JOURNAL OF BACTERIOLOGY, Jan. 1972, 447-449

Vol. 109, No. 1
Printed in U.S.A.

Temperature Dependence of Sex-Factor Maintenance in *Escherichia coli* K-12

J. STADLER AND E. A. ADELBERG

Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut 06510

Received for publication 27 September 1971

Wild-type sex factors of *Escherichia coli* K-12 can be cured from host cells by growth at temperatures above 42 C. This phenomenon is obscured by reinfection of cured cells if the culture is allowed to reach cell densities above 10^8/ml. The rate of episome loss is dependent on the genetic (chromosomal) background of the host.

In F′ strains of *Escherichia coli*, the loss of the sex factor (F) can be demonstrated by plating cells on a medium suitable for the detection of genetic alleles present on the episome. For example, cells which carry F-*lac*$^+$ and a mutant *lac* locus on the chromosome will produce *lac*$^+$ colonies if the episome is retained, and *lac*$^-$ colonies if it is lost (1). That the entire episome has been lost in the latter case can be verified by appropriate tests for F-governed properties (e.g., adsorption of male-specific phages, activity as a donor in conjugation, etc.).

Cells carrying either temperature-sensitive sex factors or chromosomal mutations which affect the maintenance of the sex factor at high temperatures have been produced by mutagenesis. When such cells are grown at low temperature (30 to 37 C), the sex factor is normally retained. When they are grown at high temperature (42 to 44 C) and tested in the manner described above, a certain fraction of the cells can be shown to produce episome-negative daughter cells at each division. In contrast, wild-type F′ cells retain the episome when grown at either low or high temperature, i.e., the episome appears to be completely heat-stable. (The term "wild-type" is used here to describe an F′ strain which has not been selected for temperature sensitivity of the episome.)

This note reports experiments which demonstrate (i) the previously undetected temperature sensitivity of wild-type sex factors, (ii) the dependence of high-temperature maintenance of a wild-type sex factor on the chromosomal background, and (iii) the relationship of the kinetics of sex factor loss to the probability of mating and reinfection at cell densities high enough to permit cell contact.

We have found that the wild-type episomes F-*gal* (F8), F-*lac* (F42), and F$^+$ (F1) demonstrate temperature sensitivity if they are kept at very low cell density (less than 5×10^4 cells/ml) while they are grown at temperatures of 42 to 44 C for several generations (Fig. 1). For the purposes of this experiment the three episomes were crossed into and examined in the same wild-type chromosomal background (AB3448). It is clear that F-*gal*, F-*lac*, and F$^+$ are steadily lost from the cultures grown at 43 C so that, at the end of four generations, the episomes appear in only 50% of the resulting populations. These episomes show no evidence of elimination from the 37 C control cultures.

Although the amount of loss is remarkably similar for each of the three episomes tested when they are examined in isogenic backgrounds, the amount of F′ loss varies greatly when a given episome is tested in a variety of chromosomal backgrounds. For example, Fig. 2 shows F-*gal* loss at 43 C in four diverse background strains. Three of these strains are wild-type, while one is known to carry an EMS-induced chromosomal mutation, *seg-1* which causes easily detectable loss of F-*gal* in clonal growth on plates incubated at 42 C. Figure 2 clearly demonstrates the presence of unselected chromosomal factors which modify the effect of high temperature on F-maintenance. These experiments were again done with cultures kept at low cell density.

It may be of some interest to compare the genetic histories of the three *seg*$^+$ strains for which F-*gal* loss is presented in Fig. 2. The lowest rate of loss occurs in a strain (AB3441) which evolved from the wild-type strain K-12 in a series of numerous steps including spontaneous mutations, nitrosoguanidine mutagenesis, and phage transductions. A slightly

