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Table II. The effect of addition of glucose (0.5%) upon respiration in the parent culture of *B. paracoli* 5099 and in small colony mutants susceptible to trypaflavine

Strain	Q _{O₂} (endogenous)	Q _{O₂} (exogenous)	Increase in the presence of glucose
Parent culture	13.1	54.9	4.2
Mutant 168	27.0	55.9	2.1
Mutant 975	20.5	60.7	2.9
Mutant 1008	28.3	46.6	1.6
Mutant 1041	22.9	23.1	1.0
Mutant 1055	23.1	72.3	3.1

the oxidative rate of mutants is more refractory to stimulation by glucose, and increases only 1.0–3.1 times, while in parent cells under similar conditions it increases 4.2 times.

An investigation of the effect of various inhibitors upon the growth of parent and mutant cultures of *B. paracoli* 5099 in the nutrient broth has produced results which are shown in Table III. It is clear that small colony mutants susceptible to trypaflavine are also more susceptible to 2 other inhibitors affecting DNA (streptonigrin and mitomycin C), and also to 2 inhibitors of protein synthesis in the bacterial cell (chloramphenicol and tetracycline).

For the examination of DNA base composition we isolated DNA from bacterial cells according to MARMUR's procedure⁴. Base composition was calculated from the

Table III. Minimal inhibitory concentrations in µg/ml for growth in nutrient broth of various inhibitors for the parent culture *B. paracoli*, 5099 and its mutants

Inhibitor	Parent culture		Mutant 168		Mutant 975		Mutant 1008		Mutant 1041		Mutant 1055	
	C		C	S	C	S	C	S	C	S	C	S
Trypaflavine	250		0.5	500	0.6	416	0.5	500	0.4	624	0.6	416
Streptonigrin	4.2		0.43	10	0.63	6.7	0.43	10	0.36	11.7	0.4	10.5
Mitomycin C	10		2	5	3	3.3	2	5	0.2	50	2	5
Chloramphenicol	10.5		1.9	5.5	2.2	4.8	2.3	4.6	2.3	4.6	2	5.2
Tetracycline	15		0.6	25	0.9	17	0.8	19	0.6	25	1.1	14

C, minimal inhibitory concentrations; S, increase of sensitivity.

Table IV. GC content in DNA of the parent culture *B. paracoli* 5099 and of small colony mutants susceptible to trypaflavine as determined from the melting temperature

Strain	Melting temperatures (<i>T_m</i>) of various inde- pendently purified samples of native DNA	Average <i>T_m</i>	% GC
Parent	88.9; 89.0; 89.0	89.0	48.0
Mutant 168	97.1; 97.0; 97.2	97.1	67.8
Mutant 975	97.8; 97.8; 97.6	97.7	69.3
Mutant 1008	97.6; 97.8	97.7	69.3
Mutant 1041	97.7; 97.8	97.7	69.3
Mutant 1055	97.7; 97.7	97.7	69.3

survival of bacteria was of the order of 0.01%. Samples of irradiated suspensions were plated upon the nutrient agar containing 1% glucose and incubated at 37°C for 7 days.

In these experiments 1310 mutants with small colonies were isolated and studied for their sensitivity to trypaflavine on gradient agar plates. In 1305 mutants the susceptibility to trypaflavine did not differ significantly from that of the parent culture, but in 5 mutants the sensitivity to trypaflavine was drastically increased, on the average by 200 times, as it can be seen from the data given in Table I. These susceptible mutants appear with the frequency of 0.4% among small colony mutants of *B. paracoli* 5099, and 5 such mutants possessing yellowish colonies (168, 975, 1008, 1041, 1055) will be described in this communication.

The respiratory capacity of the mutants with small colonies listed in Table I was studied with the aid of the Warburg technique at 37°C, and the results of these measurements are given in Table II. It can be seen that

melting temperature (*T_m*) of purified samples of native DNA according to the formula⁵: $T_m = 69.3 + 0.41 (G + C)\%$. Table IV gives information on GC content in DNA of the parent culture *B. paracoli* 5099 and of small colony mutants susceptible to trypaflavine. It is of considerable interest that in mutants GC content in DNA is increased to 68–69% as compared with 48% 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 IV by rabbit sera, immunized either by parent culture *B. paracoli* 5099 or by culture of mutant 168. It is therefore possible to conclude that drastic increase of sensitivity to trypaflavine can be instrumental in the isolation of some very rare mutants with altered GC content, which are induced by UV-radiation in cultures of *B. paracoli* 5099⁶.

Выводы. Весьма редкие мутанты *Bacterium paracoli* 5099 с измененным нуклеотидным составом ДНК, индуцированные путем воздействия ультрафиолетовой радиации, могут быть выделены с помощью градиентных агаровых пластинок содержащих трипафлавин.

G. F. GAUSE, A. V. LAIKO,
YU. V. DUDNIK, E. M. NETYKSA
and G. V. KOCHETKOVA

*Institute of New Antibiotics, Academy of Medical Sciences,
Moscow (USSR), 22nd March 1967.*

⁴ J. MARMUR, J. molec. Biol. 3, 208 (1961).

⁵ J. MARMUR and P. DOTY, J. molec. Biol. 5, 109 (1962).

⁶ The parent culture (ATCC 23280) as well as one of the mutants (ATCC 23281) are now available in the American Type Culture Collection.