structures, coupled with a quite facile consideration of the physicochemical attributes of these components, can help to bring a new dimension to our understanding of organismic behavior.

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Induction of Mutants with Altered DNA Composition: Effect of Ultraviolet on Bacterium paracoli 5099

Abstract. *The culture of* Bacterium paracoli *5099 represents a favorable system for induction of mutants with altered DNA base composition. The frequency of induction of these mutants by ultraviolet radiation is strongly dose-dependent, and has a peak at the ultraviolet exposure equal to 860 ergs. On both sides of this maximum the rate of appearance of mutants decreases, and with the exposures less than 350 and more than 1900 ergs per square millimeter, mutants with altered DNA base composition do not appear at all.*

Investigation of mutants with altered DNA base composition in bacteria has been hindered by the rarity of these events, and a summary of previous studies has been published (*1*). A system has become accessible in which such mutants are induced with greater frequency, and therefore can be reproduced routinely (*2*). Work with this system has made it possible to conclude that the frequency of induction of mutants with altered DNA base composition strongly depends on the dosage of ultraviolet radiation, as outlined in this report.

For the investigation we used *Bacterium paracoli* 5099 from the Type Culture Collection of U.S.S.R. (State Control Institute of Medical Biological Preparations of the Ministry of Health, Moscow). As a source of ultraviolet radiation (2540 Å) we used an ultraviolet lamp giving a dose rate of 11.53 erg sec^{-1} mm^{-2}; distance from the target was 33 cm. Bacteria were grown on nutrient agar at 37°C for 24 hours and suspended in water to the density 10^9 cells per milliliter; the suspensions were placed in dishes in layers 1-mm thick, and irradiated for various intervals between 30 and 165 seconds. Samples of irradiated suspensions were plated on nutrient agar that contained 1 percent glucose and were incubated at 37°C for 7 days.

We had observed earlier (*2*) that mutants appearing as small yellowish colonies on agar plates were approximately 200 times more sensitive to the action of trypaflavine than the cells of the parent culture, and that there is complete correlation between the drastic increase of sensitivity to trypaflavine and distortion of DNA base composition in the cells of mutants. We therefore used "gradient" agar plates containing trypaflavine (10 μg/ml in the upper layer) in order to evaluate the

Table 1. Guanine-cytosine (percent GC) content in DNA of the parent culture *B. paracoli* 5099 and of small yellowish-colony mutants susceptible to trypaflavine as determined from the melting temperature (T_m). Fifty mutants (1416 to 2570) were induced by ultraviolet. Two mutants (266 Fu and 426 Fu) were induced by 5-fluorouracil. Mutants 73 and 161 are representatives of a group of 13 mutants induced by ultraviolet in the parent culture resistant to kanamycin.

Strain	T_m (°C)	GC (%)	Strain	T_m (°C)	GC (%)
5099 (parent)	89.0	48.0	2450	97.5	68.8
1416	97.4	68.5	2451	98.2	70.5
1417	97.4	68.5	2452	97.5	68.8
1707	97.5	68.8	2453	97.5	68.8
1799	97.6	69.0	2454	97.9	69.8
1926	97.5	68.8	2455	97.8	69.5
1927	97.3	68.3	2456	97.8	69.5
1975	98.2	70.5	2457	97.4	68.5
2074	96.7	66.8	2458	98.5	71.2
2244	97.5	68.8	2459	97.6	69.0
2263	97.7	69.3	2462	98.2	70.5
2264	98.2	70.5	2464	97.7	69.3
2312	97.5	68.8	2467	98.0	70.0
2329	97.7	69.3	2468	97.5	68.8
2341	98.5	71.2	2469	98.1	70.2
2346	98.1	70.2	2472	97.8	69.5
2393	97.6	69.0	2484	97.8	69.5
2420	98.0	70.0	2488	97.9	69.8
2421	98.6	71.5	2497	97.8	69.5
2423	97.5	68.8	2503	98.1	70.2
2430	97.8	69.5	2519	97.6	69.0
2434	97.7	69.3	2528	97.3	68.3
2439	98.2	70.5	2570	98.5	71.2
2442	97.4	68.5	266 Fu	97.8	69.5
2444	98.0	70.0	426 Fu	97.2	68.0
2446	97.9	69.8	73	97.9	69.8
2448	97.5	68.8	161	98.0	70.0
2449	97.5	68.8			

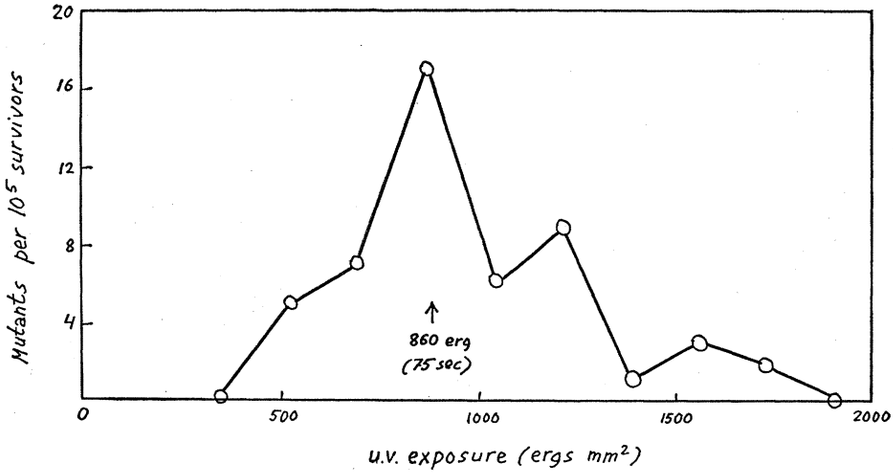

Fig. 1. Yield of mutants with altered DNA base composition at different exposures to ultraviolet radiation.

susceptibility to this agent of mutants with small yellowish colonies isolated from agar plates. In this study 3000 petri dishes were used, and 50 mutants susceptible to trypaflavine were isolated (Table 1).

