DNA NUCLEOTIDE COMPOSITION AND SENSITIVITY OF BACTERIA TO γ-RAYS

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The attention of investigators has been attracted to the phenomenon of radiosensitivity of bacteria during recent years for many reasons. Bacteria are a convenient test object for analysis of the mechanisms determining radiation injury, and for the study of processes at the molecular, subcellular, and cellular levels.

The resistance of bacteria to the action of radiation varies not only from genus to genus, but also within the same species or even within the same strain, and it depends on the conditions of irradiation and of cultivation after irradiation, and so on [2, 10, 15, 16].

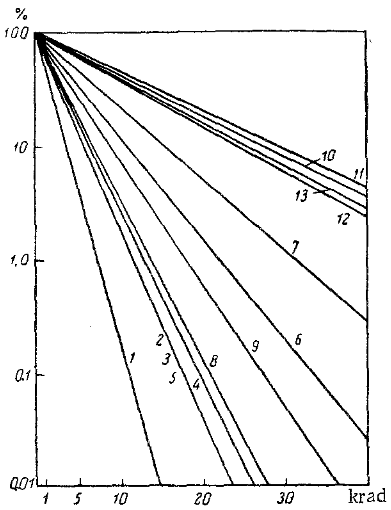

Fig. 1. Relationship between survival of bacteria (in percent) and dose of γ-ray irradiation (in krad). Here and in Fig. 2: 1) Alcaligenes faecalis; 2) E. coli 1; 3) E. coli S-1-Shch; 4) E. coli 613; 5) E. coli 613 (streptomycin-resistant); 6) E. coli K12; 7) E. coli K12 Hfr thy; 8) S. dysenteriae flexneri 550; 9) S. typhi (Ty-2); 10 mutant E. coli K12; 11) mutant E. coli Hfr thy; 12) mutant S. dysenteriae flexneri 550; 13) mutant S. typhi (Ty-2).

The causes of differences in the radiosensitivity of the cells of closely related species have not been explained. There is much experimental evidence to suggest that the lethal action of radiation is due to damage primarily of the nuclear structures of the cells, of their DNA [11]. However, the analysis of the mechanisms of radioresistance is hampered by the difficulty of differentiating between the primary and secondary effects of radiation as well as by processes of recovery after injury.

Some investigators have associated differences in the radioresistance of bacteria with qualitative and quantitative changes in the DNA and RNA of the bacteria. However, these results are conflicting [1, 3, 9].

Kaplan [12] found that with an increase in the number of GC-pairs in the DNA of bacteria, their sensitivity to irradiation increases. However, Moseley found that the highly resistant species M. radiodurans also contains many GC-pairs in its DNA [14].

The object of the present investigation was to determine the presence or absence of correlation between the nucleotide composition of the DNA and the radioresistance of pathogenic and nonpathogenic strains of the enteric group of microorganisms.

EXPERIMENTAL

The following strains were used: Escherichia coli K12 (prototroph), E. coli K12 thy, Shigella dysenteriae 550, Salmonella typhosa Ty-2, and mutants obtained from these strains by the action of increasing doses of γ-rays [6], E. coli 1 and the mutant E. coli S-1-Shch obtained from it by experimental mutation [5], E. coli 613, and an antibiotic-fast strain of E. coli 613 resistant to streptomycin. *

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TABLE 1. Nucleotide Composition of DNA of Bacteria

Microorganisms	Rel. proportions of bases (in molar percentages)				Pur / Pyr	6-keto / 6-amino	(G+C) / (A+T)
	G	A	C	T			
S. dysenteriae fl. 550	25,0	24,9	24,8	25,3	1,00	1,01	0,99
S. dysenteriae fl. 550 (radioresistant)	21,4	28,7	21,6	28,3	1,00	0,99	0,75
S. typhosa Ty-2	26,6	23,3	26,7	23,4	1,00	1,00	1,14
S. typhosa Ty-2 (radioresistant)	20,8	28,8	21,0	29,4	0,99	1,01	0,72
E. coli K-12	24,1	26,8	24,6	24,5	1,03	0,96	0,95
E. coli K-12 (radioresistant)	17,7	32,8	17,6	31,9	1,02	0,99	0,55
E. coli K-12 Hfr thy	24,3	25,8	24,5	25,4	1,00	0,99	0,95
E. coli K-12 Hfr thy (radioresistant)	19,6	31,7	18,1	30,6	1,05	1,01	0,61
E. coli 1	25,6	24,3	25,8	24,3	1,00	1,00	1,05
E. coli S-1-Shch	32,5	17,3	33,0	17,2	0,99	0,99	1,9
Alcaligenes faecalis	30,9	19,5	30,8	18,8	1,01	0,99	1,61
E. coli 613	24,2	25,7	24,6	25,5	1,00	0,99	0,96
E. coli 613 streptomycin-resistant	24,9	25,2	25,1	24,8	1,00	1,00	1,00

TABLE 2. Nucleotide Composition of DNA of Bacteria Determined by the Melting Point Method and by Paper Chromatography

