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A COMPARISON OF ENVIRONMENTAL INFLUENCES ON THE RESISTANCE OF STRAINS B AND B/r OF *ESCHERICHIA COLI* TO RADIATION AND TO FURACIN

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SUMMARY

1. Strains B and B/r of *Escherichia coli* have been grown in both complete and synthetic media to determine whether the resistance to Furacin could be modified by the conditions of culture.

2. Similar experiments have also been carried out to test whether the resistance to ultraviolet irradation could be affected by the cultural conditions.

3. The results show (a) that it is possible to eliminate in part the difference in sensitivity to radiation between the two strains, (b) that the sensitivity to Furacin cannot be modified by alteration of the cultural conditions.

4. It is suggested that this difference between the pattern of resistance to radiation and that to Furacin might be due to two different sequences of pathways in the phenotypic expression of the mutation.

INTRODUCTION

SZYBALSKI AND NELSON[1] reported that the radiation-resistant strain of *Escherichia coli*, B/r, was about forty times more resistant than the sensitive strain, B, to many nitrofuran derivatives such as Furadroxyl and Furacin. Mutation of B to B/r was found to be accompanied by increased resistance to Furacin[2]. ALPER AND GILLIES[3] reported a set of post-irradiation, environmental conditions, in which B and B/r showed a similar resistance to ultraviolet light. The purpose of the present investigation has been to determine whether resistance to Furacin is similarly altered by the conditions of culture.

MATERIALS AND METHODS

A synthetic medium[4] and a complete medium (containing 0.8% of nutrient broth and 15% agar) were used for this investigation. Strains B and B/r of *E. coli* were kindly supplied by Dr. E. M. WITKIN, of the State University of New York. Inocula for all the experiments were taken from a single slant to avoid any genetic variability

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being introduced. The strains were grown under aeration for 24 h in a nutrient broth of 0.8% containing 0.5% NaCl. From the stationary phase thus obtained, 0.1 ml was grown under aeration for a further period of 2.5 h in 10 ml of nutrient broth to obtain the logarithmic growth phase. The cultures were then washed twice in saline, and resuspended in physiological saline (0.85%).

Samples (of 3 ml each) were taken for irradiation at each dose level. For irradiation, a 2537 Å ultraviolet germicidal lamp was used, that gave 5.6 ergs/mm²/sec. For each dose level, an appropriate dilution of the irradiated culture was made and plated out for survival counts. Each irradiation experiment was repeated four times and the average results are indicated in Fig. 1.

The various concentrations (in μg/ml) of Furacin (obtained from Eaton Laboratories, Norwich, N.Y.) required to give different inhibition values in both B and B/r, chosen by adopting the gradient plate technique of SZYBALSKI[5], were for B, as follows: 0.1, 0.15, 0.2, 0.3, 0.4, 0.5 and 0.6 and for B/r 5, 9, 13, 17, 21, 25, 29 and 33.

The resistance of B and of B/r to Furacin was determined by a plate-pouring technique[6]. An appropriate dilution of the culture (0.1 ml in volume) was mixed with 15 ml of nutrient agar, or of agar containing synthetic salts, to which an appropriate concentration of Furacin solution had been added earlier. The whole mixture was plated out in a petri dish. Precautions were taken not to have the agar warmer than 45°. The plates after hardening were incubated for 40–50 h at 32°.

RESULTS AND DISCUSSION

Resistance to ultraviolet irradiation

Cells of both strains irradiated at the logarithmic phase of growth were plated out on both the nutrient and the synthetic media. On the synthetic medium the growth of both strains was slower. Strain B showed the maximum lethal effect of radiation in conditions which were optimal for growth, whereas B/r showed the maximum resistance to radiation in these conditions (Fig. 1).

