Chapter 43

BACTERIAL GENETICS: CLONES

Lecturer—*J. LEDERBERG*

PRE-LECTURE ASSIGNMENT

1. Quickly review notes for the previous lecture.
2. Suggested readings:
   a. General genetics textbooks
      Snyder and David: Chap. 26, pp. 401-407.
      Srb and Owen: Chap. 24, pp. 534-537.
      Winchester: Chap. 23, pp. 318-321.
   b. Additional references
      Bryson, V., and Szybalski, W. 1955. Microbial drug resistance. Adv. in Genet., 7: 1-46.
      Lederberg, J., and Lederberg, E. M. 1952. Replica plating and indirect selection of bacterial mutants. J. Bact., 63: 399-406.
      Luria, S. E., and Delbrück, M. 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics, 28: 491-511. Reprinted in "Papers in microbial genetics", selected by J. Lederberg. 1951. Madison: University of Wisconsin Press.

LECTURE NOTES

A. Motivations for research with bacteria are:
   1. their importance in general ecology, agriculture, and disease;
   2. the very large populations which are easily handled in the laboratory;
   3. their simple cell structure.
      a. Escherichia coli contains two or four nuclei, chemically defined as masses of DNA, each containing about six million nucleotide units.
      b. DNA content of a mouse cell is about five billion units.
      c. Although the morphological mechanism of nuclear division in bacteria is still controversial, the exact replication of DNA occurs each cell division.

B. Vegetative (asexual) reproduction
   1. This is the most important means for increasing bacterial numbers.
   2. A clone (see also Chap. 35) is a population of individuals all derived from a single cell by vegetative reproduction.
   3. Barring mutation or genetic recombination, all clonal members are genetically identical.
   4. Bacteria multiply rapidly.
      a. E. coli divides about each half hour.
      b. One such cell, in suitable nutrient medium, will produce a population of $N = 2^{2t} = 2^n$ individuals in t hours, or n generations.
      c. Thus, from a single ancestor, 30 generations (requiring 15 hours) would produce about 10 billion organisms.

C. Methods for isolating a single bacterium
   1. Directly, by the tedious but exact procedure of micromanipulation
   2. Indirectly, by dilution
      a. When a fluid suspension of bacteria is sufficiently diluted, a sample spread on agar will contain relatively few bacteria.
      b. Each such cell will be located on the agar at random and give rise to a visible clonal colony (Fig. 43-1, top left plate).
   3. Indirectly, using the simple innoculating loop
      a. A sample of a broth culture is streaked upon fresh agar.
      b. At some places single cells will have been deposited some distance apart, yielding separate colonies (Fig. 43-1, top right plate).

D. Typing bacteria by clonal phenotype
   1. Individual cells show few morphological variations -- like presence or absence of flagella.
   2. Because there is not enough material per