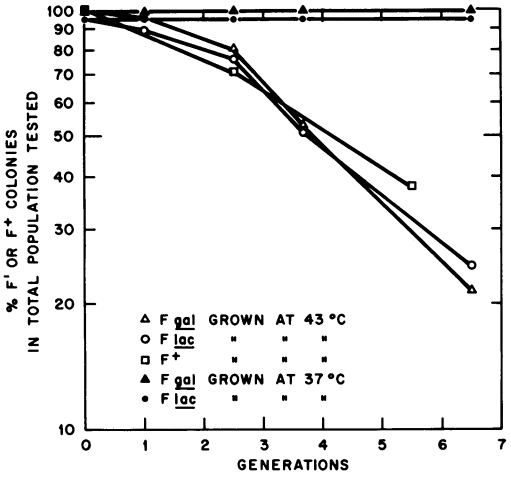

FIG. 1. *Heat-induced loss of three wild-type episomes in cultures with isogenic backgrounds. All cultures were grown with shaking in 10 ml of complete broth (2) in 125-ml flasks. At 0', prewarmed 43 C and 37 C broth was inoculated at 2 × 10³ cells/ml from an overnight culture grown at 37 C. During the growth of each culture, the cell concentration was kept below 5 × 10⁴/ml by repeated dilution with broth warmed to the proper temperature. F-gal and F-lac cultures were sampled at hourly intervals and dilutions plated on MacConkey's indicator agar so that the resulting colonies could be examined for loss of ability to ferment galactose or lactose. All plated samples were incubated at the control temperature, 37 C. Forty to fifty gal⁺, gal⁻, lac⁺ and lac⁻ colonies from the 6.5-generation samples were regrown and scored for maleness using R-17. In each case the loss of the sugar fermentation marker was entirely correlated with the loss of maleness, thus confirming episomal loss. The F⁺ culture was plated on complete agar at 0', 2.5, and 5.5 generations and incubated at 37 C. Random samples at each time point were scored for the presence of F⁺ by regrowth and plating with R-17. In the case of the F-gal and F-lac cultures, a sample population of 1,000 was examined for each point plotted. For the F⁺ culture, a sample population of 100 was examined for each point plotted.*

higher rate of loss is seen in strain AB2605, which differs from *E. coli* K-12 by the single mutation, *galT12*. The highest rate of loss, approaching that of the *seg-1* mutant, occurs in strain AB3447, which differs from AB2605 by an EMS-induced mutation in the *lac* locus. It is not possible to tell which events were responsible for the differences in episomal stability shown by these three genetic backgrounds.

Figure 3 presents a comparison of F-*gal* (AB 3447) loss at 43 C in a low-density culture maintained at 10^3 to 5×10^4 cells/ml (A), a parallel culture inoculated at 2×10^3 cells/ml and allowed to grow undiluted to approximately 2×10^8 cells/ml (B), and a culture grown from the same initial inoculum source but kept by dilution at 2×10^8 to 6×10^8 cells/ml and in log-phase growth for several hours (C).

The high-cell-density, log-phase culture (C) showed no F-*gal* loss at all. The undiluted low-cell-density culture (B) showed "recovery" of F-*gal* after reaching 6×10^5 cells/ml. As before, the culture maintained at low cell density (A) throughout the course of the experiment showed continuous F-*gal* loss.

The "recovery" of *gal*⁺ subclones in culture (B) is not attributable to the appearance of large numbers of mutant temperature-resistant episomes nor to the integration of the *gal*⁺ allele into the chromosome. A sample of the *gal*⁺ subclones which appeared in samples taken after the culture reached high cell density was tested for maleness with R-17 male-specific phage and for heat sensitivity by a further round of growth at high temperature and low cell density. Of the 52 *gal*⁺ colonies tested, all were R-17 sensitive and, like the original culture, heat-sensitive for the *gal*⁺ character.

