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Mini Review

Bacteriophage phiX174 Uses Spike to Enter into the Adhesion Zone of *E. coli* C

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Abstract

From the middle of 1950 two strains of gram-negative bacteria *E. coli* K-12 and the *E. coli* C have been extensively used in developing Molecular Genetics. These two bacteria have a single chromosome (DNA bio-macromolecule of length 4736 Kb) in CCC form (Form I) but need to be converted into its replicative form (Form II) to study DNA replication. Significantly the DNA sequences of these two strains don't differ except the presence of xylitol operon in *E. coli* C. The xylitol operon changes the shape of *E. coli* C, size and also sensitivity to the bacteriophage phiX174. Dr Arthur Kornberg used this phage genome of length 5.3 Kb but without the knowledge of adhesion zone. This adhesion zone is the site where two membranes and cell wall of *E. coli* C are fused together. Before the entry into the adhesion zone of *E. coli* C, the phage phiX174 sends bio-signal via spike to get into the adhesion zone. After the entry into the adhesion zone the phage genome in CCC DNA (Form I) is converted into its replicative form (Form II), then multiplies and before maturation the Form II becomes Form I then encapsulation. Mature phage particles are released by rupturing the bacterial cell wall.

Keyword: *Bacteriophage phiX174; E. coli C and E. coli K-12; Adhesion Zone*

Introduction

The two strains of gram-negative *E. coli*, *E. coli* K-12 and *E. coli* C have mostly been used in the development of bacterial genetics. Morphologically *E. coli* K-12 is cylindrical and *E. coli* C is spherical but their chromosomes are of the same length (approximately 4736 Kb) except Xylitol operon in *E. coli* C. In order to make it easier to follow I have drawn a diagram to show supercoiled configuration of chromosomes of *E. coli* C and *E. coli* K-12 and their attachment to the cell wall (Figure 1). Xylitol is a low calorie five carbon sugar-alcohol (neither sugar nor alcohol). Before the entry into the adhesion zone of *E. coli* C, the phage phiX174 sends bio-signal via spike to get into the adhesion zone [2,3]. Interestingly the presence of xylitol operon confers on *E. coli* C a natural growth pattern unlike binary fission of *E. coli* K-12. The gram-negative bacterium *E. coli* C grows in three phases, pre-competent, competent and post-competent like Gram positive bacterium *Streptococcus pneumoniae* [1]. Professor Arther Kornberg used the replicative form (RF) DNA of the phage phiX174 (length 5.3 Kb in a single stranded CCC form) to study DNA replication but without the knowledge of different DNA polymerases besides pol A1 [4,5].

Result

In 1960 Dr Aurthur Kornberg shared Nobel prize with Dr Severo Ochoa with the growth of molecular biology. What is more, Dr. Kornberg had used the bacteriophage ΦX174 and its host *E. coli* to study DNA replication at a molecular level but without the knowledge of bacterial

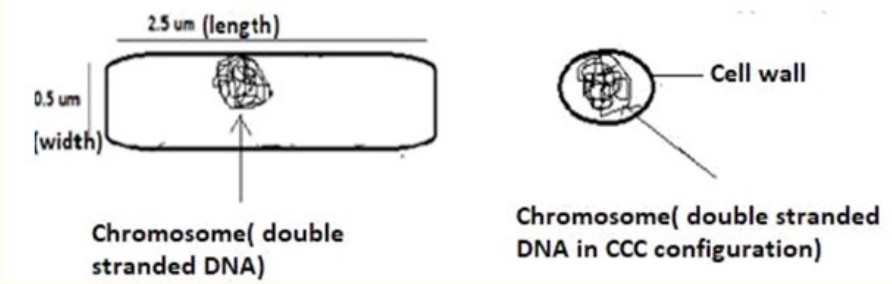

Figure 1: *Comparison between shapes of E. coli K-12 (Left) and E. coli C (Right).*

genetics [4,5]. Unfortunately, he didn't mention whether he used the *E. coli* C or *E. coli* K-12. I therefore decided to work with Dr. R. K Poddar who had recently returned from the USA after his post-doctoral experience in the field of bacteriophage genetics (1963). Prior to this there was no opportunity to gather hands on experience in the field of bacterial genetics. I prepared the growth media both nutrient agar and minimal agar and started bacterial culture from the agar stab of *E. coli* C. In this Institute (Saha Institute of Nuclear Physics) there was not even any water bath to grow *E. coli* C with shaking [6]! My mentor was mostly absent for his health problem but allowed me to order few chemicals and water bath.

Differential effect of X irradiation on *E. coli* C and *E. coli* C with bacteriophage ΦX174

Overnight culture of *E. coli* C was diluted 1000-fold in nutrient broth and then allowed to grow for 2 hours. Then exposed to different doses of irradiation. After the adsorption of ΦX174 delivery of its genome (CCC DNA) via spike into the adhesion zone [3,7]. What is this adhesion zone? Both membranes (outer and inner) and the cell wall (peptidoglycan) of *E. coli* C bacteria are fused together where the SS DNA becomes double stranded using the enzyme PolA1 to convert into its replicative form (RF DNA). Our article published in 1965 in BBA has demonstrated how the X-irradiation could distinguish the phage ΦX174 genome of length 5.3 Kb phage genome from its host *E. coli* C genome of length 4736 Kb (double stranded DNA, covalently closed circle) [7]. At the same dose of irradiation, the replication from the origin of *E. coli* C genome stops but the phage ΦX174 RF DNA continues by using the DNA polA1 required as shown in figure 2. Subsequently we have accepted the presence of adhesion zone in *E. coli* C but not in *E. coli* K-12.

