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Mutants of *Staphylococcus aureus* with Increased Sensitivity to Ultraviolet Radiation

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Nitrosoguanidine (NG) mutagenesis of *Staphylococcus aureus* resulted in the isolation of eight mutants exhibiting 3 to 28 times greater sensitivity to ultraviolet (UV) radiation. These mutants were further characterized by their ability to repair UV-irradiated bacteriophage, to act as recipients in the transduction of antibiotic resistance, and their sensitivity to NG. Based on the available data, six of these mutants are reduced in their ability to perform host-cell reactivation. One of the remaining two mutants may be deficient in post-replication repair.

Knowledge of cellular processes for the dark repair of deoxyribonucleic acid (DNA) has come primarily from studies utilizing mutants of gram-negative bacteria which are radiation sensitive as a result of specific DNA repair deficiencies. These studies have involved the characterization of two general mechanisms for DNA dark repair, namely excision repair and post-replication or recombinational repair; each of these is accomplished by a number of enzymatic steps (for reviews, see 4, 19, 22). Radiobiological techniques in *Staphylococcus aureus* research have centered on the use of ultraviolet (UV) radiation in metabolic studies (2) and the characterization of bacteriophage (6, 12-14). However, up to this time no information has been available on the capability of *S. aureus* to perform DNA dark repair.

The objective of the present study was to characterize the DNA dark repair mechanisms operative in *S. aureus*.

MATERIALS AND METHODS

Bacterial strains. The mutants described in this paper were derived from *S. aureus* strain 112 (11). *S. aureus* strain U9 (*pase*, *tet*, *ol*, *nov*) [10] which served as the donor for transduction experiments produced penicillinase (*pase* marker) and was resistant to novobiocin (*nov* marker), oleandomycin (*ol* marker), and tetracycline (*tet* marker). All cultures were maintained at 4 C on Brain Heart Infusion (BHI; Difco) agar slants.

Bacteriophage and transductions. Phage 80 of the International Typing Series, maintained, propagated, and titrated as described previously (10), was used for transduction and phage UV-inactivation experiments. Transductions were performed by the method of Kloos and Pattee (7) with the addition of 10^{-3} M $CaCl_2$ to the transduction suspensions. Selective media for the antibiotic-resistance markers have been described (10, 11).

Isolation of UV-sensitive mutants. An 18-hr BHI agar slant culture of *S. aureus* strain 112 was harvested in 5 ml of citrate solution (0.85% trisodium citrate, *p*H 5.0) and resuspended in citrate solution containing 500 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml (NG; K and K Laboratories, Plainview, N.Y.). After incubation at 37 C for 1 hr, the cells were resuspended in 5 ml of BHI. A 1-ml sample of this suspension was added to 75 ml of BHI contained in a 250-ml nephelometer flask which was incubated with shaking at 37 C until an approximate doubling in optical density occurred (final OD_{540} = 0.6). The cell suspension was then diluted and plated on BHI agar to yield about 70 colonies per plate. Screening for UV-sensitivity was performed as outlined by Setlow et al. (17) with the following modifications: UV doses of 210 and 390 ergs/mm^2 were used for both primary and secondary screening. Isolates producing no growth at the high UV dose and no growth, or extremely poor growth, at the low UV dose were selected for further study.

UV irradiation of bacteria and bacteriophage. Cultures to be irradiated were incubated with shaking in a 250-ml nephelometer flask containing 75 ml of BHI. Exponentially growing cells (OD_{540} = 0.5) were harvested by centrifugation, washed three times, and resuspended in 10 ml of ice cold saline (0.5% NaCl). In order to disrupt cellular aggregates which prevail in broth cultures of *S. aureus*, the cell suspension was held in an ice bath and sonically treated at 50% output for 1 min (needle probe, Biosonik II ultrasonic generator, Bronwill Scientific Co., Rochester, N.Y.); primarily unicellular populations were so obtained. The suspension was diluted in cold saline to a titer of ca. 10^7 cells/ml as determined by a Petroff-Hauser counting chamber and phase contrast microscopy. A 30-ml volume of the cell suspension was transferred to the bottom half of a glass petri dish (100-mm diameter) and agitated continuously during irradiation. At intervals, samples were withdrawn, diluted, and plated. All irradiations of cells and bacteriophage and subsequent assays of their viability were performed under subdued illumination to avoid photoreactivation. The UV source was a Champion G15T8, 15 watt germicidal lamp; the dose rate was

