TRANSFORMATION OF RADIATION SENSITIVE STRAINS OF *MICROCOCCUS LYSODEIKTICUS*[1]

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The isolation from strains of *Micrococcus lysodeikticus* of nucleases capable of removing thymine dimers from ultraviolet (UV) irradiated DNA (Carrier and Setlow, 1966; Takagi *et al.*, 1968; Grossman *et al.*, 1968) has prompted the isolation of UV sensitive mutants of *M. lysodeikticus*.

This paper describes the properties of three selected mitomycin sensitive mutants, which are UV^-. It also describes the transformation of these mutants with wild type DNA and the characteristics of the transformants obtained.

Materials and Methods

Strains and Media--*M. lysodeikticus* #4698 and bacteriophage B_4 were obtained from the American Type Culture Collection.

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Cells were grown in brain heart infusion (BHI) agar or broth (Difco), at 32°. In BHI, the doubling time of all strains was 60 min. For host cell reactivation (hcr) experiments, irradiated B_4 was plated with cells grown for 6 hr using the medium described by Naylor and Burgi (1956).

Isolation of Mutants--Approximately 5 X 10^8 cells were spread on BHI plates and irradiated with a UV dose of 1 X 10^5 ergs/mm^2 or treated with 100 µg/ml of N-methyl N'-nitro N-nitrosoguanidine (NTG) (Adelberget et al., 1965). The isolation of UV^- mutants of M. lysodeikticus following NTG treatment has been reported recently by Feiner (1967). Cells sensitive to 0.05 µg/ml of mitomycin-C (M^s) were selected by replica plating (Okubo and Romig, 1966) and examined for radiation sensitivity. Spontaneous erythromycin resistant (E^r) mutants were isolated from the wild type strain and from one UV^- mutant by plating approximately 1 X 10^9 cells on BHI plates containing 1 µg/ml of the antibiotic.

Transformation--For M^r transformation, 16-hr broth cultures of UV^- cells (viable count 1 X 10^8 cells/ml) were diluted tenfold into fresh broth, and incubated with shaking in the presence of 30 µg/ml of wild type DNA isolated according to the method of Marmur (1961). After $3\frac{1}{2}$ hr, the cells were plated on agar containing 0.05 µg/ml of mitomycin C (Kyowa Hakko Kogyo Co., Ltd. Japan) and incubated at 32° for 3 to 5 days. For transformation to E^r resistance, recipient cells were incubated in the presence of transforming DNA for 90 min, spread on plates containing 20 ml of agar and incubated for 6 hr. The plates were then overlaid with 20 ml of agar containing 2 µg/ml of erythromycin (Lilly Co.).

Radiation--A 15-watt germicidal lamp was used as a source of UV. The output, 42 cm from the source, was 12 ergs/

mm^2. For X-irradiation a 100 kv Phillips source, fitted with a 0.78 mm aluminum filter was used.

Results

Characterization of UV^- Mutants--M^S mutants obtained after NTG or UV treatment were checked for sensitivity to UV and X radiation, hcr, and sensitivity to 0.05 M methylmethane sulfonate (MMS) (Searashi and Strauss, 1965). The properties of three UV^- mutants compared to the UV^+ wild type are shown in Table I. Of the mutants listed in Table I, #8 is typical

Table I. Characteristics of M. lysodeikticus Wild Type and UV Sensitive Mutants

	Strains			
Agent	Wild Type	#8	#6	#25
	% Survival			
UV - 7 X 10^2 ergs/mm^2	54	1.4	0.2	0.15
X ray - 6.2 X 10^3 rads	23	1.2	20	6
MMS - 0.05 M (30 min)	3.8	0.04	3.5	3.1
UV irradiated B_4	hcr+	hcr+	hcr^-	hcr^-
Mitomycin - 0.05 µg/ml for spontaneous revertants		+	+	-

of a large class that is sensitive to both UV and X radiation, sensitive to MMS and hcr^+. Mutant #6, which is X ray^+, MMS^+ but hcr^-, represents a class found less frequently. One other mutant, #7, resembling #25 has been found in a total of 48 UV^- mutants examined. These two strains, #7 and #25, have the interesting property of being X ray^- but MMS^+. No spontaneous M^r, UV^+ revertant could be isolated from either #7 or #25.

Transformation of UV^- Mutants--Transformation of the three UV^- mutants succeeded with cells that had grown in broth for 14-18 hr (viable count 1-3 X 10^8 cells/ml). A ten- to twentyfold dilution of the cells into fresh broth at this point increased the frequency of transformation. A similar finding has been reported by Kloos (1968) working with adenine transformation of M. lysodeikticus. Studies of DNA uptake with P^{32} labeled DNA (specific activity 3.5 X 10^4 c/min/mμmole) showed very little DNA uptake by cells grown for less than 12 hr. Maximal DNA fixation occurred with cells grown for 16 to 24 hr. The mean number of M^r transformants obtained from the three UV^- mutants (per 10^7 recipient cells) is shown in Table II. The frequency of erythromycin transformants from these strains was tenfold lower.

Characterization of Transformants--Fifteen M^r transformants of strains 8, 6 and 25 were examined for UV and X ray sensitivity, and sensitivity to MMS. All the transformants had achieved resistance to these agents comparable to that of

Table II. Transformation of M^s, E^s, UV^- Mutants of M. lysodeikticus

Donor DNA	Marker	Transformants from recipient strain No. 8	No. 6	No. 25
Wild type	M^r	9 X 10^3	8 X 10^2	6 X 10^3
" " and 10 μg/ml DNase	M^r	-	-	-
UV^+, M^r, E^r	E^r	5 X 10^2	70	3.2 X 10^2
25	E^r	6 X 10^2	90	2.6 X 10^2
Wild type	E^s	-	-	-

the wild type strain. The transformants of #6 and #25 had also become hcr+. Ten E^r transformants of strains 8, 6 and 25 were also checked for UV resistance. The E^r transformants obtained from these three strains were still UV^- and M^s.

In order to confirm the absence of linkage between the associated M^s, UV^- marker and the E marker, mutant #6 was transformed with wild type E^r DNA. Plates containing 50-400 M^r transformants were transferred to E plates by replica plating. Only six colonies out of 2500 tested (0.24%) showed simultaneous resistance to the two antibiotics.

Discussion

The finding that strain #4968 of M. lysodeikticus is capable of adsorbing and integrating purified DNA makes possible the genetic mapping of another organism of biochemical interest.

It has been possible to transform M^s, UV^- mutants with wild type DNA; resistance to 0.05 g/ml of mitomycin was used as a selective marker. The M^r transformants examined appear to have acquired the radiation resistance and all associated properties of the wild type strain.

The high degree of correlation between mitomycin and UV sensitivity in M. lysodeikticus, already observed in Escherichia coli (Terawaki and Greenberg, 1966) and Bacillus subtilis (Okubo and Romig, 1966; Mahler, 1966) points to a requirement for dark repair enzymes in the repair of this DNA cross-linking agent. It also provides a simple selective tool for the isolation and transformation of UV^- strains of M. lysodeikticus.

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