Effect of Mild Heating on *Salmonella typhimurium* Containing an R Factor

By D. G. MACPHEE*

School of Biological Sciences, University of East Anglia, Norwich, NOR 88 C

(Received 28 December 1972)

MacPhee (1972) reported that derivatives of *Salmonella typhimurium* strain LT2 carrying the drug-resistance transfer factor R-Utrecht are substantially more resistant than their wild-type parent strains to the lethal effects of ultraviolet (u.v.) radiation, ionizing (γ) radiation, thymine starvation, methyl methane sulphonate (MMS) treatment and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) treatment. Since all of these agents are believed to cause the formation of single-strand gaps in deoxyribonucleic acid (DNA) (Strauss, 1968; Witkin, 1969), testing was extended to mild (52 °C) heat treatment which may also cause DNA strand breaks in *Escherichia coli* (Bridges, Ashwood-Smith & Munson, 1969; Sedgwick & Bridges, 1972; Woodcock & Grigg, 1972).

The strains used were *Salmonella typhimurium* SL1156 (LT2 *trpD*r) and SL1156/R-Utrecht (MacPhee, 1972). All liquid cultures were grown with shaking at 37 °C in nutrient broth (Oxoid, London, SE1 9HF), and all plates were incubated at 37 °C. Heat and NTG experiments were carried out in broth or in minimal E salts (Vogel & Bonner, 1956) with no added carbon source. Oxoid Eosin Methylene Blue Agar (Levine) was supplemented with 1 % (w/v) galactose to allow differentiation of GAL⁺ and GAL⁻ strains.

In preliminary experiments to determine whether R-Utrecht had any effect on survival after mild heating, exponential phase bacteria grown at 37 °C to about 2×10^8 /ml were treated in their growth medium in a water bath at 52 °C. Samples (0.5 ml) of each culture were withdrawn at intervals, diluted in 0.85 % (w/v) NaCl, and plated on nutrient agar (Oxoid). The results were extremely inconsistent, since the absolute survival values for any one strain varied markedly from experiment to experiment, even although conditions were apparently identical before, during and after treatment (e.g. in three separate experiments with strain SL1156, survival values of 0.09, 0.9 and 0.3 % were obtained after 60 min at 52 °C). In addition, it was noted that in some experiments R⁺ and R⁻ bacteria showed approximately equal survivals whereas in others the R⁺ bacteria seemed to be somewhat more sensitive. Unsuccessful attempts were made to improve reproducibility by resuspending exponential or stationary phase organisms in fresh broth or in minimal E salts prior to heat treatment.

In all of the above experiments, strains SL1156 and SL1156/R-Utrecht were heated separately in screw-capped 1 oz bottles, and it had to be assumed that the actual temperature inside each bottle rose uniformly and rapidly to 52 °C and remained relatively constant for the required length of time. This may be an unjustifiable assumption, since minor but critical variations in temperature within the bottle might tend to mask any intrinsic difference in sensitivity between the two strains. To overcome this problem, mixtures of R⁺ and R⁻ bacteria were therefore subjected to mild heating in a single container. The relative proportions of R⁺ and R⁻ bacteria in the surviving population could not be determined simply

* Present address: Department of Genetics, La Trobe University, Bundoora, Victoria, 3083, Australia

by plating on media with and without an antibiotic to which the R factor confers resistance because the results might be distorted simply because two different plating media are involved, or because transfer or loss of the R factor occurs during mild heat treatment. An alternative method, which allows survivors of the two original strains to be distinguished after growth on a single post-treatment plating medium was therefore used. This method is simple, requiring only that the R⁺ and R⁻ bacteria differ by a single chromosomal mutation (for example, ability to utilize galactose) which does not influence their sensitivity to the lethal agent involved. *Salmonella typhimurium gal* mutants were obtained by selecting spontaneous phage-resistant mutants with a mixture of phages P22.c2 and Felix 0 and then testing for ability to utilize galactose (MacPhee & Stocker, 1969). Of twenty phage-resistant mutants of strain SL1156 five were found to be galactose-negative. One of them, designated SL1156 *gal-1101*, was used in subsequent experiments.

The effect of R-Utrecht on the sensitivity of *Salmonella typhimurium* to mild heating was then tested using mixed cultures of strains SL1156 *gal-1101* and SL1156/R-Utrecht. Samples (0.1 ml) taken from stationary phase broth cultures of each strain were added to a 1 oz bottle containing 9.8 ml of minimal E salts and the resulting mixture was placed in a water bath at 52 °C. Samples (0.5 ml) were withdrawn at intervals, diluted in 0.85 % (w/v) NaCl and plated on nutrient agar to provide an estimate of overall survival. Following incubation at 37 °C overnight, 200–300 of the colonies appearing on these plates were picked with sterile toothpicks and stabbed into EMB-galactose medium so that the proportions of GAL⁺ and GAL⁻ bacteria among the survivors of the heat treatment could be determined. (It would have been simpler to plate the organisms on EMB-galactose directly after the heat treatment, but preliminary experiments showed that plates of EMB-galactose spread with identical samples often gave widely divergent survival results presumably because some component of the medium was capable of interfering with the recovery of heat-treated organisms.)