measured by a Blak-Ray UV meter equipped with a J-225 short wavelength sensor (Ultra-Violet Products, Inc., San Gabriel, Calif.). Bacteriophage lysates to be irradiated were diluted in a 30-ml volume of suspending medium (20) to a final titer of ca. 10^{10} plaque-forming units/ml.

Determination of NG sensitivity. Cells to be tested for NG sensitivity were prepared as for irradiation except that, after sonic treatment, the titer was adjusted to ca. 10^8 cells/ml in citrate solution, *p*H 5.0, containing 100 μg of NG/ml. This suspension was incubated at 37 C and samples withdrawn and plated for viable cell counts at 15-min intervals.

RESULTS

UV irradiation of cells. Of 32 isolates selected after NG mutagenesis, only eight exhibited consistent UV sensitivity through primary and secondary screening while maintaining growth rates that did not differ significantly from strain 112. Figure 1 presents the inactivation curves obtained when strain 112 and these UV-sensitive (UVS) mutants were exposed to UV irradiation at a dose rate of 26 ergs per mm^2 per sec. Each curve represents the average of at least two separate experiments. Strain 112 characteristically exhibited a broad-shouldered inactivation curve and a D_{37} value of 56 ergs/mm^2, as calculated from the exponential slope. At this dose rate, however, the UVS mutants were inactivated too rapidly to be differentiated (Fig. 1). This problem was resolved by increasing the distance from the UV source to the cell sample, thereby reducing the dose rate to 4 ergs per mm^2 per sec. At this reduced level of irradiation (Fig. 2) all of the mutants exhibited multiple-hit survival curves with the exception of UVS-1; this mutant is clearly the most sensitive to UV radiation and is inactivated exponentially. The D_{37} values for the UVS mutants, calculated from the exponential slopes of the curves, range from 2 ergs/mm^2 for UVS-1 to 19 ergs/mm^2 for UVS-8 and UVS-9.

UV irradiation of phage 80. The ability of the mutants to repair UV-irradiated phage 80 was examined by determining viable phage titers after irradiation of the phage at a dose rate of 26 ergs per mm^2 per sec. These results (Fig. 3) represent the average of at least two separate experiments for each strain and reveal similar survival curves for irradiated phage plated on strains 112, UVS-1 and UVS-8. In contrast, the remaining six mutants were unable to support efficient plaque formation unless the phage were exposed only to a comparatively low UV dose. Calculations from the slopes of the curves indicate at least an 8 times difference between strain 112 and mutant UVS-4 in ability to repair irradiated phage. Plating efficiencies of unirradiated phage did not

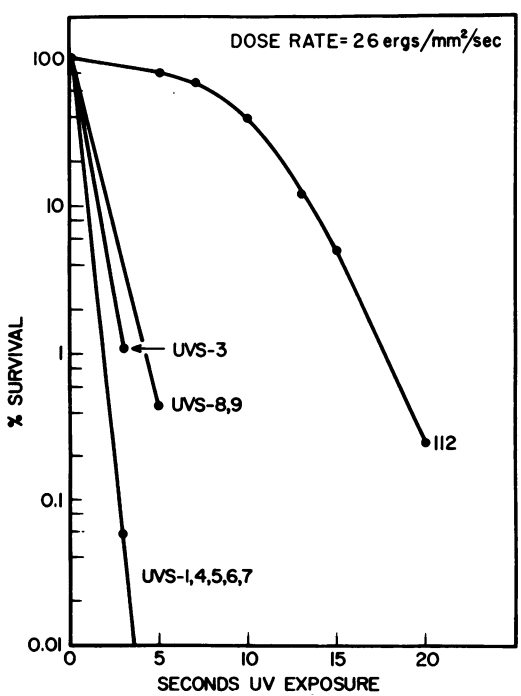

FIG. 1. *The inactivation of S. aureus strain 112 and eight ultraviolet-sensitive (UVS) mutants by UV radiation at a dose rate of 26 ergs per mm² per sec.*

