PHOTOREACTIVATION OF ULTRAVIOLET-IRRADIATION DAMAGE IN *STAPHYLOCOCCUS AUREUS*

BERNARD ADKINS, JR. AND WENDALL E. ALLEN*

*Inhalation Toxicology Research Program, Northrop Services, Inc.,
Research Triangle Park, North Carolina 27709*

**Department of Biology, East Carolina University,
Greenville, North Carolina 27834*

(Received September 10, 1981)

This study reports the capacity of *Staphylococcus aureus* strain 7–8 to undergo photoenzymatic repair of UV-irradiation induced damage and compares it to the photoreactivation (PR) response of *Escherichia coli* strain B. *Staphylococcus aureus* showed greater inhibition by UV irradiation than *E. coli*, consistent with its higher adenine and thymine content of DNA. *Staphylococcus aureus* showed an enhanced rate of photoreactivation with no lag in initiation of the PR response at low PR doses compared to *E. coli*. Maximum PR capacity of both cultures was about equal and occurred in cultures incubated at 23–25°. The PR responses at 11–12 and 35–37° for *S. aureus* and *E. coli* differed although both were capable of PR at each of these temperatures. The PR response of *E. coli* was directly related to the dosage of PR light (J/m²); however, the photoenzymatic capacity of *S. aureus* was not directly responsive to continued decrease in light intensity. The capacity of *S. aureus* to undergo liquid holding recovery (LHR) occurred at 23–25° (not at 11–12° or 35–37°), whereas *E. coli* underwent LHR at 11–12° and 23–25° but not at 35–37°. The LHR response of *S. aureus* was somewhat more effective than *E. coli* and did not show the direct response to increased liquid-holding period as did *E. coli*.

Most of the knowledge of cellular repair processes to date has resulted from studies of ultraviolet-sensitive mutants of gram-negative bacteria. The characterization of some of these processes, including: deoxyribonucleic acid (DNA) dark repair (or liquid holding recovery), excision repair; recombinational repair, post-replication repair; and photoreactivation; has involved enzymatic studies (1–3). Ultraviolet (UV)-irradiation research on *Staphylococcus aureus* has included metabolic studies (4), mutagenesis studies (5), transduction studies (6),

excision repair studies (7), and recombinational repair studies (8, 9). IKENAGA and co-workers (10) described the photoreactivation repair of UV-irradiation damage in *Staphylococcus epidermidis*. However, to date, no information is available on the capacity of *S. aureus* to undergo photoreactivation of UV-induced damage.

This study was performed to define the parameters of the photoreactivation response of *S. aureus* to UV-induced damage and to compare any response of *S. aureus* to the established photoreactivation capacity of *E. coli*. The capacity of *S. aureus* and *E. coli* to repair UV-induced damage would be expected to have some relation to their situation in nature and may have evolutionary implications.

MATERIALS AND METHODS

Cultures. The culture of *Staphylococcus aureus* strain 7-8 used throughout this investigation was isolated from the infected teat of a cow with mastitis. The culture of *Escherichia coli* strain B (ATCC 11303) was purchased from American Type Culture Collection, Rockville, MD. The cultures were maintained at 4° on Trypticase Soy Agar (TSA), Baltimore Biological Laboratories (BBL), Baltimore, MD, containing 0.5% Yeast Extract (Difco).

Ultraviolet-irradiation technique. The cultures of *S. aureus* and *E. coli* were inoculated separately into 10 ml of BBL Trypticase Soy Broth (TSB) containing 0.5% Yeast Extract and were incubated at 37° for 18–24 hr. The cells were collected by centrifugation at $1,500 \times g$ for 10 min, washed twice with 10-ml portions of sterile phosphate buffer (0.01 M, pH 7.0) to remove nutrients and resuspended in 10 ml of the same buffer. Samples from each cell suspension were removed prior to all irradiations and the viable cell titers/ml were determined. Samples were diluted with 0.85% sterile saline to yield approximately 30–300 colony-forming units/ml and pour plates were prepared by adding 20 ml of melted and colled TSA, fortified with 0.5% Yeast Extract, to 1-ml amounts of appropriate dilutions in sterile petri plates. All plates, regardless of sample treatment, were incubated in the dark at 37° for 48 hr prior to colony counting. The pour plate technique was used to determine the viable cell titers for all experiments.

Suspensions of cells in buffer, containing approximately 10^9 bacteria/ml, were equally divided into two aliquots prior to exposure to UV irradiation. All irradiation procedures and subsequent incubations were performed in the dark. Each aliquot was transferred to a sterile glass petri plate. The cell suspension in one plate was irradiated at 254 nm (Sylvania Electric Products, Inc., germicidal lamp, Model G815, 8 W) at room temperature (22–25°) on an electric rotary shaker (1 cycle/sec) 12 cm below the UV lamp. The other aliquot was treated in the same manner but was not irradiated. After treatment, viable cell titers of each aliquot were determined.

