A METHOD FOR SELECTING RADIATION-SENSITIVE MUTANTS OF ESCHERICHIA COLI

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RADIATION resistant cells such as *E. coli* B/r can be selected by using radiation to kill the more sensitive organisms (WITKIN 1947). A radiation sensitive mutant of *E. coli* B has also been found among the survivors of irradiated bacteria without the use of any selective procedure (HILL 1958; HILL and SIMSON 1961). Although this mutant, B_{s-1}, was obtained from among 12 survivors of UV-irradiation, the present authors failed to find any sensitive K-12 mutants among 500 progeny of survivors of UV-irradiation.

ELLISON, FEINER and HILL (1960), found that T1 bacteriophage plated with almost equal efficiency on *E. coli* B and B_{s-1}, but that after UV-irradiation, T1 plated with a much lower score on B_{s-1} than on B. The mutant B_{s-1} evidently lacks some factor that is present in *E. coli* B and that will reactivate the UV-irradiated T1. This property can be used as the basis of a selective procedure to obtain radiation sensitive mutants of the same class. This paper will describe the procedure used and some of the properties of the sensitive mutants obtained from *E. coli* K-12.

MATERIALS AND METHODS

To facilitate the subsequent genetic analysis of the loci involved in the change in radiation sensitivity, a female strain of bacteria was chosen that has suitable markers for use in mating experiments. The strain *E. coli* K-12 AB1157, which was obtained from DR. E. A. ADELBERG, was isolated by DR. S. N. BURNS and has the following characteristics: Sm^R, $T1^s$, F^-, TL^-, pro^-, lac_1^-, gal_2^-, λ^s, his^-, ara_2^-, xyl^-, mtl^-, arg^-, thi^-, $T6^R$. In the present paper this strain will be referred to as UV^R and the sensitive muntants will be called UV^s. These abbreviations have the following meanings: Sm^R—streptomycine resistant, $T1^s$—sensitive to T1 phage, F^-—mating type, TL—threonine and leucine, *pro*—proline, *lac*—lactose fermenting, *gal*—galactose fermenting, λ^s—sensitive to λ phage, *his*—histidine, $T6^R$—resistant to T6 phage, *ara*—arabinose fermenting, *xyl*—xylose fermenting, *mtl*—manitol fermenting, *arg*—arginine, *thi*—thiamine.

Mutations were induced in the bacteria by exposure to nitrous acid. Bacteria were harvested from an overnight growth on agar nutrient plates, suspended in buffered saline, spun and resuspended. 3×10^8 bacteria were spun again and resuspended in 0.015 M nitrite in acetate buffer at pH 4.5. After treatment in this solution for 50 minutes at 22°C, the cells were diluted into buffer at pH 6.8, spun and resuspended in nutrient broth at 10^6 per ml, of which 2×10^3 per ml re-

tained the capacity for colony formation. They were then incubated for ten hours with aeration at 37°C, during which time the cells recovered from the posttreatment lag and passed through about ten divisions. This permitted the segregation of mutants and phenotypic expression. The cells were then plated on Difco nutrient agar with 500 to 1,000 progeny of the survivors per plate and incubated for four hours at 37°C, at which time the colonies were not quite large enough to be visible. Then 2.5 ml of 0.6 percent agar with 0.7 percent NaCl at 46° was poured very gently over the colonies. The soft agar contained 5×10^8 T1 phage which had been exposed to a dose of 700 ergs per mm^2 of UV light at 2537A. The plates were then incubated for 16 to 24 hours. Surviving colonies were picked and streaked on nutrient agar to separate surviving bacteria from T1 phage and single colony isolates were made and grown in nutrient broth. These were then tested for ability to show plaques with T1 phage and also for sensitivity to UV as judged by the survival of colony forming ability.