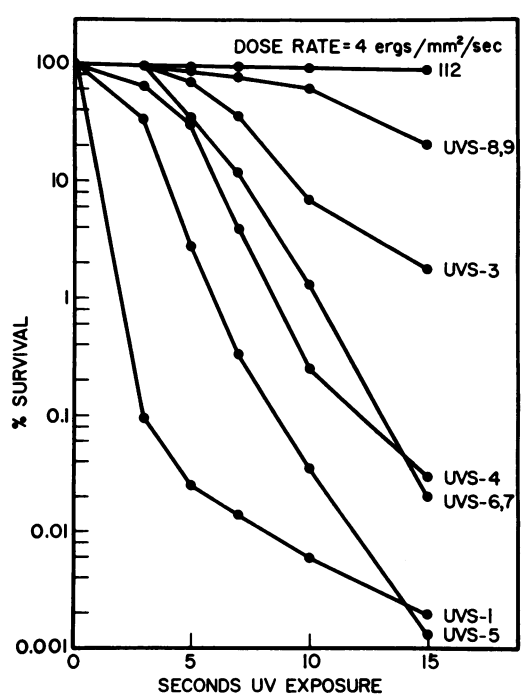

FIG. 2. *The inactivation of S. aureus strain 112 and eight ultraviolet-sensitive (UVS) mutants by UV radiation at a dose rate of 4 ergs per mm² per sec.*

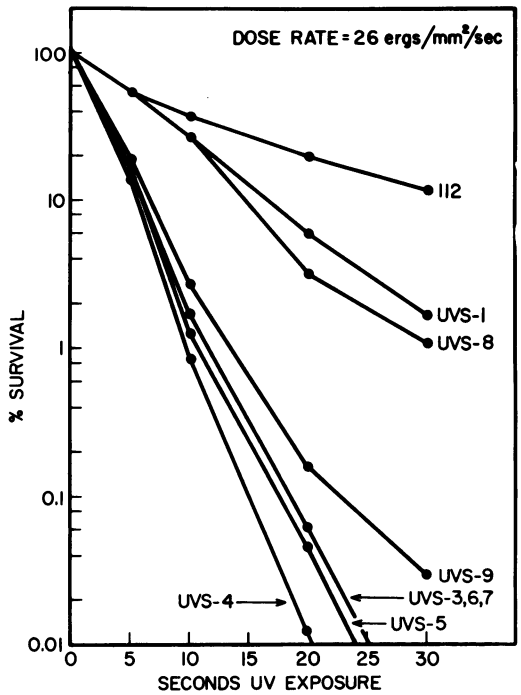

FIG. 3. *The survival of UV-irradiated phage 80 when plated on S. aureus strain 112 and eight ultraviolet-sensitive (UVS) mutants.*

vary significantly among the mutants.

Transduction of antibiotic resistance. To obtain some measurement of recombinational ability in the UVS mutants, transduction frequencies were determined for the *nov*, *ol*, and *tet* markers using phage 80 propagated on *S. aureus* U9 (*pase*, *tet*, *ol*, *nov*) [10]. These results are summarized in Table 1; each value is the average of at least two separate experiments. Whereas a few strains exhibited reduced transduction frequencies for some markers, the most interesting observation is the consistent inability of UVS-1 to serve as a recipient of the *nov* marker. In addition, UVS-1 also exhibited reduced transduction frequencies for the *ol* and *tet* markers, thus establishing it as the poorest recipient in this transduction system.