The lethality of the UV-irradiation dose employed was determined by meas-

uring the viable cell titer at 10-sec intervals during irradiation.

Photoreactivation technique. Immediately after irradiation at 254 nm, the cell suspensions were divided equally into sterile test tubes (Pyrex) and placed in a constant-temperature chamber for photoreactivation (PR). This chamber consisted of a square chromatographic Pyrex jar (3-gallon, Fisher Scientific Company) enclosed on three sides by light-reflective aluminum foil. The styrofoam top (1 inch thick) contained holes to permit suspension of tubes in the PR chamber. The desired temperature was maintained by adjusting the ratio of hot to cold water flowing into the chamber from a common nozzle.

The cells to be photoreactivated were immediately exposed to white light from a quartz-iodide lamp (Sylvania Electric Products, Inc., Type BCK, 500 W) housed in a slide projector. The wavelength range emitted by this lamp was approximately 300–750 nm. The Pyrex glassware used throughout the process absorbed all wavelengths below approximately 275 nm (11). The time required for PR was varied experimentally. All extraneous light was excluded from the laboratory.

Nonphotoreactivated (NPR) samples were covered with light-impermeable aluminum foil and treated exactly as the photoreactivated sample. After the specified time of light exposure had elapsed, viable cell titers of PR and NPR suspensions were determined.

Liquid-holding recovery (LHR). A sample of the UV-irradiated cell suspension was transferred to a sterile test tube, covered with light-impermeable aluminum foil, and placed in the PR chamber. This sample was held before plating for the same length of time as the PR-exposure period. Capacity of the cells to undergo LHR was determined at three temperature ranges: 11–12°, 23–25°, and 35–37°. These LHR samples served as NPR controls for these three temperatures. Samples exposed to these temperatures were removed after a specified time and viable cell titers determined.

Effect of light intensity on PR. Light intensity was controlled by using glazed glass filters (white light) and a blue filter (peak transmittance at 450 nm, CBS Blue-450, obtained from Carolina Biological Supply Company, Burlington, NC). Samples were photoreactivated by exposure for 60 min to the following light intensities: white light, full intensity; white light, 50% full intensity; white light, 1% full intensity; blue light-filtered, full intensity. Controls consisted of samples not exposed to light (NPR). Viable cell titers of PR and NPR samples were determined.

Calculations. All light intensities (UV, white light and blue light) were determined with a radiometer (Model 65, Yellow Springs Instrument Company).

The percent PR (% PR) was determined as follows:

$$\% \text{ PR} = \frac{A - B}{C - B} \times 100 .$$

A is the average viable cell count from cell suspensions irradiated with UV

light and subsequently exposed to white or blue light for the specific time periods. *B* is the average viable cell count from cell suspensions irradiated with UV and held in the dark for the same time period as *A*. *C* is the average viable cell count from cell suspensions not irradiated with UV light and not exposed to white or blue light, but held in the dark for the same time period as *A*.

The percent LHR (% LHR) was determined as follows:

$$\% \text{ LHR} = \frac{B-D}{B} \times 100.$$

D is the average viable cell count from suspensions immediately after UV irradiation.

All % PR values were normalized to 0.1 % survival (*X*) as follows:

$$X = \frac{(0.1\%)(\% \text{ PR})}{D}.$$

All % LHR values were normalized to 0.1 % survival as above by substituting %LHR values into the equation for % PR values.

RESULTS

Figure 1 shows the effect of UV-irradiation time (seconds) on the percent

Fig. 1.

Fig. 1. Survival of *Escherichia coli* and *Staphylococcus aureus* under UV irradiation (254 nm) at 24°.

Fig. 2.

Fig. 2. Effect of length of exposure to white light at 23–25° on the PR of *Escherichia coli* and *Staphylococcus aureus*.

Table 1. Photoreactivation dosages.

Intensity and color	PR period (min)	Dosage ($\times 10^{-8}$ J/m ²)
Full, white light	5	2.76
	10	5.52
	15	8.28
	20	11.0
	30	16.6
	40	22.1
	50	27.6
	60	33.1
	90	49.7
	120	66.2
	150	82.8
	180	99.4
50% full, white light	60	16.2
1% full, white light	60	0.36
Filtered-full intensity, blue light	60	0.36

survival of *Escherichia coli* strain B and *Staphylococcus aureus* strain 7-8. The results indicated UV-irradiation dosages of 3.15×10^{-10} J/m² (45 sec) for *E. coli* and 1.75×10^{-10} J/m² (25 sec) for *S. aureus* were required for 99.9% lethality.