The role of the preincubation before adding the UV-irradiated phage in the selective procedure is to permit growth until micro colonies are formed. It is visualized that a number of UV-irradiated phage will adsorb to each colony and will be killed if it is of the sensitive strain. Although some of the bacteria may be killed by the injured phage, others will survive and grow to form a visible colony. If the bacteria in the microcolony are UV^R, however, the phage will be reactivated and will have the opportunity to multiply in the colony and to kill all the cells.

A total of 14 UV-sensitive mutants of K-12 referred to as UV_1^s, UV_2^s, etc., were obtained from about 2×10^4 nitrous acid treated bacteria spread on 50 plates. The properties of these mutants were tested by measuring the survival of UV-irradiated T1 when plated on each mutant. The results are shown in Figure 1. Survival of the colony forming ability of the bacteria after UV-irradiation was also determined with the results shown in Figure 2. The results of tests carried out on *E. coli* B and *E. coli* B_{s-1} which was obtained from DR. R. HILL, are also included in the figures.

The efficacy of this method of selection was tested in reconstruction experiments of two types with UV^R and UV^s bacteria and were incubated for periods of 0, 2, 4 and 6 hours. Soft agar containing UV-irradiated T1 in varying amounts was then poured over the micro colonies. After incubating the plates for a further 18 hours, the surviving colonies were scored. The results are shown in Figure 3. These experiments, however, were liable to show an exaggerated degree of selection against the UV^R strain because the cooperative production of phage in nearby colonies might raise the phage titre in the soft agar to the level where no colonies of any kind survived. To demonstrate the importance of this effect, plates were spread with either 2,000, 300 or 100 UV^s and varying numbers of UV^R bacteria. The plates were then incubated for four hours before adding soft agar containing 5×10^8 T1 phage that had been exposed to 700 ergs/mm^2 of UV light. The numbers of surviving colonies was counted after the plates had been incubated for a further 18 hours. The results given in Figure 4 show that the

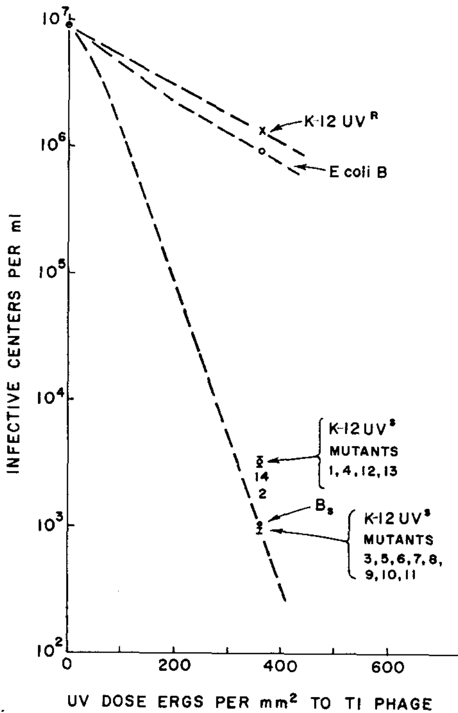

FIGURE 1.—The host effect on the survival of UV-irradiated T1 bacteriophage. The T1 phage were irradiated in buffer with 2537A light from a low pressure mercury germicidal lamp and were plated in soft agar seeded with 0.25 ml of an overnight broth culture of the bacteria and then incubated overnight at 37°C. Aliquots of the same stocks of irradiated phage were used with the different hosts, so that small differences in the survival of the irradiated phage on the various hosts could be detected.

