THE STIMULATION OF GENE RECOMBINATION IN ESCHERICHIA COLI

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The existence of recombination of genetic factors and their segregation into prototroph recombinants in *Escherichia coli* has been reported by Lederberg and Tatum (1946*a*,*b*), Tatum and Lederberg (1947), and Lederberg (1947, 1949). The significance of such findings, both to bacteriology and genetics, is apparent. Haas *et al.* (1948) observed that irradiation of the recombining strains with ultraviolet light would cause a marked increase in the number of prototrophs over that found when the strains are grown together without irradiation. The correlation between the increase in recombination rate and the percentage of filamentous "large forms" induced by the radiation suggested the possibility that these large forms might be related to sexual recombination in these bacteria. Stone, Wyss, and Haas (1947) reported that ultraviolet irradiation of a nutrient substrate in which bacteria were subsequently grown would induce mutations similar to those found by direct irradiation of the same organism. Further studies with hydrogen peroxide, catalase, and catalase poisons (Wyss *et al.*, 1947, 1948) suggested that organic peroxide might be involved in the mutagenic process. These findings have been confirmed in part and extended to *Neurospora* by Dickey *et al.* (1949) and Wagner *et al.* (1950). It was the purpose of this work to determine whether these observations on increased mutation rates could be extended to the recombination phenomenon between mutant strains of *E. coli* K-12.

EXPERIMENTAL RESULTS

The mutants of *E. coli* used were strains Y-53 and 58–161 (kindly furnished by Dr. J. Lederberg). Strain Y-53 requires threonine, leucine, and thiamine and does not ferment lactose; strain 58-161 requires biotin and methionine. Nutrient agar was used as a complete medium for determining total cell counts, and asparagine glucose mineral salt agar was used as a minimal medium for determining prototroph formation. The ultraviolet light used was a Hanovia double U, SC 2537, quartz lamp operating at 120 milliamperes.

In experiments with direct irradiation, separate nutrient broth tubes were inoculated with Y-53 and 58-161. After 3 hours' growth the cultures were centrifuged, washed, and resuspended in a small amount of sterile physiological saline to give a very turbid suspension. The two cultures were then mixed in essentially equal amounts and 1:10 and 1:M dilutions were made. After 1 ml of each of these dilutions was pipetted onto the surface of a series of plates of minimal agar, the plates were irradiated at a distance of 47 cm from the light source for time intervals ranging from 0 to 30 seconds. After 3 hours' further

incubation the plates containing the 1:M dilution were layered with nutrient agar to determine the total cell count, and the plates containing the 1:10 dilution were layered with more minimal agar to determine the number of prototrophs. The colony counts after 24 hours are reported in table 1. As the time of irradiation is lengthened, prototroph formation increases to a maximum and subsequently declines. Exposures to radiation that are without effect on the total number of organisms markedly affect the prototroph count.

For indirect irradiation experiments, 50-ml aliquots of nutrient broth were irradiated in a large petri plate at a distance of 47 cm from the lamp. The time of irradiation was varied from 0 to 90 minutes. After irradiation the broth was transferred aseptically to a sterile flask and inoculated with 0.1 ml of a log phase culture of Y-53 and the same amount of a similar culture of 58–161. After 24 hours' incubation in the irradiated substrate, the resulting cultures were plated in appropriate dilutions in both nutrient agar and minimal agar for total count and prototroph count. Similar procedures were followed for the peroxide treatment; in such experiments aliquots of nutrient broth were treated with from

TABLE 1

Effect of direct irradiation on the rate of recombination

TIME OF IRRADIATION	TOTAL COUNT IN MILLIONS	PROTOTROPHS	PROTOTROPHS/10 M CELLS
seconds			
0	84	40	5
5	98	817	83
10	64	750	118
15	69	580	84
30	48	243	50

0 to 25 ppm hydrogen peroxide and allowed to stand 1 hour before inoculation with the two mutant strains.

Tables 2 and 3 show the results of the effects of irradiated substrate and peroxide-treated substrate on recombination rate. In both cases the rate increases to a maximum and subsequently declines.

Wyss *et al.* (1948) found that the addition of subinhibitory concentrations of sodium azide to cultures of *Micrococcus pyogenes* would induce the formation of antibiotic-resistant mutants and suggested that the mutagenic action of this iron porphyrin poison was probably due to increased peroxide formation and accumulation in the metabolism of the poisoned cell. Table 4 shows that, when the mutants are grown together in broth containing subinhibitory concentrations of azide, a definite effect is observed. It is possible that treatments more vigorous than optimum induce a larger percentage of lethal mutations and thus obscure the appearance of the specific phenotypes.

