AMERICAN JOURNAL OF MEDICAL AND CLINICAL SCIENCES, 2024
VOL 9, NO. 4, PAGE 1 – 3

CASE REPORT

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Loss of Antibiotic Resistance Transposons if grown in the absence of Antibiotics

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ABSTRACT

In a recent article I have proven F is not a single replicon but co-integrate of two complete replicons – F and R. These two replicons are not allowed to function simultaneously but their individual functional states are stringently controlled by the transposon Tn 1000(5.7 Kb). Besides these two replicons we have previously mentioned the Ent plasmid P307. In 1977 we characterized the antibiotic resistance plasmid pCG86 in ETEC isolated from the stool of a piglet with diarrhoeal disease. The genes for heat-labile enterotoxin and heat-stable enterotoxin were also carried by the same plasmid pCG86. In the year 1975 we received this same bacterial isolate of Carlton Gyles (Department of Veterinary Microbiology and Immunology, University of Guelph, Ontario Canada) from Professor S. Falkow (University of California) who saved the stab at room temperature but without adding antibiotics. The same strain can't grow in the presence of antibiotics. After the discovery of transposons, I felt the re-interpretation of this heteroduplex molecule formed between pCG86 and Ent P307 is absolutely necessary.

ARTICLE HISTORY

Received 21 May 2024
Accepted 28 June 2024
Published 04 July 2024

KEYWORDS

Foregut duplication cysts, Mediastinal tumors, Necrotic Lymph Node.

Introduction

In a recent article I have proven F is not a single replicon but co-integrate of two complete replicons – F and R (1). Figure 1 shows that the F plasmid carries two complete replicons- F1A and F1C and one incomplete replicon F1B.

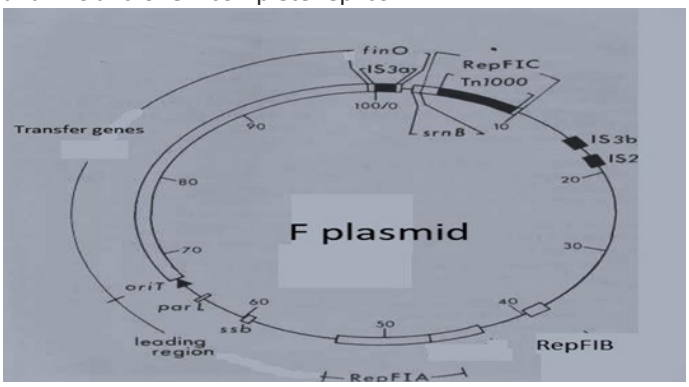

Figure 1: F plasmid with replicons Rep F1A, Rep F1B, Rep F1C and three insertion sequences IS2, IS3 and Tn 1000.

Two F plasmids or two F prime plasmids are incompatible because F1A carries inc loci and F1C or R plasmid does not carry such inc loci. In fact, F1C is the replicon of R plasmid and does not revert back to F plasmid. In addition to these two replicons F1B is not a complete replicon but it has other functions (Palchaudhuri S,unpublished data). F1C is the replicon of R plasmid (pCG86). The insertion sequences are IS2, IS3 are used in the formation of stable Hfr and the Insertion sequence Tn 1000 also forms Hfr which is highly unstable. Unlike antibiotic resistance transposons Tn 1, Tn 2, Tn 3, Tn 4.....Tn 10, the transposon Tn 1000 controls the functional states of Rep F1A and Rep F1C including their copy number. They prevail in a monocopy state. I have observed if Tn 1000 is present in E. coli K-12 carrying the multicopy cloning vector pBR322 then Tn 1000 reduces the copy number of pBR322 [2-4].

Method

EM / heteroduplex analysis. Heteroduplex molecule, formed between F plasmid and R plasmid following the procedure of Sharp et al in 1973 but later in mapping the location of Tn 1000 in F plasmid this procedure of Sharp et al was modified by Palchaudhuri et al. [5-7].

Results/Discussion

F1A with inc loci is apparently responsible for the incompatibility between the two F or two F prime plasmids. Therefore, the two replicons are never allowed to function simultaneously but their functional states are stringently controlled by the transposon Tn 1000. Besides these two replicons we have previously accepted as if the Ent plasmid P307 is another new replicon but now we want to make it clear **that the Ent plasmid does not carry any new replicon but** it is really the deletion derivative of the antibiotic resistance plasmid pCG86 [8-10].