The results of nine separate experiments on the effect of increasing the dose of PR light at 23-25° on PR of *E. coli* and *S. aureus* are given in Fig. 2. *S. aureus* exhibited a greater rate of PR than *E. coli*. *E. coli* showed an initial drop in response to photoreactivating light compared to *S. aureus*. The PR values for both cultures increased rapidly during the first 90-min exposure period. Both samples exhibited only gradual increases in PR after 90 min. Maximum PR percentages for both cultures were not significantly different.

The effects of temperature variations on the liquid-holding recovery of *E. coli* and *S. aureus* from UV-irradiation damage are shown in Table 2. All LHR values were normalized to 0.1% survival. *E. coli* exhibited 0.01% LHR after 15 min, 0.09% LHR after 30 min and 0.13% LHR after 60 min at 23-25°. *S. aureus* exhibited 0.05% LHR after 15 and 30 min and 0.08% LHR after 60 min at 23-25°. Neither culture exhibited LHR at 35-37° after 15 min. *E. coli* exhibited 0.11% LHR at 11-12° after 15 min while *S. aureus* exhibited no LHR at this temperature.

The effects of temperature on PR are shown in Fig. 3a for *E. coli*. All PR values represent average values of five separate experiments at 11-12° and 35-37° and nine separate experiments at 23-25° and were normalized to 0.1% survival. *E. coli* exhibited an initial decrease in PR that persisted for about 15 min for all three temperature ranges. The maximum PR exhibited by *E. coli* at 23-25° was 5 times greater than at 35-37° and 25 times greater than at 11-12°. The rate of

Table 2. Liquid-holding recovery (LHR) of *Escherichia coli* and *Staphylococcus aureus*.

Temperature range (°)	LHR period (min)	% survivors	% LHR	
			<i>E. coli</i>	<i>S. aureus</i>
11-12	15	0.1	0.11	0
23-25	15	0.1	0.01	0.25
	30	0.1	0.09	0.05
	60	0.1	0.13	0.08
35-37	15	0.1	0	0

Fig. 3a.

Fig. 3a. Effect of temperature on PR of *Escherichia coli*.

Fig. 3b.

Fig. 3b. Effect of temperature on PR of *Staphylococcus aureus*.

PR was greatest at 23-25° and least at 11-12°.

Figure 3b shows the effects of temperature variations on PR of *S. aureus*. All PR values represent average values of five separate experiments at 11-12° and 35-37° and nine separate experiments at 23-25° and were normalized to 0.1% survival. The maximum values exhibited by *S. aureus* at 23-25° were 3 times greater than at 11-12° and 6 times greater than at 35-37°. The rate of PR was greatest at 23-25°. There was no significant difference in the PR rates at the other temperatures.

The PR values for both *E. coli* (Fig. 3a) and *S. aureus* (Fig. 3b) were highest at 23-25°. *E. coli* exhibited an initial lag in PR at all temperatures, but *S. aureus* did not. *E. coli* exhibited approximately the same PR at 35-37° as *S. aureus* at

Table 3. Effect of light intensity on PR of *Escherichia coli* and *Staphylococcus aureus* at 23–25°.

Dosage (Intensity and color) (J/m ²)	% survivors	% PR (60 min)	
		<i>E. coli</i>	<i>S. aureus</i>
3.31×10^{-7} (Full intensity, white light)	0.1	4.37	15.47
1.62×10^{-7} (50% full, white light)	0.1	0.22	0.75
3.60×10^{-9} (1% full, white light)	0.1	0.10	1.00
3.60×10^{-9} (Filtered-full intensity, blue light)	0.1	0.10	0

this temperature. *S. aureus* exhibited 8 times the PR at 11–12° than *E. coli* at this temperature. The lowest PR values for *E. coli* were observed at 11–12° and for *S. aureus* at 35–37°.

The effects of varying light intensity on PR at 23–25° are given in Table 3. The intensities and dosages are for white and blue light. All PR values were normalized to 0.1% survival. The PR values for *E. coli* decreased with the decreases in dosage. The percent PR values for *S. aureus* decreased with the decreases in dosage, but with no significant decrease between 50% full intensity and 1% full intensity (white light). There was a 20-fold decrease in the % PR for both *E. coli* and *S. aureus* upon exposure to one-half the light intensity. The PR values for *S. aureus* were 4–10 times greater than those for *E. coli* for all light intensities (white light) except for blue light. *S. aureus* did not exhibit PR with blue light.

