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RADIATION-INDUCED MUTAGENICITY AND LETHALITY IN SALMONELLA TYPHIMURIUM

M. Işildar and G. Bakale
Division of Radiation Biology, Department of Radiology, Case Western Reserve University, Cleveland, Ohio 44106, USA

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The mutagenic and lethal effects of ionizing radiation on the histidine-deficient auxotrophs of Salmonella typhimurium that were developed by Ames and co-workers [Ames et al., Mutat. Res. 31, 347 (1975)] to test the mutagenicity of chemicals were studied to improve our understanding of radiation damage to DNA. The auxotrophs may be divided into two groups — one which is sensitive to base-pair substitutions and another sensitive to frameshifts. These groups are composed of parent-daughter pairs in which the chemical mutagenicity enhancing plasmid, pKM101, is absent in the parent strain and present in the daughter. Thus, comparison of the radiation response of the base-pair substitution-vs frameshift-sensitive strains and of the parent-daughter pairs provides new information on radiation-induced mutagenicity and lethality.

Co-60 γ-radiation at 18 Gy/min and 250 kV x-rays at 0.85 Gy/min were used to irradiate the bacteria to a total dose ranging from 4 to 60 Gy. For mutagenicity testing, 0.1 ml aliquots of stationary-phase culture were plated as described by Ames et al (loc. cit.) in their plate-incorporation test. The bacterial strains were irradiated on the plates 40 minutes after plating. For viability measurements the culture was diluted by a factor of 2.5×10^5 and 0.1 ml of this dilute culture was plated on minimal plates fortified with a histidine concentration tenfold greater than used in mutagenicity testing. Viability determinations were conducted concurrently with mutagenicity tests for each strain. After irradiation, all plates were incubated at 37°C for 48 hours and the number of revertants to protrophy was counted.

Qualitatively, irradiation of the frameshift-sensitive strains TA98 and TA2637 which carry the pKM101 plasmid doubled the absolute number of induced revertants whereas irradiation of the base-pair substitution sensitive TA100 strain which also carries pKM101 plasmid produced nearly no change in the number of induced revertants. A nearly negligible radiation effect on the mutation rate was observed for all parent strains which include the base-pair substitution strain TA1535 and the frameshift strains TA1537 and TA1538. Consideration of the effects of radiation-induced cell killing on mutation frequency, which is the absolute number of induced revertants corrected to 100 percent survival, indicated that all plasmid-containing strains exhibit a greater radiation-induced mutagenicity than their parent strains. To ensure that the survival corrections were valid, the radiation dose response of revertants with respect to killing was determined and found to coincide with the radiation response of non-revertants.

A quantitative measure of the preceding results is summarized in Table 1 in which the radiation dose required to induce a doubling of the survival-corrected mutation frequency, which is referred to as mutation doubling dose

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Table 1. Mutagenicity doubling dose, MDD, and LD_{50} of Salmonella strains irradiated by Co-60 γ-rays.*

	Tester Strains (Parent-Daughter)					
Radiation Response	TA1535 - TA100		TA1538 - TA98		TA1537 - TA2637	
MDD, Gy	25	17	48	5.5	20	8.0
LD_{50}, Gy	18	22.5	43	14	24	30

* Same values were obtained for 250 kV x-rays except for x-irradiated TA1535 for which MDD=40 Gy and TA100 for which MDD=23 Gy and LD_{50}=30 Gy.

or MDD, is listed for each of the three parent-daughter tester strains. Also included in Table 1 are the LD_{50} values (dose required to induce 50 percent lethality) for the same strains. The LD_{50} values indicate that the presence of the pKM101 plasmid did not uniformly sensitize the daughter strains to the lethal effects of radiation as it did to mutagenic effects but did so only for the TA1538-TA98 pair.

The differences in the radiation responses of the TA1535 and TA100 tester strains for γ- and x-irradiations which are noted in Table 1 are attributed to differences in the dose rates of 18 Gy/min and 0.85 Gy/min, respectively. This behavior contrasts with the other tester strains for which no dose-rate effect was observed for the survival dose response of TA1535 and for both survival and mutagenicity responses of TA1538, TA98, TA1537 and TA2637.

The preceding results can be compared to the work of MacPhee [Mutat. Res. 45, 1 (1977)] and Imray and MacPhee [Int. J. Radiat. Biol. 40, 111 (1981)] who studied the radiation-induced survival and mutagenic responses of S. typhimurium strains with pKM101 and other plasmids. There is qualitative agreement between MacPhee's results and this work; however, the mutagenic and survival responses of four strains common to both studies were five- to tenfold greater in our work. This difference in radiation sensitivities between MacPhee's results and ours may be attributed to a difference in the time lag between plating and irradiating which we found strongly influenced the radiation dose response.

Our results indicate that frameshift strains are more sensitive than base-pair substitution strains to the mutagenic action of ionizing radiation, but this generality does not apply to the lethality responses between the two types of strains.

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