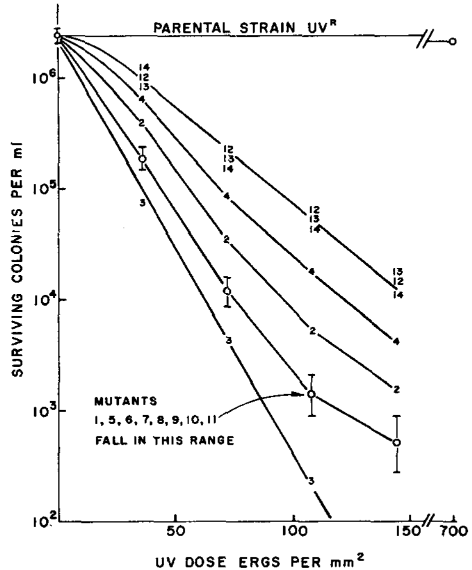

FIGURE 2.—The survival of colony forming ability of UV-irradiated *E. coli* K-12 and of the 14 nitrous acid induced UV^s mutants. The bacteria were irradiated in buffer and then spread on nutrient agar plates and incubated overnight at 37°C.

numbers of UV^s bacteria that survive decrease with increasing numbers of UV^R cells on the same plate.

RESULTS

The results shown in Figures 3 and 4 indicate that conditions can be obtained in which all the micro colonies of the parent strain of bacteria are killed by UV'd bacteriophage, while a proportion of the sensitive mutants survive. It appears from Figure 4 that the cooperative action of colonies in producing sufficient phage to kill the sensitive mutants becomes increasingly important when there are more than about 1,000 UV^R bacteria per plate, so that the number of bacteria that can be screened for sensitive mutants on one plate is limited.

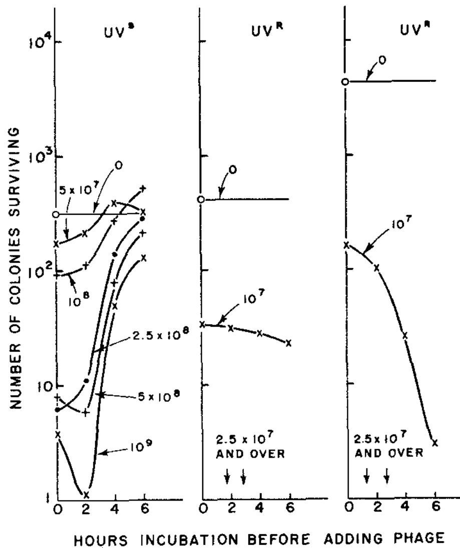

FIGURE 3.—The numbers of *E. coli* K-12 UV^S and UV^R colonies that survive attack by UV-irradiated T1 bacteriophage in soft agar as a function of the number of phage added and the time of incubation of the bacteria before adding them. The phage were exposed to a UV dose of 700 ergs per mm^2 at 2537A. After adding the phage, the plates were incubated overnight at 37°C. The number against each line denotes the number of phage added to each plate. The initial numbers of bacteria are indicated by the lines marked zero to indicate no added phage.

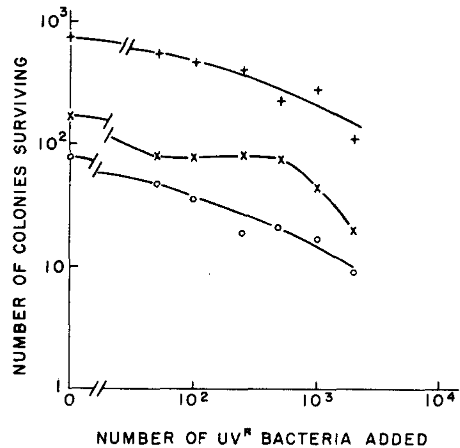

FIGURE 4.—The numbers of colonies that survive the addition of UV-irradiated T1 phage. The plates were seeded with 2000, 300 or 100 UV^S and a variable number of UV^R bacteria and incubated for four hours. Then they were covered with soft agar containing 5×10^8 T1 bacteriophage that had received a dose of 700 ergs per mm^2 of UV light of 2537A and then incubated overnight at 37°C.