The heteroduplex molecule formed between F ins and Ent P307 shows two insertion loops at 58.8 Fand 52.5. Since Fins is used as a reference molecule in the heteroduplex molecule is present in Ent P307. Ent P307 is a cryptic plasmid but a deletion derivative of pCG86 [2,3]. This heteroduplex was published in J Bacteriology in 1975 by Santos et al without the knowledge of transposons. Now with the knowledge we like to claim that

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Ent P307 is the deletion derivative of R plasmid and therefore incompatibility with F plasmid should not arise. We have to remember that the. antibiotic resistance transposon differs from the transposon Tn1000 of F plasmid. Significantly the transposon Tn 1000 controls the copy number of pCG86 or its deletion derivative Ent P307. Both pCG86 and Ent P307 prevail in a monocopy state in *E. coli* K-12 or in ETEC. I want to mention there is no difference between ETEC and E.coli K-12 except disease producing ability.

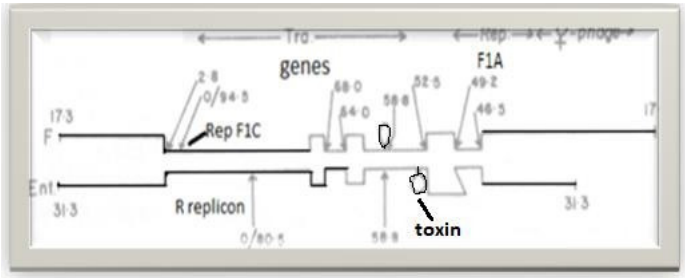

Figure 2: Heteroduplex formed between Fins and Ent P307.

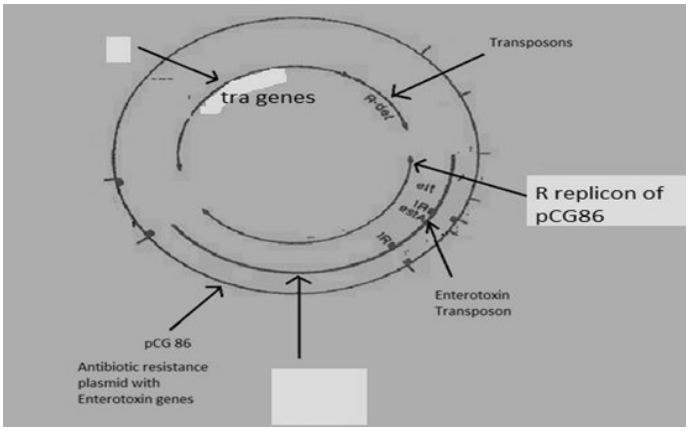

Figure 3: Homology between antibiotic resistance plasmid pCG86 and Ent P307(both carry the same replicon).

In 1975 work we have characterized the plasmid Ent p307 which was evolved as a deletion derivative of the pCG86 with R replicon (**RepF1C**) [5]. Evidently the Ent p307 is the deletion derivative of pCG86 but retained the enterotoxin genes (both heat stable and heat unstable). However, its incompatibility classification was not necessary. We must not forget that Carlton Gyles sent the same **ETEC** with pCG86 also to his mentor Professor S. Falkow but it became antibiotic sensitive being in his laboratory or when I grew this strain in nutrient broth and tested for its sensitivity to ampicillin or tetracycline but does not grow (unpublished data) [2,5]. Many years later we have recognized them as transposons.

In the year 1977 Gyles, Palchaudhuri and Maas have published an article in **SCIENCE (1977)** showing the plasmid pCG86 carries antibiotic resistant characters ampicillin and tetracycline and also enterotoxin genes for both heat stable and heat labile [2]. Since most ETEC isolates from the stools of piglets carry antibiotic resistance genes as well as genes for enterotoxin production [2,9]. In fact, we want to make it clear that **there is no enterotoxin replicon** but Ent plasmid P307 carries genes for the production of enterotoxin and also lacking a few tra-genes. What is more, F is never a single replicon but co-integrate of two replicons – F and R. From self-annealing experiments of Mazaitis et al, they found that the plasmid pCG86 contains two DNA segments bounded by inverted repeats suggesting the presence of two transposons [8]. One of these segments carries the gene for tetracycline resistance and the other carries the gene for heat stable enterotoxin production. We also found that about half of pCG86 is completely homologous with Ent P307.