Wild-type episomes are capable of high-frequency sexual transfer at high temperatures. The "recovery" of F-*gal* in the population of culture (B) occurs only under conditions which would allow for F' transfer, that is, when the cell concentration becomes greater than 10^5 cells/ml. At that density, the cell which has

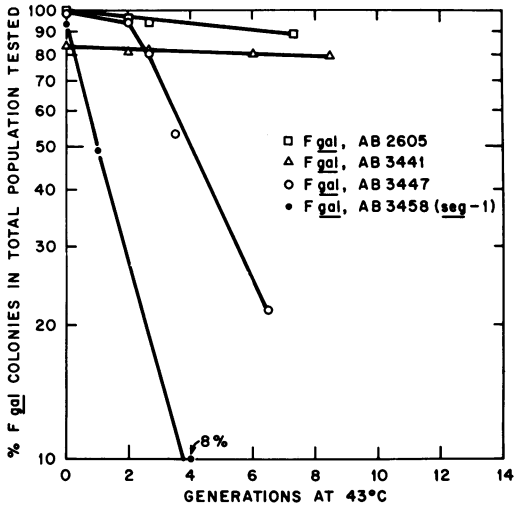

FIG. 2. *Heat-induced loss of F-gal in cultures with diverse genetic backgrounds. Culture methods and sample sizes are as described for F-gal in Fig. 1.*

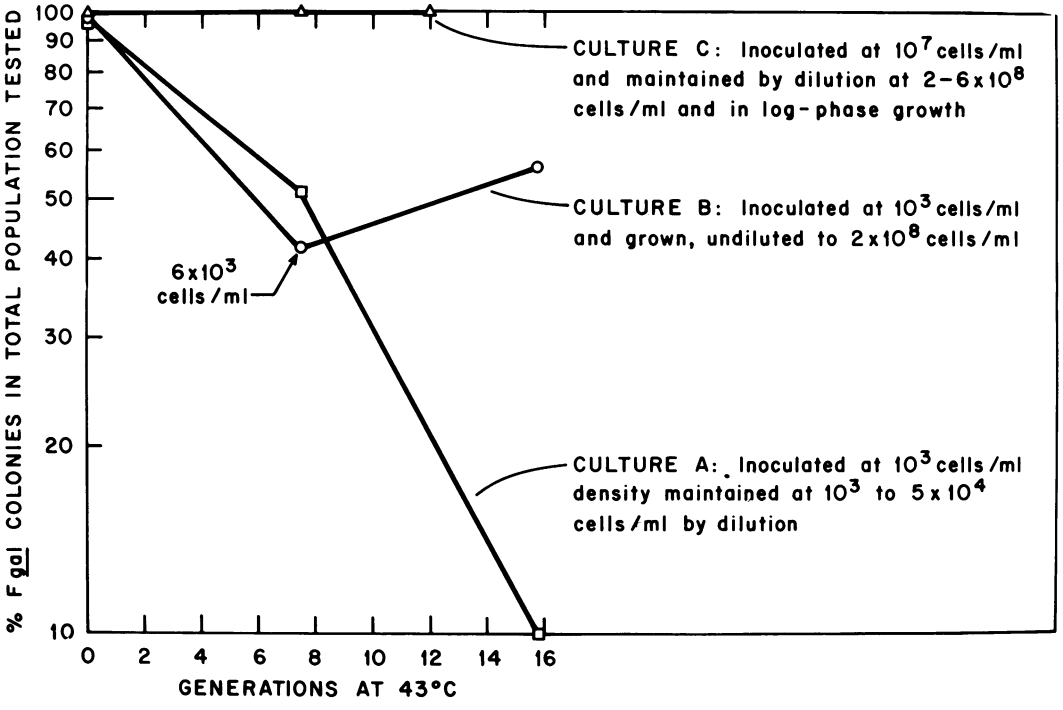

FIG. 3. *Heat-induced loss of F-gal in cultures of various cell densities.*

recently lost its episome can act as a recipient F^-. Thus, the reappearance of F-*gal* in the increasingly dense population may be inferred to occur because the rate of F′ transfer exceeds the rate of F′ elimination during growth at high temperature.

The best explanation for the previously assumed heat stability of wild-type episomes is that, in the past, they have been examined only under culture conditions in which F′ loss attributable to heat sensitivity was masked by the ability of the F^- cells to recover F′ by sexual transfer.

This investigation was supported by Public Health Service grant no. 15336 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Jacob, J., S. Brenner, and F. Cuzin. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329-348.
2. Luria, S. E., and J. W. Burrous. 1957. Hybridization between *Escherichia coli* and Shigella. J. Bacteriol. **74**: 461-476.