DISCUSSION

GATES (12, 13) has reported the action spectra for UV inactivation in bacteria. JAGGER (14) reported that bacteria were generally susceptible to inactivation by low doses (10^1 to 10^4 erg/mm²) of UV-irradiation. All UV dosages employed in the experiments were within this range. The UV dosage required for 99.9% lethality of *E. coli* strain B represented 1.8 times the dosage for the same lethality of *S. aureus* strain 7–8 (Fig. 1).

Variation existed in the responses of different cultures of *Staphylococcus* to UV-irradiation doses. UV irradiation of *S. aureus* strain 7–8 with 1.75×10^{-10} J/m² was 99.9% lethal. GOERING and PATTEE (7) reported UV irradiation with one-third of this dosage was 99.5% lethal to *S. aureus* strain 112. IKENAGA et al. (9) reported UV irradiation with 6.5×10^{-11} J/m² (approximately one-quarter of the above dosage) was 99% lethal to *S. epidermidis*.

The effect of UV dosage on delay of growth and division of *E. coli* strain B has been presented by TAKEBE and JAGGER (15) and KANTOR and DEERING (16).

Growth and cell division were inhibited by 31.5 to 157.5 times less dosage than the UV dosage 99.9% lethal for our *E. coli* strain B. Radiation-resistant *E. coli* strain B/r was inactivated by UV-irradiation with 2.0×10^4 erg/mm² (10). This was a 6.4 times greater dosage than that proving 99.9% lethal for *E. coli* strain B. The wavelengths employed in all of the above studies were 230–295 nm.

Results of the survival of *E. coli* and *S. aureus* under UV irradiation indicated that *E. coli* was more resistant than *S. aureus*. SINGER and AMES (17) reported the DNA of *Staphylococcus* contained approximately 66% adenine (A) plus thymine (T) and the DNA of *Escherichia* contained approximately 49% A plus T content. BEUKERS and BERENDS (18) reported the primary effect of UV irradiation of DNA was the production of thymine dimers. Subsequent replication of the DNA was halted at the dimer. This was lethal to the cell. These differences in DNA base ratios indicated that *S. aureus* would be expected to be more sensitive than *E. coli* to UV irradiation. This was indicated by the results observed in Fig. 1.

S. aureus had a significantly greater capacity than *E. coli* for repair of UV damage. Both UV radiation and white light are foreign to the natural habitat of *S. aureus*. It is possible that the PR capacity of *S. aureus* observed in these experiments was the manifestation of a mechanism normally utilized for a different purpose by this internal pathogen, suggesting that *S. aureus* has not been an internal parasite for very long. *S. aureus* would require a more efficient repair mechanism than *E. coli* to compensate for the greater effects of UV irradiation on cells with a high thymine content in their DNA.

The % PR obtained for *E. coli* B in our experiments could not be compared with the results of other workers since either the % PR was not determined in their experiments or the PR dosage was not given. There are no reports in the literature of PR of *S. aureus*. Our data indicated *S. aureus* exhibited 0.3 times greater PR than *E. coli* for the same dosage.

E. coli exhibited LHR capacity over a wide temperature range (11–25°, Table 2). *E. coli* exists naturally at all of the experimental temperatures tested. Lethal replication of UV-damaged DNA may have occurred at 35–37° during the short LHR period and thus accounted for the 0% LHR at that temperature. Lethal replication of DNA did not occur during the short LHR period at the lower temperatures used.

The LHR capacity exhibited by *S. aureus* occurred within a narrowly defined temperature range below optimum temperatures for growth. Apparently, *S. aureus* must also be maintained under non-growth conditions to allow time for repair of the UV-induced damage before the lethal error can be replicated. This accounted for the 0% LHR obtained at 35–37°. *S. aureus* normally exists within the narrow temperature range of the host organism, which may account for its inability to undergo LHR at a temperature as low as 11–12°.

The LHR capacity of both *E. coli* and *S. aureus* was measured for 15–60 min at 23–25° (Table 2). *S. aureus* exhibited approximately the same LHR over

this holding period. *E. coli* exhibited a tenfold increase in % LHR during the 15–60 min holding period. These results indicated that *E. coli* exhibited greater LHR than *S. aureus*.

Results of the effect of temperature on PR of *E. coli* and *S. aureus* are given in Fig. 3a and 3b, respectively. The maximum PR for both cultures was obtained at 23–25°. The results indicated that the PR capacity of *S. aureus* was not as temperature dependent as that of *E. coli*. The PR capacity of both *E. coli* and *S. aureus* was less at 11–12° and 35–37° than at 23–25°. This indicated these temperatures were below and above the temperature for maximum PR. The PR capacity of *S. aureus* was lowest at the elevated temperature range.