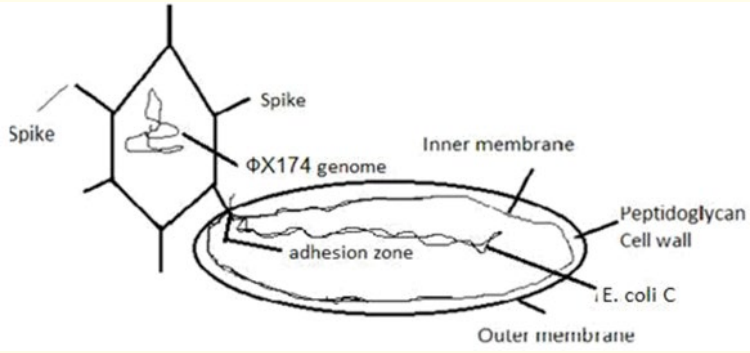

Figure 2: *Entry of ΦX174 genome into the adhesion zone of E. coli C.*

When *E. coli* C and the *E. coli* C infected by the phage ΦX174 exposed to specific dose of X-irradiation, *E. coli* C does not multiply but the infected *E. coli* C is induced showing the lysis! Thus, the differential effect of X-irradiation helped me to distinguish between the host *E. coli* C RNA and ΦX174 RNA. My thesis work showed that the 16S ribosomal RNA had been affected [8]. Even in the nutrient agar medium the plaques formed but not any clear plaques. If left in the same incubator for a longer period, the few bacterial colonies of a giant size apparently nutrients are derived from the lysates for the cross-feeding. My interpretation was that the nutrients released by the lysis of *E. coli* C in a solid nutrient agar fed the *E. coli* C who escaped the lysis by ΦX174! Nobel Prize winner A Kornberg studied DNA replication but he isolated the DNA polymerase from a mass culture without the knowledge about different types of DNA polymerases [6]. I counted these phage particles and surprised to discover that one phage may produce about few hundred particles in a few hours! After incubation for additional days, few bacterial colonies grew out even in the turbid plaques of the bacteriophage ΦX174.

This data led me to conclude the phage ΦX174 is a semi lysogenic unlike lambda bacteriophage which is lysogenic (Figure 3). The phage phiX174 uses its spikes to enter into the adhesion zone of *E. coli* C unlike the bacteriophage lambda that uses its tail to infect *E. coli* K-12 lacking adhesion zone.

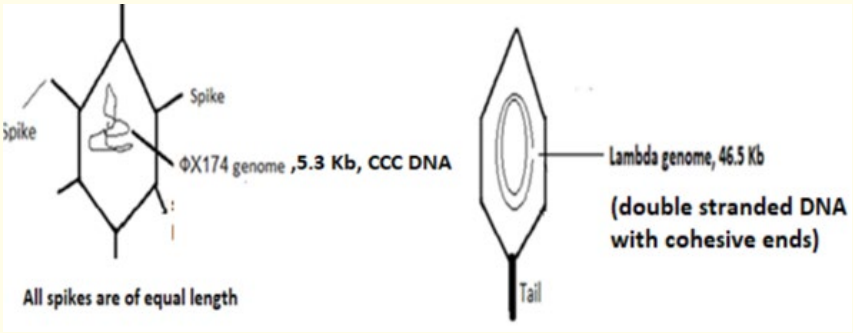

Figure 3: *Difference between lysogenic phage lambda and semi lysogenic phage ΦX174.*

Discussion

Morphologically *E. coli* K-12 and *E. coli* C differ in size and shape but *E. coli* C also becomes sensitive to the phage phiX174. This phage ΦX174 contains approximately 5.3 Kb genome (single stranded covalently closed circular) and is capable of making 11 proteins. Molecular Biology and bio-macromolecule helped me to develop bacterial genetics [6,8]. ΦX174 is a semi-lysogenic and adheres to enter into the adhesion zone of *E. coli C*. Professor Arthur Kornberg initially showed the presence of DNA polymerase PolA1 but subsequently he discovered few more polymerases PolA2, PolA3, PolA4 and PolA5 in *E. coli* K-12 and also in *E. coli* C. We must not forget that the chromosomes of these two bacteria don't differ in producing all the polymerases but the DNA replication of bacteriophage phiX174 PolA1 as reported by DR. Kornberg who failed to recognized there are few polymerases not just polA1 [9].

Conclusion

There is a difference between bacteriophage ΦX174 and bacteriophage lambda. The phage ΦX174 multiplies only in *E. coli* C with adhesion zone but not in *E. coli* K-12 which is lacking adhesion zone.

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