

humans. In 2004 review article professor Sanford A. Lacks has made a great mistake (in all his articles) by his statement that the artificial transformation of *E. coli* K-12 and the natural transformation of *S. pneumoniae* is similar [25]. Probably he has been biased by the gene cloning technique of Dr. S. N. Cohen [8-10]. Fragments of *E. coli* K-12 DNA (double stranded) are forcibly introduced into a recipient artificially made competent by growing in the presence of 0.01 M CaCl<sub>2</sub> followed by a thermal shock. Major problem is the difference between such "Artificial transformation and the Natural transformation" as observed by Dr. F. Griffith in 1928. *S. pneumoniae* grows in three phases, pre competent, competent and post competent [2]. *E. coli* K-12 does not grow in chains but *Pneumococci* grow in chains [2]. In natural transformation, there is no role of exogenous DNA fragments. This is purely a physiological process as in humans. The oval shaped pre-competent bacterium becomes competent diplococcic. In this process why the question of entry of exogenous DNA (single or double strand) arises? Such a misconception is carried over for almost 50 years starting with Avery et al. [26]. They have never made it clear that there is a difference between tiny DNA fragments (TCA insoluble) and DNA bio-macromolecule of Watson and Crick, 1953 Nobel Prize [27]. Bio-macromolecule of length 4734 Kb is the whole chromosome of *E. coli* K-12 and 2200 Kb approximately is the chromosome of *S. pneumoniae* [28-30]. Previously Cohen et al. have not made it clear that there is a big difference between a transposon and antibiotic resistant determinant. Now we have accepted that the r-det component is a collection of transposons flanked by the IS1 sequence [31]. Previously Dr. N. Kleckner has published a Review Article in Annual Review of Genetics (Vol 15, 1981) without the knowledge about the integration of F-plasmid into its host chromosome *E. coli* K-12 and formation of F-prime plasmid (Type-1). The Type-2 uses transposons (non-homologous and Rec-A independent) [32].

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