These observations are in agreement with those of WITKIN[7] and STAPLETON *et al.*[8]. But on the synthetic medium B showed a ten-fold increase in resistance to radiation, whereas B/r showed only a slight decrease in its resistance at lower doses and a twenty-fold decrease at higher doses. The data of WITKIN[9] indicated that she could obtain only a ten-fold increase in the resistance of B on minimal medium. My experiments did not eliminate completely the difference between B and B/r in resistance to irradiation by ultraviolet light. Only a partial agreement with the report of ALPER AND GILLIES[10] could be found. This failure of the two strains to produce a similar resistance on synthetic medium might be due to heterogenic differences[11] in the strains used by me and those used by ALPER AND GILLIES[11].

Resistance to Furacin

As reported earlier[1], B/r was found to be more resistant than the parent strain to the inhibitory action of Furacin, when plated on a complete medium. As no significant difference was obtained in the values for cell-inhibition, only the average results of ten experiments are given in Figs. 2 and 3. The data indicate that at low concentrations there was no appreciable difference in the sensitivity of B to Furacin between the two media, while only a ten-fold difference was noticed at higher concen-

trations. In the case of B/r the difference in resistance to Furacin between the media was not statistically significant. To summarise the results, it was found that the sensitivity of B and of B/r to Furacin could not be modified by modifying the conditions of culture.

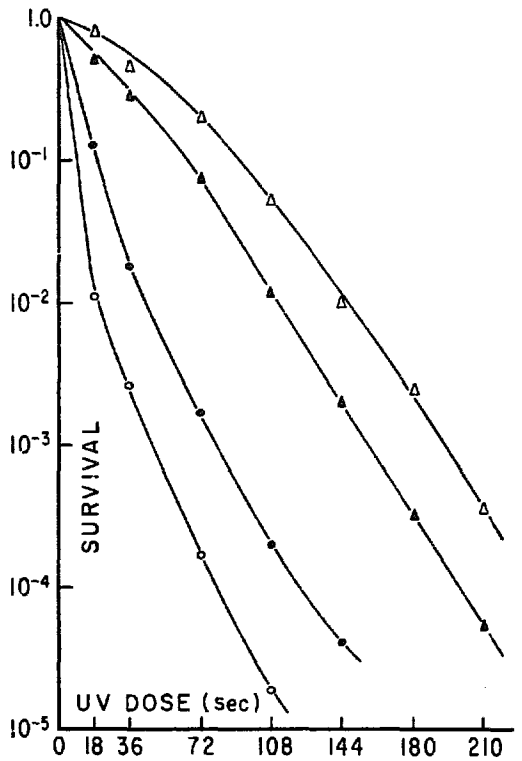

Fig. 1. Survival of *E. coli* strains, B and B/r, after ultraviolet irradiation on two media (mean value from four experiments). O—O, B on nutrient medium; ●—●, B on synthetic medium; △—△, B/r on nutrient medium; ▲—▲, B/r on synthetic medium.

The survival curves for B obtained for irradiation with ultraviolet light were exponential on both media, whereas the inhibition curves were sigmoid. In the case of B/r the curves obtained on the two media for both ultraviolet irradiation and Furacin-treatment were of the same pattern.

Only the pattern of resistance of these two strains to these two agents was comparable. In the case of ultraviolet light, the irradiated culture contained at the time of plating out a mixture of dead, partially damaged but recoverable cells, non-recoverable cells, and resistant cells. The resistant cells would begin growing immediately, whereas in the damaged cells the growth was delayed.

In the Furacin tests, all the cells were in the logarithmic growth phase; their growth was only temporarily arrested at the time of plating. The damage caused, here, was a slow but continuous process as the drug was incorporated in the growth media. The dividing cells were also subjected to the action of the agent, whereas with ultraviolet light, the cells being in buffer, cell division was temporarily arrested during irradiation. Since the growth on the synthetic medium was slow, more cells could resist the inhibitory action of Furacin. But both the strains suffered much in the enriched medium.

The concentration of cells plated out in higher concentrations of Furacin could not influence the selective inhibition of the sensitive cells, as the cells would be in

contact with the drug from all sides due to the plate-pouring technique. If plated out on the surface then one could expect a different result due to overcrowding. That this was true was shown by the fact that, when higher concentrations of the cultures were plated out on the surface of the medium containing Furacin, cells with different sizes against a cloudy background were observed.