In 1973 Dr Stanley Cohen had a brief telephone conversation with Professor WK Maas and asked him about the location of R plasmid replicon. Later on, I had a long conversation with Professor Maas on why Stanley Cohen wanted to know the replication origin of antibiotic resistance plasmid [6]. Around this time Professor Maas and I were discussing about the strength of EM heteroduplex technique but my problem was availability of Electron Microscope. In 1974 Professor Maas had solved the problem by helping me to learn the technique as well as its application in my project [5]. In a recent article I have shown F plasmid was never a single replicon but precisely a co-integrate of two replicons F replicon and R replicon [1]. Why does such a question arise? An heteroduplex molecule formed between the F and Ent P307 shows two insertion loops; the two insertion loops are almost of the same size but located at two different distances of F plasmid (unpublished data). After a year I was happy to confirm that the second insertion loop formed in heteroduplex molecule, due to heat stable enterotoxin in Ent P307 and therefore this loop was previously ignored.

EM- heteroduplex analysis shows DNA sequence relationship between the two DNA bio-macromolecules, F plasmid and the antibiotic resistance plasmid R [1]. Sharp et al remained silent about the location of R replicon even in the absence of F plasmid replicon (F1A) and its incompatibility loci but showed the homology with RepF1C [8]. The transposon Tn1000 was still present but allowed the F1C to function stringently in a monocopy state.

I have just discovered that the antibiotic resistance transposons if stored in the absence of their selection pressure is lost. The plasmid pCG86 was evolved in ETEC pathogen responsible not only for the piglet's diarrheal disease in Canada but also allowed it to survive in the presence of antibiotics abused in veterinary medicine.

Doses of drugs and duration of treatment varied depending on the recovery of pigs, therefore, I was excited to observe the loss of antibiotic resistance characters (transposons) when the R plasmid pCG86 was transferred into our laboratory bacterium *E. coli* K-12(711, Falkow) and stored in an agar medium (stab) but without any antibiotics. The antibiotic resistance transposons are selectively lost when its growth environment completely free from antibiotics.

Evidently Dr. Carlton Gyles sent the same bacterial strain ETEC pCG86 carrying plasmid to his mentor Professor S. Falkow stored it in an agar stab at room temperature in 1975 [13]. Professor Maas received the same **stab(P307)** from Professor Falkow in the same year. The Ent p307 is carrying genes necessary for the production of enterotoxin (both heat labile and heat stable) but its replicon is R replicon and originated in an *E. coli* K-12

derivative 711 after its transfer and storage in the agar stab without any antibiotics.

I spent years with Professor W.K. Maas at NYU school of Medicine as his Research associate but he was always thinking to classify all these plasmids of Gram- negative bacteria by their incompatibility but the confusion arises when I discovered that the plasmid Ent P307 is compatible with F plasmid [5,11].

We have described an unusual conjugative plasmid pCG86 present in ETEC, isolated from a piglet with diarrhoea. This plasmid pCG86 carries genes for heat-labile enterotoxin and heat-stable enterotoxin and also carries genes for resistance to tetracycline (Tc), streptomycin (Sm), sulphonamides (Su)and mercury (Hg).Now these antibiotic resistance characters are recognised as transposons. Interestingly heat stable enterotoxin is also a transposon.

In a recent article we have made it clear that fertility factor F of *E. coli* K-12 needs to be recognised as co- integrate of two major replicons, F plasmid replicon and R plasmid replicon. Fortunately, the **transposon Tn1000 (gamma delta) of length 5.7Kb plays an extremely important role in the genesis of R plasmid from F plasmid but irreversibly**. In 1960, the presence of such an R factor in the pathogen Shigella flexneri, (close kin of *E. coli* K-12) begins antibiotic resistance crisis but abuse of in-vitro gene cloning technique makes it global. This pathogen (Shigella flexneri) was also identified in the stool of a female patient suffering from her serious abdominal pain in a reputed hospital of Tokyo [10,12]. Their clinicians were forced to accept the truth that the causative pathogen is no longer sensitive to ampicillin and obviously her abdominal pain continued and it was the beginning of antibiotic resistance crisis [7,9]. Figure 1 shows schematically the locations of F replicons (RepF1A, RepF1B) and RepF1C (R replicon), F1A is a complete F replicon and F1C is a complete R replicon. The transposon Tn1000(mobile DNA element) stringently controls the functional state of F and R plasmid.

Acknowledgement

I appreciate Tripti Bhattacharya for her sincere effort to help me in preparing these figures.

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