S. aureus exhibited a narrow temperature range requirement for LHR, but the PR capacity was active over a broad temperature range. These results indicated the LHR and PR systems were different for *S. aureus*. This is in disagreement with what has been reported for another species. CASTELLANI et al. (19) reported the LHR and PR systems were apparently identical in *E. coli* strain B. *S. aureus* may have lost its LHR capacity at low and high temperatures, but still have PR capacity at these temperatures. Whether or not these results reflect some type of evolutionary trend might be clarified by further investigation of LHR and PR capacities of different cultures of *S. aureus* to determine if this temperature relationship is a constant feature of members of this species.

The data in Table 3 substantiate our results that showed an enhanced PR capacity of *S. aureus* over *E. coli*. The exposure time used was not sufficient for the occurrence of maximum PR of either *E. coli* or *S. aureus*. The results indicated that the PR capacity of *E. coli* varied directly with the light intensity, whereas *S. aureus* does not demonstrate this direct relationship. The PR capacity of *S. aureus* was identical at 50% and 1% full intensity. The PR capacity for both *E. coli* and *S. aureus* was greatly reduced with the reduced intensity of PR light; however, the failure of *S. aureus* to undergo additional reduction in PR values with further reduction in light intensity deserves comment. *S. aureus* would appear to have enhanced PR capacity, as compared to *E. coli*, in nature where limited dosages of light could exist.

RUPERT (20) reported that *E. coli* exhibited maximum PR within a wavelength range of 310–400 nm. Similarly, IKENAGA et al. (10) reported that *S. epidermidis* exhibited from 5 to 9 times greater PR at 313–365 nm (near UV) than at 436 nm (violet-blue light). We predicted reduced or no PR response by *E. coli* and *S. aureus* to blue light (450 nm) based upon the above reports. *S. aureus* did exhibit a lack of PR capacity to the blue light, and *E. coli* did exhibit reduced PR with blue light (Table 3). These response differences indicated that we were in fact observing PR in *S. aureus* and *E. coli*.

REFERENCES

- 1) L. GROSSMAN, A. GRAUN, R. FELDBERG, and I. MAHLER, *Ann. Rev. Biochem.*, **44**, 19 (1975).

- 2) P. HANAWALT, *Photochem. Photobiol.*, **79**, 179 (1975).
- 3) H. HARM, *In Photochemistry and Photobiology of Nucleic Acids*, ed. by S. Y. WANG, Vol. II, Academic Press, New York (1976), p. 219.
- 4) G. GAUSE and G. KOCHETKOVA, *J. Gen. Microbiol.*, **29**, 317 (1962).
- 5) W. YOTIS, J. BERTUCCI, T. FITZGERALD, and E. YOTIS, *Yale J. Biol. Med.*, **45**, 150 (1972).
- 6) S. RUBIN and E. ROSENBLUM, *J. Bacteriol.*, **108**, 1192 (1971).
- 7) R. GOERING and P. PATTEE, *J. Bacteriol.*, **106**, 157 (1971).
- 8) M. KRIVANKOVA, J. PILLICH, and E. JANOVSKA, *Int. J. Radiat. Biol.*, **20**, 69 (1971).
- 9) R. GOERING, *Mut. Res.*, **60**, 279 (1979).
- 10) M. IKENAGA, M. PATRICK, and J. JAGGER, *Photochem. Photobiol.*, **11**, 487 (1970).
- 11) K. SMITH and P. HANAWALT, *Molecular Photobiology: Inactivation and Recovery*, Academic Press, New York (1969).
- 12) F. GATES, *Science*, **68**, 479 (1928).
- 13) F. GATES, *J. Gen. Physiol.*, **41**, 31 (1930).
- 14) J. JAGGER, *Photochem. Photobiol.*, **3**, 451 (1964).
- 15) M. TAKEBE and J. JAGGER, *J. Bacteriol.*, **98**, 677 (1969).
- 16) G. KANTOR and R. DEERING, *J. Bacteriol.*, **94**, 1946 (1967).
- 17) C. SINGER and B. AMES, *Science*, **170**, 822 (1970).
- 18) R. BEUKERS and W. BERENDS, *Biochem. Biophys. Acta*, **41**, 550 (1960).
- 19) A. CASTELLANI, J. JAGGER, and R. SETLOW, *Science*, **143**, 1170 (1964).
- 20) C. RUPERT, *In Molecular Mechanisms for Repair of DNA*, ed. by P. HANAWALT and R. SETLOW, Part A, Plenum Press, New York (1975), p. 73.