The results of two mild heating experiments are shown in Fig. 1 (lower curves). In one case the proportion of GAL⁺ bacteria in a mixed GAL⁺R⁺ and GAL⁻R⁻ culture decreased significantly during exposure to 52 °C, and in the other case the proportion of GAL⁻ bacteria in a mixed GAL⁻R⁺ and GAL⁺R⁻ culture decreased during the treatment. The overall survival percentages in these and other experiments with the same pairs of strains still varied considerably (see Fig. 1), but the proportion of R⁺ bacteria among the survivors always decreased. R⁺ bacteria therefore seem to be more sensitive to mild heating than R⁻ bacteria. Similar results were obtained whether mixtures of GAL⁺R⁺ and GAL⁻R⁻ bacteria or GAL⁻R⁺ and GAL⁺R⁻ bacteria were used, so that the presence of the *gal-1101* mutation in one of the two strains does not appear to affect sensitivity to mild heating. This point was tested directly by exposing mixtures of the R⁻ strains SL1156 and SL1156 *gal-1101* to mild heat, and it was found that the percentage of GAL⁻ bacteria among the survivors changed only very slightly (± 3 % at most) during treatment for up to 2 h. Transfer of the R factor between the two strains during treatment of mixed R⁺/R⁻ cultures was not detected and therefore must have occurred at a frequency of less than 0.3 % (if at all), while loss of the R factor was detected at a frequency of only 1–2 %. Neither phenomenon is therefore likely to affect interpretation of the data significantly.

From these results, it seems clear that R-Utrecht causes a decrease in resistance of *Salmonella typhimurium* to mild heating, although it causes an increase in resistance to a number of other agents which induce the formation of DNA strand breaks (MacPhee, 1972). Since the method used for the heating experiments is quite different from the one used for the other agents, the new method was checked when an increase in resistance was

Fig. 1. Percentage of R⁺ bacteria among the survivors after treatment with mild heat (52 °C) or nitrosoguanidine: ■, mixture of GAL⁺R⁺ and GAL⁻R⁻ bacteria treated at 52 °C; ●, mixture of GAL⁻R⁺ and GAL⁺R⁻ bacteria treated at 52 °C; ○, mixture of GAL⁺R⁺ and GAL⁻R⁻ bacteria treated with NTG (50 µg/ml). Figures in brackets are estimates of overall percentage survival in each mixed culture.

expected on the basis of previous data (MacPhee, 1972). Mixtures of SL1156/R-Utrecht and SL1156 *gal-1101* were treated with NTG at 50 µg/ml in minimal E salts, diluted and plated on nutrient agar, and replica-plated to EMB-galactose as previously described. The results in Fig. 1 (upper curve) show that the proportion of GAL⁺ (and hence R⁺) organisms in the surviving population increased with time of exposure to NTG; the R factor caused the expected increase in resistance. Significant increases in resistance to MMS and u.v. treatment were also found by this method, which may therefore be generally suitable for determining the relative sensitivities of strains of *Salmonella typhimurium* to bactericidal agents (and should be particularly useful when the difference in sensitivity is small).

The explanation for the increased sensitivity of bacteria carrying R-Utrecht to mild heating could involve any of several proteins which may be specified by the plasmid (e.g. a heat-inducible nuclease, or a heat-sensitive inhibitor of nuclease action), or perhaps an alteration in the structure of the membrane resulting from possession of the plasmid. It would therefore be of some interest to determine whether other types of plasmid also increase sensitivity to mild heating.

The increased resistance of bacteria carrying R-Utrecht to radiation and chemical

treatment is thought to be due to one or more plasmid genes whose products function in repair of single-strand gaps (MacPhee, 1972). R⁺ bacteria should therefore have an increased capacity to repair DNA strand breaks produced by heating at 52 °C (Bridges *et al.* 1969), but this is clearly not the only factor involved in recovery from mild heat treatment since it is the R⁻ bacteria which are more resistant. One possible explanation for these results, suggested by the recent finding of Sedgwick & Bridges (1972) that DNA strand breakage is not a general occurrence after treatment at 52 °C in all strains of *Escherichia coli*, is that mild heating does not lead to significant amounts of DNA strand breakage in the strains of *Salmonella typhimurium* used in this study. In such circumstances, any additional capacity for repair of DNA strand gaps provided by the R factor would not be expected to increase resistance to treatment at 52 °C. Whether or not this explanation is correct, the data presented here indicate that the general correlation between sensitivity to mild heating and ionizing radiation found among various strains of *E. coli* and postulated to result from use of the same processes to repair or mitigate the effects of both types of damage (Bridges *et al.* 1969) does not extend to the R-Utrecht repair system in *S. typhimurium*.

This work was carried out while I held a Research Fellowship from the Medical Research Council. I thank Dr C. H. Clarke for providing facilities and for many stimulating discussions, Mrs P. Howes for excellent technical assistance, and Dr J. Greenberg for his advice during preparation of the manuscript.

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