Strain	Temperature (in deg)	Melting point method (GC-pairs, in percent)	Paper chromatography (GC-pairs, in percent)	(G+C) / (A+T)
E. coli K 12 Hfr thy	90	50,5	49,7	0,95
E. coli K 12 Hfr thy (radioresistant)	85	38	37,7	0,61
Staph. aureus	81,5	30	31	0,45
Staph. aureus (antibiotic-resistant)	81,5	30	30,2	0,43

The survival curves were plotted by the usual method. Irradiation of the cells washed from an 18 h culture in the stationary phase of growth was carried out in phosphate buffer, pH 7.0, in aerated flasks by means of an experimental γ-ray apparatus (Co^{60}; dose rate 10 krad/min). The number of surviving cells was determined from the number of colonies on Hottinger's nutrient agar. DNA was isolated from the bacteria by a modification of the Schmidt–Thannhauser method, and the bases were separated by radial paper chromatography. High-polymer native preparations of DNA were obtained and their melting point determined by the method of Marmur and Doty [13], with some modifications.

EXPERIMENTAL RESULTS

It will be seen in Fig. 1 that the curves of survival of all the bacteria studied were exponential, demonstrating the one-stage mechanism of action of γ-rays. For all the mutants of the bacteria obtained by the method of multistage screening the survival curves were very close and the values of LD_{90} were almost identical with those of the sensitive strains (LD_{90} 25-30 krad). Strains E. coli 1, E. coli 613, E. coli 613 (streptomycin-resistant), and also the strain E. coli S-1-Shch obtained by experimental mutation possessed similar LD_{90} values (6.0-6.5 krad). The strain Alcaligenes faecalis was the most sensitive to irradiation (LD_{90} 3.5 krad).

The nucleotide composition of the bacteria determined by circular paper chromatography is given in Table 1. The results show that the composition of the DNA from the investigated bacteria mainly obeys Chargaff's rule ($\frac{\text{Pur}}{\text{Pyr}}$ and $\frac{G + T}{A + C}$ close to 1). The fact was noted that strains with increased radioresistance gave a lower $\frac{G + C}{A + T}$ ratio.

However, despite their identical radiosensitivity, the strain E. coli 1 and the variant E. coli S-1-Shch obtained from it differed considerably in their coefficient of specificity (in the former this was 1.05, so that its DNA was of the AT-type, in the latter it was 1.9, so that the DNA of this strain was decidedly of the GC-type). The nucleotide composition of the streptomycin-resistant strain of E. coli was unchanged by comparison with that of the original strain of E. coli. The nucleotide composition of the DNA was also determined on the basis of the melting point. The nucleotide composition was calculated by the Marmur–Doty formula: M.p. = 69.3 + 0.41 · (G + C) [13].

As an example, the results are given for four strains determined by two methods (Table 2).

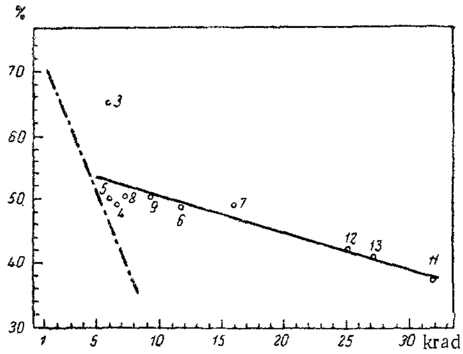

Fig. 2. Relationship between radioresistance (LD_{50} in krad) and nucleotide composition of bacteria (TC-pairs, in percent).

The results obtained by the two methods agreed, but the the circular chromatography method revealed finer differences in the nucleotide composition. Determination of the coefficient of specificity of the high-polymer DNA and after hydrolysis of the DNA into bases by the two methods proved conclusively that the results were reliable, despite the fact that certain modifications had to be introduced for isolating the DNA from the different strains. Comparison of the radioresistance of the bacteria (LD_{90}) and the coefficient of specificity of their DNA revealed a general principle: the more resistant strains contained a larger number of AT-pairs of their DNA (Fig. 2).

It is clear from Fig. 2 that the straight line describing the correlation between radioresistance and contact of GC-pairs had a somewhat different slope from the line given by Kaplan [12]. Besides, strain E. coli S-1-Shch was identical in its radioresistance with strain E. coli 1, but differed from it considerably in its nucleotide composition, so that a deviation from the general principle was observed. Furthermore, the bacteria of one species (E. coli), although possessing the same nucleotide composition, differed in their radiosensitivity.

Changes in the nucleotide composition of the DNA in alkali-forming mutants by comparison with the original strains were reported as long also as in 1956 [7]. Similar changes have been observed by other authors [1, 4, 8]. For this reason, the present findings indicating an increase in the number of AT-pairs in mutants obtained by γ-ray irradiation in increasing doses are confirmed by the investigations cited above.

The general principle can therefore be stated that more radioresistant strains contain more AT-pairs in their DNA. However, some strains do not obey this rule (E. coli S-1-Shch and M. radiodurans).

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