Inactivation of cells by NG. Figure 4 presents the inactivation curves obtained when exponentially growing cells of *S. aureus* strain 112 and the UVS mutants were incubated in the presence of 100 μg of NG/ml in citrate solution (*p*H 5). Of all the mutants, only UVS-1 exhibited marked inactivation as evidenced by a 10^4-fold drop in viable cells after incubation with NG for 1 hr. All of the other mutants survived this treatment in a manner comparable to strain 112, sustaining about a 50% decrease in viable cells over the same time period. An unexpected result was obtained when control experiments were performed to determine whether citrate solution (*p*H 5), by itself, had any effect on cellular viability. It was observed that strains which were resistant to NG inactivation suffered no decrease in viable cells after incubation in citrate solution (*p*H 5). Conversely, UVS-1 was inactivated by about 50% under these same conditions. Comparatively speaking, UVS-1 is as susceptible to inactivation

TABLE 1. *Transduction frequencies of the nov, tet, and ol markers in Staphylococcus aureus strain 112 and eight ultraviolet-sensitive (UVS) mutants using phage 80/U9 (pase, tet, ol, nov)*

Recipient	Frequency of transduction[a]		
	nov	*tet*	*ol*
112	8780 (100)	18650 (100)	5710 (100)
UVS-1	0 (0)	6990 (37	626 (11)
UVS-3	7330 (84	27850 (149)	6260 (110)
UVS-4	3560 (41)	3380 (18)	1605 (28)
UVS-5	9160 (105)	7225 (39)	6452 (113)
UVS-6	11890 (137)	20950 (112)	6300 (110)
UVS-7	10290 (118)	37400 (201)	4560 (80)
UVS-8	1340 (15)	22500 (121)	15410 (270)
UVS-9	2860 (33)	12000 (64)	3620 (63)

[a] Expressed as number of transductants per 10^9 plaque-forming units. Numbers in parentheses are relative transduction frequencies calculated using the frequency of 112 as 100%.

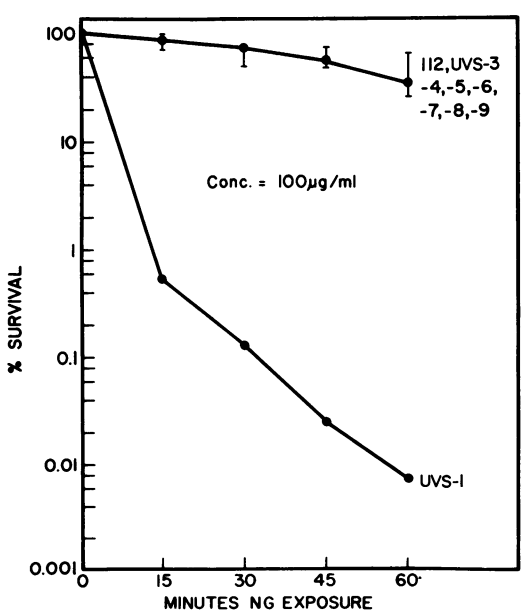

FIG. 4. *The inactivation of S. aureus strain 112 and eight UV-sensitive mutants by 100 μg of nitrosoguanidine (NG)/ml of 0.85% trisodium citrate solution (pH 5).*

by mild acidic treatment as are the remaining mutants when incubated in citrate solution (*p*H 5) containing 100 μg of NG.

DISCUSSION

The mutants which were isolated during this study exhibited UV sensitivities ranging from 2.8 to 28 times that of strain 112. Criteria used to further characterize these mutants included: (i) ability to repair irradiated bacteriophage, (ii) participation in the transduction of antibiotic resistance, and (iii) sensitivity to NG. A comparative summary of these results is presented in Table 2.

When irradiated phage 80 was plated against most of the UVS mutants, the efficiency of plating was significantly lower than that obtained in strain 112. This observation strongly suggests a decreased capability of these mutants to repair irradiated phage DNA. Mutants UVS-1 and UVS-8, however, were only slightly reduced in their repair of irradiated phage. These two mutants and strain 112 (Fig. 3) exhibit phage inactivation curves similar to those reported for strains of other species capable of host-cell reactivation (HCR; reference 15), and on this basis we consider them to be HCR$^+$. The remaining mutants are clearly less effective in their capability for phage repair and are probably HCR$^-$ (Table 2). Although questions remain concerning some aspects of DNA dark repair processes, current evidence points to excision repair as the fundamental mechanism of host-cell reactivation (15). This suggests that mutants UVS-1 and UVS-8 may possess capabilities for excision repair comparable to strain 112; therefore, the UV sensitivities of these mutants may be due to deficiencies in some other aspect of DNA dark repair (e.g., post-replication repair). Conversely, the UV sensitivities of the HCR$^-$ mutants may be a consequence of a decreased capacity for excision repair. The ability of these mutants to perform excision repair can best be confirmed by radioisotope studies.

