Artificial elimination of F factor in Bact. coli K-12.

A few years ago, Lederberg and his co-workers (1952; 1953) found that in Bact. coli K-12 there was a difference in sex-compatibility between the two mating cells, one having a transmissible agent, F, and the other lacking this agent. Hayes (1953b) demonstrated that these mating types were very stable heritable characters, but, the accidental disappearance of the F factor was found by Lederberg et. al. (1952), and by Hayes (1953a). The study reported here reveals that it is possible to obtain F- cells at will from F+ cells under certain environmental conditions. This phenomenon may be called "F elimination". The environmental factor responsible for F elimination is the cobalt or nickel concentration in the medium (Co(NO₃)₂, CoCl₂, etc.; Ni(NO₃)₂, NiSO₄, etc.). The first method of artificial elimination of F factor is called the "direct" method. A culture of F+ cells was inoculated into peptone medium (peptone, 10g. and glucose, 2g. in 1 liter distilled water), and incubated overnight at 37°C. After 24hrs., 0.15ml. of 0.4M. cobalt solution was added to 3 ml. of the culture (the final concentration was 20mM.) which was incubated for 24 hrs. more. This treated culture was then spread on nutrient agar containing 40mM. of sodium citrate, which was used for detoxification of cobalt, and single colonies grown on the medium were isolated at random. By this procedure, it was found that many colonies were converted into F- For example, in the mutant K-12 strain 58-161 and the derived strains from 58-161, 10-30 % of the colonies were converted into F-. However, all of the F- were sensitive to cobalt. The frequency of conversion varies according to the strain of Bact. coli K-12 used. The second method is called "resistant isolation", in which cobalt-
medium containing cobalt in increasing concentrations. A slow and smooth increase of resistance was observed. Starting from a concentration of 0.5 mM., in which strains derived from the original Bact. coli K-12 are able to grow, resistant strains tolerating even 20 mM. were obtained. These resistant strains were plated on nutrient agar containing 20 mM. of cobalt, and 10 well grown colonies were isolated at random. In mutant strains 58-161 and Y-40, for example, all 10 colonies were F− whereas in the W-1485 strain, only 4 colonies were F−. However, drug resistance and the F− character of these strains could be separated; cobalt sensitive strains derived from the resistant were F− as before, and cobalt resistent strains received the F factor from F+ bacteria to become F+ themselves. The mating behavior of the F− strains corresponded to that of the F+ strain described by Hayes (1952a; b) and by Lederberg et. al. (1952). By the cobalt treatment, other characteristics, e.g. nutritional requirements, sugar fermentation, phage-resistance, lysogenicity, indol formation, shape, gram staining, and antibiotic resistance, were not changed. The F+ trait is very stable in successive serial passages in ordinary media such as broth, peptone, and minimal medium. Therefore, in the F+ clone, the replication of the F factor may be closely concerned with bacterial reproduction. This hereditary conversion of mating type within a clone gives rise to the question of the basis of the change and the mechanism by which the F− strains occurred. There are two well-recognized possibilities: (a) F elimination may be due to the selection of spontaneous gene mutation; (b) it may be induced by the nickel or cobalt in the medium. From the experimental data, the following may be concluded; (1) F elimination occurs even in
occurrence of F elimination is about 100,000 times more frequent than that of other bacterial mutants; (3) F+ and F- strains are equally susceptible to the killing action of cobalt or nickel as judged by viable counts, and F- trait is not linked with cobalt or nickel resistance. These conclusions seem to exclude possibility (a). The conjecture used to explain the contradiction in the stability and instability of the F factor is that it is a non-lytic symbiotic agent, as reported Cavalli et al. (1953), and Hayes (1953a). It may behave as a cytoplasmic factor in one state in the course of its life cycle. In this state, the sensitivity of the F factor to cobalt or nickel may be greater than that of other cytoplasmic factors or of nuclear genes.

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