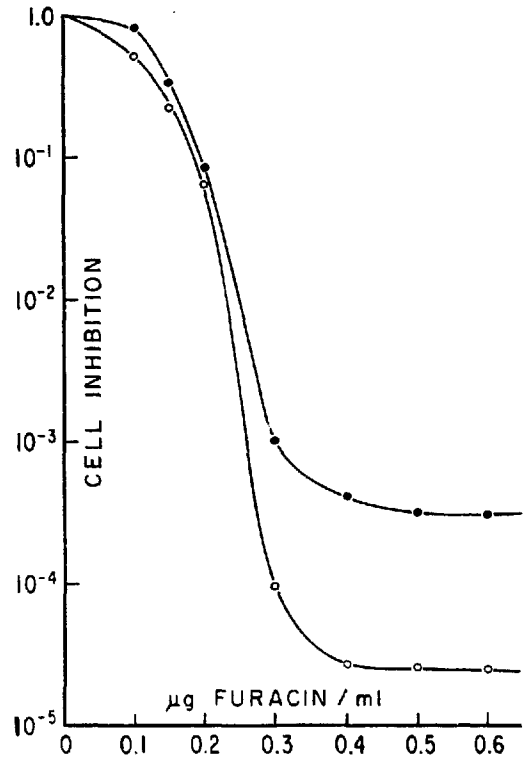

Fig. 2. Cell-inhibition by Furacin in *E. coli*, strain B (mean value from ten experiments). O—O, on nutrient medium; ●—●, on synthetic medium.

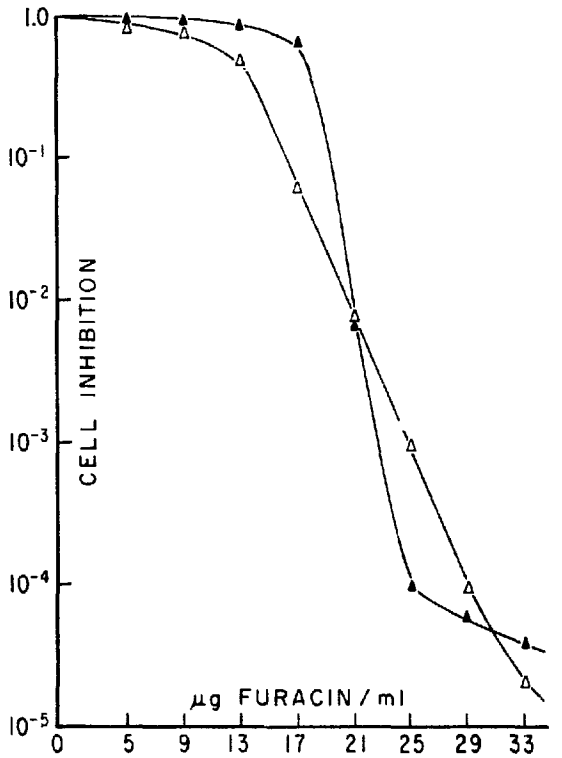

Fig. 3. Cell-inhibition by Furacin in *E. coli* strain B/r (mean value from ten experiments). △—△, on nutrient medium; ▲—▲, on synthetic medium.

If the genes for resistance to radiation and to Furacin are the same, then one would normally expect a similar pattern of behaviour to be observed on both the media. In the case of strain B, however, the resistance to Furacin was found to increase slightly on the synthetic medium. Instead of getting an expected decrease in resistance, B/r cells showed a slight increase in resistance as did the B cells. However, the B/r strain showed a decrease in ultraviolet resistance when plated on synthetic medium.

This difference might be interpreted as follows: The mutation for resistance to radiation and to Furacin could be a primary effect. The phenotype of these mutations would depend much on the interaction with the environmental conditions, this being a secondary effect. In the case of resistance to radiation, the phenotype could be modified to some extent by cultural conditions, whereas the same conditions of culture could fail to modify the resistance to Furacin. In other words, the sequence of pathways for these two types of resistance might be different.

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