The mutagenic effect of both irradiated substrate and peroxide-treated substrate can be negated by the action of catalase (Wyss *et al.*, 1948). Table 5 shows a similar effect of catalase on the recombination rate increase that results from the treatment of the substrate with ultraviolet or hydrogen peroxide. A final

TABLE 2

Effect of irradiation of the substrate on the rate of recombination

TIME OF IRRADIATION	TOTAL COUNT IN MILLIONS	PROTOTROPHS	PROTOTROPHS/10 M CELLS
minutes			
0	225	250	11
15	280	370	14
30	135	1,100	82
45	130	2,200	170
60	200	1,050	53
90	64	310	48

TABLE 3

Effect of H_2O_2-treated substrate on the rate of recombination

PPM H_2O_2	TOTAL COUNT IN MILLIONS	PROTOTROPHS	PROTOTROPHS/10 M CELLS
0	225	250	11
5	170	740	43
10	60	500	83
15	58	820	140
20	70	130	18
25	88	65	8

TABLE 4

Effect of sodium azide on the recombination rate

CONCENTRATION OF AZIDE	TOTAL COUNT IN MILLIONS	PROTOTROPHS	PROTOTROPHS/10 M CELLS
μg/ml			
0	420	200	5
100	13	73	56
300	2	3	15
500	4	2	5

TABLE 5

Effect of catalase in negating increased recombination rate

TREATMENT	TOTAL COUNT IN MILLIONS	PROTOTROPHS	PROTOTROPHS/10 M CELLS
Control	470	480	10
Control + catalase	530	300	6
Substrate, irradiated 45 minutes	520	1,500	29
Substrate, irradiated 45 minutes, catalase added	620	630	10
15 ppm H_2O_2	510	1,300	26
15 ppm H_2O_2, catalase added	770	900	12

concentration of 0.05 per cent catalase "Sarett" (kindly furnished by Vita Zyme Laboratories, Chicago) was used. The catalase was added after irradiation or

peroxide treatment, and 30 minutes was allowed for the enzyme reaction to go to completion before inoculation. Whether the slight reduction of the normal rate of prototroph formation by the addition of catalase to untreated cell suspensions is significant has not been determined.

The possibility exists that the apparent increase in recombination rate was due to an increased back mutation rate of the mutant *E. coli* strains or was connected in some way with the release of cell nutrients from injured cells. Therefore the individual strains were subjected to treatments similar to those that gave the increased prototroph formation. The strains were then plated in media containing all but one of the multiple factors required for growth. In no case was any back mutation for even one factor observed; the spontaneous and the induced back mutation rate were both less than could be detected by our methods. Thus the increased prototroph formation seems to be due to an actual increased rate of genetic recombination.

DISCUSSION

The recombination within a single cell of genetic factors originally found in separate individual cells necessitates the acceptance of a concept of some type of genetic transference between bacterial cells. A mechanism similar to the transformation found in the pneumococcus by Avery *et al.* (1944) or in *E. coli* by Boivin (1947) and Wyss (1950) has not been demonstrated. Haas *et al.* (1948), as well as Dienes (1946), and others consider that the filaments often observed in cultures may be involved in a sexual stage of the bacterial life cycle. This suggests the possibility of conjugation between bacterial cells. We found more of the large forms in treated substrates but were unable to establish a correlation between the appearance of these forms and prototroph formation because of the time lag in these experiments.

The increase of prototroph formation could be due to one or both of two factors. The treatment could cause increased transfer of genetic material between the two strains, either by the induction of more sexual forms, by liberation of more of a diffusible transforming substance, or by recombination by the organisms of desirable genetic properties in an attempt to overcome undesirable side reactions that result from the treatment. The latter is almost inseparable from selection, but no evidence of selective factors has been found in our experiments. A second possibility is that the degree of genetic interchange between cells may not alter, but the frequency of crossing over may increase. It is possible that an exchange of nuclei occurs and that a form of autogamy exists in the bacterial cell, since these organisms appear to be multinucleate.

In any case the treatments that caused increased prototroph formation also induced nonspecific mutations. It might be considered that such treatment causes a general genetic instability. It is also possible that some apparent mutations in bacteria might be only the uncovering of a recessive allele by crossing over. This could happen if bacteria were diploid, and there is some evidence that they are (Lederberg, 1949).

SUMMARY

The rate of formation of prototrophs between mutant strains of *Escherichia coli* K-12 can be increased by direct irradiation of the cells, irradiation of the substrate in which the organisms are grown, treatment of the nutrient substrate with hydrogen peroxide, or growth in the presence of sodium azide. Induced back mutation of the strains did not occur with the treatments used; thus the increase of prototrophs must be due to increased recombination.

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