TABLE 2. *A comparative summary of four characteristics in Staphylococcus aureus strain 112 and eight ultraviolet-sensitive (UVS) mutants*

Strain	Relative UV[a] sensitivity	HCR[b]	NG[c] sensitivity	Average[d] relative transduction frequency
112	1.0X	+	–	100%
UVS-1	28.0	+	+	16
UVS-3	9.6	–	–	114
UVS-4	11.0	–	–	29
UVS-5	14.0	–	–	86
UVS-6	10.0	–	–	120
UVS-7	10.0	–	–	135
UVS-8	2.8	+	–	135
UVS-9	2.8	–	–	53

[a] D_{37} of strain 112 divided by D_{37} of each mutant, where D_{37} is the dose in ergs/mm^2 needed to reduce survival on the exponential part of the curve (Fig. 1 and 2) to 37% (*see* reference 18).

[b] +, Host-cell reactivation equivalent to that of strain 112; –, host-cell reactivation significantly lower than strain 112. Data from Fig. 3.

[c] +, Nitrosoguanidine (NG) sensitivity significantly greater than strain 112; –, NG sensitivity equivalent to that of strain 112. Data from Fig. 4.

[d] Data taken from Table 1.

Whereas host-cell reactivation may provide insight into the capabilities of bacterial strains for excision repair, studies on recombinational or post-replication repair (4, 16) require, by definition, a system based on the exchange or recombination of DNA. Since transduction is the only known mechanism of genetic exchange in *S. aureus*, the ability of the mutants to act as recipients of antibiotic resistance was examined. In general, the data obtained do not clearly differentiate the mutants; however, UVS-1 not only failed completely to accept the *nov* marker, but also exhibited clearly reduced transduction frequencies for the *tet* and *ol* markers. The lowered transduction frequencies obtained with UVS-1 were not accompanied by a change in phage sensitivity. UVS-1 is clearly the most UV sensitive of all the mutants, although retaining its ability to repair irradiated phage 80 (Table 2). The question arises, therefore, whether the reduced transduction frequencies of UVS-1 might be related to a deficiency in post-replication repair, a relationship observed in recombination deficient (*rec*$^-$) mutants of other bacterial species (1, 3). If recombination-deficient mutants of *S. aureus* also exhibit impaired activities as recipients during transduction, the presence of multiple repair deficiencies may exist in those UVS mutants which are HCR$^-$ and exhibit reduced transduction frequencies. This situation may pertain to mutants UVS-4 and UVS-9. The question of multiple mutations must be taken into consideration after any mutagenesis procedure, however, and is particularly important when NG is used.

Recent evidence indicates that a loss of excision repair capability does not significantly increase bacterial sensitivity to NG (5, 8, 22). Conversely, mutants impaired in post-replication repair do exhibit increased inactivation by NG (5, 21, 22). This is more easily understood in light of recent work by Olson and Baird (9) which indicates that, in addition to its alkylating activity, NG produces single-strand breaks in DNA. Of all the mutants, only UVS-1 is more sensitive to NG than strain 112 (Fig. 4), again lending sup-

port to the suggestion that the UV sensitivity of this mutant may reflect a deficiency in post-replication repair. Some support for this proposal is also found in the marked resemblance between the UV inactivation curves of strain 112 and UVS-1 compared to those of *Escherichia coli* K-12 and strain AB2463 *rec-13* (5).

In summary, mutants UVS-3, 4, 5, 6, 7, and 9 exhibit a decreased ability for host-cell reactivation although differing from one another in terms of either their UV sensitivities or their transduction frequencies, or both. Of the remaining two mutants, UVS-1 may be deficient in post-replication repair, whereas UVS-8 is somewhat of an enigma since its UV sensitivity is not accompanied by decreased host-cell reactivation or by significantly reduced transduction frequencies.

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