Among survivors of the selective procedure, 14 out of 26 were UV^S, while eight were T1 resistant, and four were T1 sensitive but UV^R. It is seen in Figures 1 and 2 that as regards sensitivity to UV, the majority of mutants fall into a single class with similar properties. Several of the mutants, however, have significantly higher resistance to UV and appear to retain some residual ability to reactivate the UV-irradiated T1 as shown by the greater number of plaques. Since tests of this kind can be carried out with a single population of irradiated phage plated on different strains of bacteria, errors in radiation dose affect the score on the different mutants in the same way. The test is, therefore, very sensitive. The higher survival obtained with the mutants, 1, 2, 4, 12, 13 and 14 in comparison with that on the remainder is significant, the differences being many times greater than statistical errors involved.

It is noteworthy that all 14 of the mutants that lacked the capacity for reactivating the UV-irradiated T1 were also much more sensitive to UV light themselves as judged by the survival of colony forming ability. The selective procedure was used a second time on one of the sensitive mutants, to see whether

a two-step mutant of greater sensitivity could be obtained. As judged by the numbers of colonies surviving, the sensitivity of this mutant to nitrous acid was about the same as that of the parental strain. In this experiment, the bacteria were treated with the same number of T1 phage that had been used before, but in this case the phage were exposed to a dose of only 250 ergs per mm^2 of UV light. None of the progeny of the survivors showed a greater sensitivity than that of the parent strain. This attempt to obtain a two-step mutant of greater sensitivity was without success.

DISCUSSION

The method and rationale outlined here for obtaining a certain class of sensitive mutants of bacteria was successful. As 12 of the 14 mutants came from a single growth tube in which surviving bacteria were allowed to divide about ten times, it is possible that they were not all of independent origin. However, less than one percent of the bacteria in this growth tube were plated, and only a small fraction, probably less than ten percent of the sensitive cells were picked for test as possible mutants, so that it is unlikely that more than a few of the 14 mutants have a common ancestry in the same mutation. As the selective procedure was not tested without the nitrous acid treatment, it was not established that the mutants were not of spontaneous origin.

This method of selecting for radiation sensitive mutants by attack by irradiated bacteriophage may be of use for screening bacteria deficient in other reactivating enzymes. It can also be used with other mutagenic agents. There is, at present, no evidence that nitrous acid affords a selective advantage over UV for producing UV^s mutants. The method may also be of use for selecting sensitive mutants of other organisms that can serve as host to a virulent phage or other virus, but because of the specificity that reactivating enzymes may show, it may be necessary for the phage nucleic acid to be of the same chemical composition as that of the host cell. The method of selection by virus attack may also be of use when searching for mutants of other genes that control products essential for virus reproduction under the particular condition set up in the experiment.

Reviewing evidence on the modification of the observed frequency of UV-induced mutations in bacteria, subjected to various treatments, WITKIN (1961) was led to the conclusion that *E. coli* contain a reactivating enzyme capable of repairing certain UV products in the bacterial DNA, provided that oxidative phosphorylation is not blocked and that there is sufficient time for the enzyme to act before DNA synthesis occurs. A satisfactory explanation of the greater sensitivity of *E. coli* B_{s-1} is that it lacks an enzyme that reactivates certain UV photoproducts in the DNA of the T1 phage or bacterium (HOWARD-FLANDERS, BOYCE, THERIOT 1962; SAUERBIER 1962). The properties of the sensitive mutants of *E. coli* K-12 are similar in certain respects to those of *E. coli* B_{s-1}, so that it is likely that the UV^s mutants are deficient in the same reactivation enzyme or enzyme system. That this reactivating enzyme is not the only factor that is involved in radiosensitivity, is shown by the fact that there are differences in the sensitivity of bacteria that are independent of the properties as a host for irradi-

ated T1 phage. For example, *E. coli* B_{s-1} is several times more radiosensitive than *E. coli* K-12 1157 UV^s, when tested by the survival of colony ability after exposure to UV light. A full discussion of this question must, however, await further investigation and will not be attempted here.

SUMMARY

A method for obtaining by selection a certain class of sensitive mutants of bacteria has been reported. The method depends upon contra-selection by irradiated T1 bacteriophage.

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