EVIDENCE OF TRANSFORMATION IN MICROCOCCUS LYSODEIKTICUS

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Micrococcus lysodeikticus has been used in the studies of dark repair, the repair of ultraviolet(UV)-irradiated deoxyribonucleic acid (DNA) without visible light in vivo, as an endonuclease specific for UV-irradiated DNA was found in the extract of this organism (Rörsh et al., 1964; Strauss et al., 1962 and 1967; Carrier and Setlow, 1967; Grossman et al., 1967; Moriguchi and Suzuki, 1967; Nakayama et al., 1967; Shimada et al., 1967; Takagi et al., 1967). In the course of our own studies, we have been hampered by the lack of a suitable system for genetic analysis in M. lysodeikticus. In this paper, we present evidence for the occurrence of a DNA-mediated transformation in one strain of M. lysodeikticus.

Materials and Methods

Bacterial strains. Two strains of M. lysodeikticus were used. One strain (S) was obtained from Dr. B. Strauss, the University of Chicago, the other strain (G), #4698 of ATCC, from Dr. L. Grossman, Brandeis University. UV-sensitive mutants from the strain S were selected after treatment of bacteria with N'-methyl-N-nitro-N'-nitrosoguanidine (Okubo et al., 1967). They were designated S1, S2, etc. Three UV-sensitive mutants derived from the strain G were supplied by Dr. Grossman, and in this paper capital "G"

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was added to his mutant number as G4, G7 and G8. A spontaneous streptomycin-resistant mutant (SM^r) was isolated from G7 by spreading 0.1 ml of a G7 culture onto nutrient agar containing 1 mg/ml of streptomycin.

Medium. Nutrient broth contained (per liter of distilled water) 10g of polypeptone, 5g of beef extract (Kyokuto), 1g of yeast extract (Difco) and 2g of NaCl; it was adjusted to pH 7.2 with NaOH. $MgSO_4$ and $MnCl_2$ were usually supplemented at a concentration of 5×10^{-3}M and 5×10^{-5}M, respectively.

Preparation of recipient cells for transformation. Bacteria were grown with shaking in nutrient broth overnight at 37°. The culture was diluted 10 fold in fresh nutrient broth and shaken for 4 hrs at 37°. At this time, the bacteria have reached the stationary phase.

Preparation of DNA. Bacterial DNA was prepared by Marmur's procedure (Marmur, 1961).

UV irradiation. The cell suspension in 0.1 M Tris-HCl buffer, pH 7.2, was exposed in a petri dish to UV at a distance of 50 cm from a 15 watt germicidal lamp (Toshiba). The dose rate was about 10 ergs/mm^2 per sec.

Results and Discussion

UV-sensitive mutants of *E. coli* and *B. subtilis* were found to be sensitive to mitomycin C (MC) (Boyce and Howard-Flanders, 1964; Okubo and Romig, 1965). We have confirmed this correlation in *M. lysodeikticus*. Fig. 1 shows the UV sensitivity of wild type strain S and of mutants derived from both strains S and G. In Fig. 2 and Table 1, those UV-sensitive mutants were also shown to be sensitive to MC. UV-sensitive mutants did not form colonies on nutrient agar containing 0.1 μg/ml of MC, in contrast to wild type. We chose this character as a selected marker in transformation experiments.

Demonstration of transformation. Cultures of mutant G7 grown in nutrient broth were incubated with DNA of wild type strain S for 4 hrs and then plated

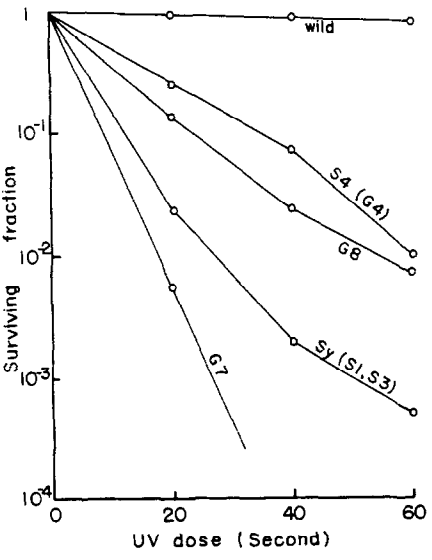

Fig. 1. Ultraviolet survival curves of various strains of M. lysodeikticus. Each strain was grown in nutrient broth and then diluted 100 fold with 0.1 M Tris-HCl buffer. The diluted sample was irradiated in a petri dish and at intervals, samples were removed and assayed for survival. Strains in parenthesis have the almost same UV sensitivity as that of the strain shown in the figure.

