Opinion

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Genesis of sex factor F in *E. coli* K-12 but the Gram-positive bacterial genetics was delayed

Volume 13 Issue 3 - 2026

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Received: March 23, 2026 | **Published:** June 18, 2026

In 1928 Dr. Fred Griffith collected blood samples from patients with lobar pneumonia and streaked on blood agar growth-medium. Then he left the petridish at 35^{0}C with or without CO_2 in the incubator.[1] After overnight incubation he observed very tiny colonies of bacteria appears to be growing but apparently this was mostly one kind of colony but too small (without the presence of any contaminants). He therefore decided to go for longer incubation: Small colonies become large but surprisingly with uneven contour.

Unfortunately, we lost him in World war-II and we forgot about his observation and his laboratory notebook for sixteen years. Fortunately, he left his notebook.

In 1944 Dr. Avery and his associates (Rockefeller University, New York City) made an attempt to understand Griffith's two colonies but without the academic preparation required.[2] Avery did not know the difference between the bacterial colonies and genetic material in his DNA insoluble precipitate. The TCA insoluble DNA fragments isolated by using alkaline pH by Avery et al.[2] are usually too small to contain any biologically inheritable characters. Watson and Crick were given Nobel Prize for the discovery of genetic material as DNA double helix.[3]

Many years later I tried to isolate DNA bio-macromolecule in the laboratory of professor W K Maas (NYU) by the procedure of Avery et al.[2] and concluded that use of high alkaline pH denatured the double stranded DNA into a single stranded form and highly fragile and defeated the purpose. After renaturation (pH 7.0) the precipitate was examined under TEM at high magnification and I observed tiny nucleotide fragments which is just nucleotide fragments but not any gene or operon. Avery et al.[2] made an attempt to isolate DNA from Griffith's colonies (Smooth and Rough) without any academic preparation required for such work.

When Avery's procedure failed, I developed a new isolation procedure for the purpose and was able to visualize F+ under TEM. Then with an adequate academic preparation I developed a new DNA isolation procedure (unpublished data) and tried to isolate F^+ from male *E. coli* K-12 F^+ collected from Joshua Lederberg. Supercoiled DNA was obtained from cleared lysate by the dye-buoyant density procedure of Radloff et al.[4] After the cell density had reached $5x10^6$ cells/ml, the bacteria were harvested by centrifugation at low temperature, washed twice with Tris buffer [0.05 M Tris [pH 8.0]. DNA spreading technique of Kleinsmith but extensively modified by Dr. S. Palchaudhuri in early 1972.[5]

In order to convert the CCC DNA into open circular form I exposed X-irradiation dosage ranging from 200-750 rads given at room temperature to nick the one of the strands of the double helix DNA (Figure 1). So that supercoiled DNA open and I can measure the DNA length. I measured most of the DNA molecules of almost same size of length 31 um but occasionally I found few sex factor DNA bio macro-molecules of length 41 um (larger than F of length 31 um).[6] The male strain of *E. coli* K-12 was used to isolate F+ was also carrying bacteriophage lambda (unpublished data).

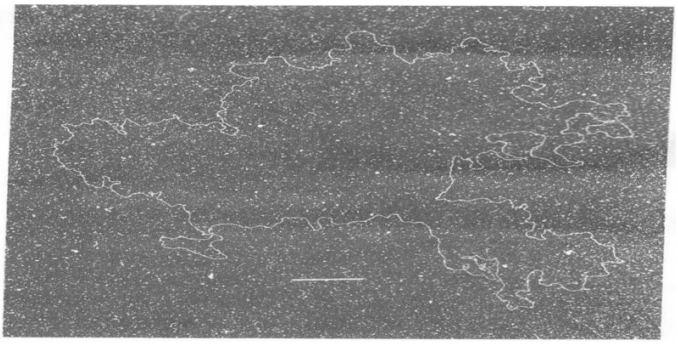

Figure 1 Dark field electron micrograph of fused F+ DNA.

Discussion

Use of X-irradiation is not only used for radio-therapy but also helped us to develop *E. coli* K-12 genetics at a molecular level. I used X-irradiation to nick one of the strands of double helix- FDNA to open the supercoiled F plasmid into its open circular form and thus I measure the molecule from the electron micrograph. DNA bio-macro molecule or *E. coli* K-12 genome never prevails as linear DNA fragments.

There is no eclipse phase as mentioned by Dr. DA Morrisson[7] but the absence of bio-macromolecule is never Avery's TCA precipitate. For the group force Gram-negative bacteria Growth-curve progress delayed. What I have proven that the isolation procedure used by Avery et al.[2] in 1944 is really not an attempt to appreciate Dr. Fred Griffith's two bacterial colonies (Smooth and Rough) but Avery et al.[2] were not prepared to appreciate the DNA is not just nucleotides does not mean bio-macromolecule.

In 2021 I have established the growth curve of Streptococcus pneumoniae, a Gram-positive serious pathogen.[8] It grows in three phases pre-competent, competent, and post-competent.

Acknowledgement

None

Conflicts of interest

None.

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Citation: Palchaudhuri S. Genesis of sex factor F in E. coli K-12 but the Gram-positive bacterial genetics was delayed. *Int J Radiol Radiat Ther.* 2026;13(3):70–71. DOI: 10.15406/ijrrt.2026.13.00457