For the examination of DNA base composition, we isolated DNA from bacterial cells according to Marmur's procedure (*3, 4*). Base composition was calculated from the melting temperature (T_m) of purified samples of native DNA as follows

$$T_m = 69.3 + 0.41\ (G + C)\ \text{percent}$$

Table 1 gives information on guanine-cytosine (GC) content in DNA of the parent culture *B. paracoli* 5099 and of 50 small yellowish-colony mutants which were induced by ultraviolet radiation and were susceptible to trypaflavine. It is of interest that in all mutants guanine-cytosine content in DNA is increased to 67 to 71 percent as compared to 48 percent of the parent culture. The study of antigenic relationships has shown that there is cross-agglutination of parent culture *B. paracoli* 5099 and of mutants listed in Table 1 by rabbit antiserum to the parent culture.

Figure 1 illustrates the dependence of the frequency of induction of mutants with altered DNA base composition (per 10^5 survivors) upon the dose of ultraviolet radiation. This frequency is strongly dose-dependent, and has a peak at the ultraviolet exposure equal to 860 erg mm^{-2}. On both sides of this maximum the rate of appearance of mutants decreases, and with exposures less than 350 and more than 1900 erg mm^{-2} mutants with altered DNA base composition do not appear at all.

An attempt was also made to investigate the fate of a marker of resistance to kanamycin in the mutants with altered DNA base composition. The minimum inhibitory concentration of kanamycin in nutrient broth for the parent strain *B. paracoli* 5099 attains 5 μg/ml. By cultivating this strain in nutrient broth containing increasing concentrations of kanamycin a resistant culture was produced, and this culture was not inhibited by kanamycin even at the concentration of 500 μg/ml. This resistant culture was irradiated by ultraviolet; 800 petri dishes were used, and 13 mutants with altered DNA base composition were isolated. These mutants were induced by the ultraviolet exposure around 860 erg mm^{-2}, and the dependence of the frequency of their appearance upon the dose of radiation was similar to that shown in Fig. 1. The resistance to kanamycin was lost in all 13 mutants with altered DNA base composition derived from the resistant parent culture, and minimum inhibitory concentration of kanamycin for mutants again attained 5 μg/ml. In accordance with current views (*5*) resistance to kanamycin depends upon the decreased permeability of cells for the antibiotic, and mechanisms of this decreased permeability are evidently lost in mutants with altered DNA base composition.

Table 2. The effect of addition of glucose (0.5 percent) upon respiration (as judged by Q_{O_2}) in the parent culture of *B. paracoli* 5099 and in small yellowish-colony mutants susceptible to trypaflavine. Mutants 1416 to 1975 were induced by ultraviolet. Mutants 266 Fu and 426 Fu were induced by 5-fluorouracil. Q_{O_2} shows the consumption of oxygen in cubic milliliters per hour, calculated for 1 milligram of the dry weight.

Strain	Q_{O_2}		Increase in the presence of glucose
	Endogenous	Exogenous	
5099 (parent)	13.0	69.1	5.3
1416	15.4	25.2	1.6
1417	20.3	36.5	1.8
1707	17.3	33.5	1.9
1799	12.0	26.3	2.2
1926	11.7	23.0	2.0
1927	10.6	17.2	1.6
1975	16.7	44.4	2.6
266 Fu	31.2	43.5	1.4
426 Fu	13.5	22.6	1.7

It is possible to induce mutants with altered DNA base composition in the parent culture of *B. paracoli* 5099 not only by ultraviolet irradiation but also by exposure to 5-fluorouracil. However, in the latter case the frequency of appearance of mutants is very low. The parent culture 5099 was cultivated at 43°C for 2 days in 300 tubes of nutrient broth containing 1 percent glucose and 3 to 160 μg of 5-fluorouracil per milliliter. When samples from each tube were plated on nutrient agar containing 1 percent glucose and incubated at 37°C, two mutants with altered DNA base composition (266 Fu and 426 Fu) were isolated from tubes containing 5-fluorouracil (160 μg/ml). Table 1 shows that guanine-cytosine content of DNA in mutants induced by 5-fluorouracil and by ultraviolet irradiation is very similar.

A characteristic feature of mutants with altered DNA base composition is related to their biochemical inflexibility (*1*). This aspect of the metabolism of mutants is shown in Table 2. The oxidative rate of mutants is more refractory to stimulation by glucose, and increases only by 1.4 to 2.6 times, whereas in parent cells under similar conditions it increases by 5.3 times.

It appears that the parent culture (*6*) of *Bacterium paracoli* 5099 represents a favorable system for the study of induction of mutants with altered DNA base composition.

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