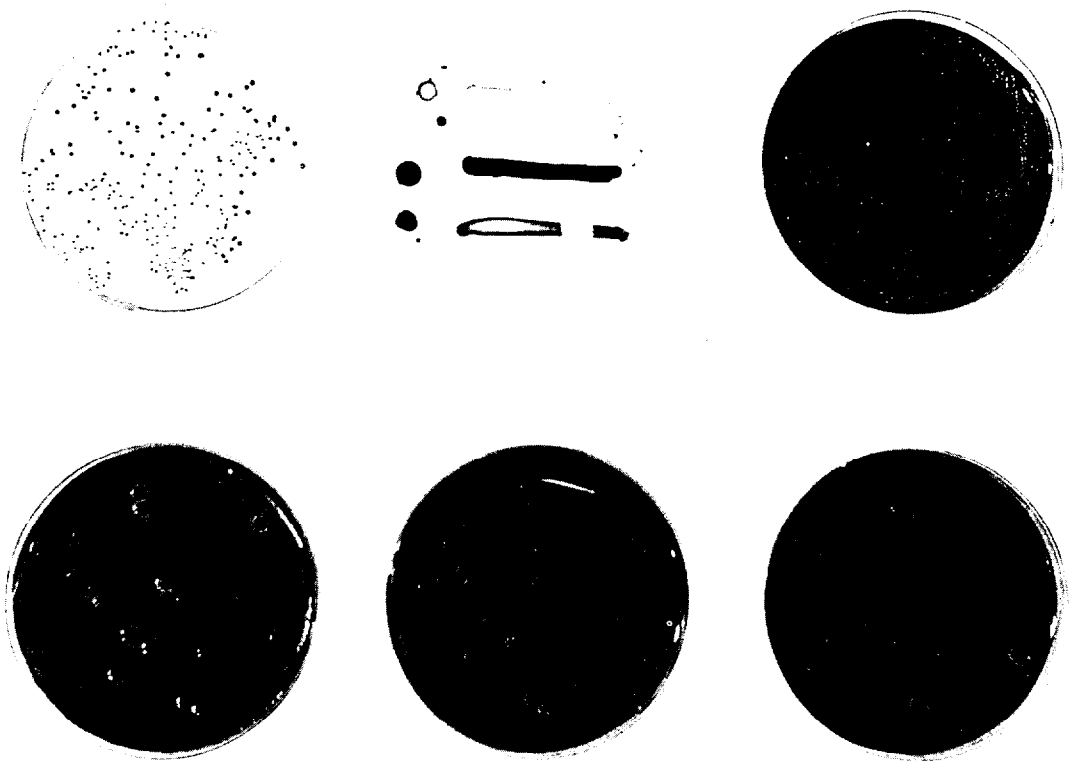

Figure 43-1

cell, a bacterium is typed from the physiological and biochemical behavior of the clone to which it gives rise.

3. The top right plate in Fig. 43-1 has an eosin, methylene blue, agar medium containing lactose.
   a. The top half was streaked with wild-type organisms, which ferment lactose via beta galactosidase, generating colored, dark, clones.
   b. The bottom half was streaked with an ultraviolet induced lactose-negative mutant which produces light clones.

E. Origin of bacterial mutants
   1. Bacteria are intimately exposed to their chemical environment.
   2. Mutations from lactose-negative to lactose-positive could be detected by appearance of dark colonies in the lower half of the top right plate in Fig. 43-1.
   3. Would the medium in that plate have induced the mutations, or would these have occurred anyway?
   4. The same question may be asked also whenever a mutant form has a selective advantage on the culture medium employed for its detection.
   5. For example, streptomycin-sensitive cells do not form colonies when plated on agar containing streptomycin. But a streptomycin-resistant mutant which occurs among them will form a visible colony.
   6. Are the mutants pre-adapted or post-adapted with respect to the detecting medium? Are they spontaneous or medium-induced?

F. Pre-adaptiveness and spontaneous nature of bacterial mutations
   1. Fluctuation test (Luria and Delbrück)
      a. The number of mutants within a clone will depend upon the amount of time there is for multiplication before the test for them is made.
      b. On the post-adaptive view, the number of mutants in different samples tested will fluctuate and form a normal distribution because of the random occurrence of mutations in the final generations exposed to the testing medium.
      c. On the pre-adaptive view, the number of mutants in different samples should form

a skewed distribution. For a very few samples should contain a very large number of mutants because mutation had occurred early in clonal life, long before exposure to the testing medium.

d. The frequency of cultures containing jackpots of mutants demonstrated these were pre-adaptive and spontaneous in origin.

2. Three clone-sampling procedures are available.

a. A single streptomycin-sensitive clone is plated on agar to produce a large number of colonies. Each colony is individually streaked across a streptomycin-containing line in the agar. All clones will grow except in the streptomycin region, but if enough clones are tested one will grow there also -- being a spontaneous, pre-adaptive, streptomycin-resistant mutant (see Fig. 43-1, top center plate).

This method is too laborious to test the pre-adaptation hypothesis.

b. Replica plating of separate colonies

An agar plate containing up to a thousand separate colonies is pressed on velvet so that a sample of each colony is left on it. The velvet is then used as a master to plant a corresponding pattern of growth on a series of additional agar plates.

The three lower plates in Fig. 43-1 show the master (left) and two of its subsidiary plates prepared this way.

A master plate not containing streptomycin can be used to make a first copy, also on drug-free agar, and then additional copies on plates containing streptomycin. On the streptomycin plates only the resistant colonies will grow.