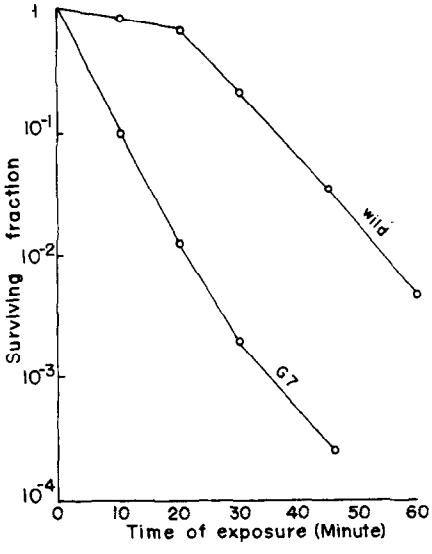

Fig. 2. Survival of cells after exposure to 0.5 μg/ml of mitomycin C. Bacteria grown in nutrient broth were harvested in logarithmic phase and resuspended in the same volume of nutrient broth containing 0.5 μg/ml of mitomycin C. At intervals, samples were removed, diluted at least 100 fold, and assayed for survival.

on nutrient agar containing 0.1 μg/ml of MC. MC-resistant colonies appeared on the plates after three day incubation at 37° (Table 2). The transformation to MC-resistance was completely prevented by treatment of the donor DNA with 1 μg/ml of pancreatic DNase in the presence of Mg-ion. As the re-

Table 1. Sensitivity to mitomycin C of the various strains

Mitomycin C concentration (μg/ml)	Wild type (s)	UV-sensitive mutants S1	S2	Sy	G4	G7	G8
0.005	+	+	+	+	+	+	+
0.01	+	+	+	+	+	+	+
0.025	+	+	+	+	+	+	+
0.05	+	-	-	-	-	-	-
0.075	+	-	-	-	-	-	-
0.1	+	-	-	-	-	-	-
0.2	+	-	-	-	-	-	-
0.3	-	-	-	-	-	-	-

Each bacterial culture was streaked onto nutrient agar containing MC, and the plates were incubated for 48 hrs. The + sign means that bacteria could grow on nutrient agar containing the indicated concentration of MC.

Table 2. Transformation of G7 to mitomycin-resistance

Incubation with	Numbers of colonies on nutrient agar containing 0.1 μg/ml of mitomycin C	Transformation frequency
wild type DNA	1270	10^{-5}
wild type DNA + DNase	0	
G7 DNA	0	

0.9 ml of G7 culture grown in nutrient broth as described in Materials and Methods was incubated with 0.1 ml of 100 μg/ml of DNA or DNA pretreated with DNase for 4 hrs and then 0.1 ml of the mixture were placed on the selective plates. Colonies were counted after 3 day incubation at 37°.

sidual growth of MC-sensitive cells was observed on nutrient agar containing 0.1 μg/ml of MC, the phenotypic expression to MC-resistance could occur even after plating.

In order to be sure that the appearance of colonies was not due to wild type contaminants in the preparation of wild type DNA, a streptomycin-resis-

tant G7 was used as a recipient. Transformants obtained were all streptomycin-resistant.

Colonies from the selective plates were purified by repeated single colony isolation and the purified clones were tested with UV. All clones tested (13 clones) were as UV-resistant as the wild type.

Transformation of G7 to streptomycin-resistance by DNA extracted from a SM^r mutant was attained at a very low frequency of 10^{-7}. Competence, an ability of bacteria to incorporate DNA molecules, appears only under narrow cultural condition in the transformation systems so far reported (Schaeffer, 1964). The growth condition used here seems not adequate for the development of maximum competence. Efforts to get good competence have been made, but up to date a definite procedure has not been established.

Table 3. Transformability of various UV-sensitive mutants of M.lysodeikticus.

Incubation mixture	Numbers of colonies on nutrient agar containing 0.1 µg/ml of mitomycin C
G7	0
G7 + DNA	702
G8	12
G8 + DNA	353
G4	1
G4 + DNA	1241
S1	0
S1 + DNA	0
S3	5
S3 + DNA	3
Sy	0
Sy + DNA	0

The procedure was the same as that described in the legend of Table 2.

The data presented above seem to satisfy the criteria required for demonstration of transformation in M. lysodeikticus. As shown in Table 3, however, transformation to MC-resistance occurred only in the strain G. Demonstration of transformation in mutants derived from the strain S has never been successful so far. The fact that not all strains of a species can be transformed has been found in almost all transformation systems so far reported, and little is known about the differences between a transformable strain and a non-transformable strain in the same species (Schaeffer, 1964). Recently, we heard that several investigators had also succeeded in

transforming M. lysodeikticus and M. radiodurans (Mahler, personal communication).

Summary

Transformation was found to occur at low frequencies in one strain of M. lysodeikticus, #4698 of ATCC. Demonstration of transformation with the other strain of the same species was unsuccessful. At present a definite procedure yielding good competence has not been established.

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