This also is too laborious for testing the pre-adaptation hypothesis.

c. Replica plating of unseparated colonies

A billion or so organisms plated on agar will form small clones so closely spaced as to show continuous growth. Replicas can be made as already described.

Subsidiary streptomycin-containing plates will show growth where there are drug-resistant mutants. On the pre-adaptive view one can return to the corresponding site on the master plate and obtain a sample which is richer in drug-resistant mutants than is a sample taken from another part of the plate. This result has been found.

d. In all of these methods only a sample of each colony is exposed to the medium that tests for mutants, making it possible to prove the testing medium has not played a direct role in producing the mutants.

G. Detection of bacterial mutants

1. Spontaneous mutants
   a. can be selected for, using deleterious agents.
   b. to nutritional independence can be detected easily among nutritional mutants plated on media lacking the required nutrients.
2. Very low mutation rates can be measured with these techniques. The lowest rate so far detected is one per one billion divisions for mutation from streptomycin sensitivity to resistance in E. coli.
3. Mutagen-induced mutants also can be detected through the use of these techniques.

H. Induced mutation

1. X-rays and many other agents are mutagenic in bacteria.
2. Novick and Szilard showed that purines like caffeine, adenine, and guanine increase, while their ribosides decrease, bacterial mutation rate.
3. No mutagen is known at present which produces a given mutation at will.
4. This reflects the fact that each gene must contain all four of the nucleotides in DNA, different genes having these in different arrangements.
5. A specific mutagen would have to recognize specific assemblages of nucleotides, being itself as complicated, chemically and structurally, as the gene it mutates.
6. Spontaneous mutation is in many respects an incident of the normal metabolism of the cell.

POST-LECTURE ASSIGNMENT

1. Read the notes immediately after the lecture or as soon thereafter as possible, making additions to them as desired.
2. Review the reading assignment.
3. Be able to discuss or define orally or in writing the items underlined in the lecture notes.
4. Complete any additional assignment.

QUESTIONS FOR DISCUSSION

43. 1. What advantages do bacteria have as material for genetic study?

43. 2. What disadvantages do bacteria have as genetic material?

43. 3. If division occurred once an hour, how many bacteria would be produced

a. after 4 hours, starting with one bacterium?
b. after 3 hours, starting with four bacteria?
c. after n-1 hours, if on the nth hour there were 2^n?

43. 4. What proportion of a clone would be mutant if one cell produced by the third division underwent a mutation, but was adaptively unchanged?

What would you expect to find in this clone if there was selection for or if there was selection against the mutant?

43. 5. Discuss the advantages and disadvantages of various techniques for obtaining a clone from a single bacterium.

43. 6. Bacterial clones have been compared to the soma produced by zygotes of multicellular organisms.

Discuss whether or not this view is justified or potentially fruitful.

43. 7. What is the virtue of the necessity of using biochemical traits in most mutation studies with bacteria?

43. 8. What morphological traits of clones would be of use in bacterial genetics?

43. 9. What disadvantage has the use of the clone for typing the parental cell?

43.10. Does the post-adaptive view of the origin of bacterial mutations ever apply? Explain.

43.11. Suppose from a single clone of streptomycin-sensitive E. coli approximately 100 bacteria are placed in each of 100 test tubes containing drug-free broth. When each test tube contains about one billion individuals its contents are poured on the surface of nutrient agar medium containing streptomycin.

a. Describe the kind of result which would prove mutations to streptomycin resistance were pre-adaptive.

b. What kind of result would prove neither the pre-adaptive nor the post-adaptive hypothesis of mutant origin?

43.12. How could you show that the streptomycin resistance seen in the two central streaks in the top center plate of Fig. 43-1 was not induced by the exposure to streptomycin?

43.13. Explain how you would proceed to detect and isolate independent mutations from methionine-requiring to methionine-independence using the techniques of replica plating

a. separated colonies, and
b. unseparated colonies.

43.14. How would you proceed to detect bacterial mutations from wild-type to threonine-requiring? from threonine-requiring to threonine-independence?

43.15. Design a specific experiment which would test whether X-rays induce mutations in bacteria.

43.16. Design an experiment using semi-solid culture medium to detect and collect mutations to motility.

43.17. Discuss Lederberg's statement that spontaneous mutation is in many respects an incident of the cell's normal metabolism.