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UV Light-Induced Survival Response in a Highly Radiation-Resistant Isolate of the *Moraxella-Acinetobacter* Group†

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A highly radiation-resistant member of the *Moraxella-Acinetobacter* group, isolate 4, obtained from meat, was studied to determine the effect of preexposure to UV radiation on subsequent UV light resistance. Cultures that were preexposed to UV light and incubated for a short time in plate count broth exhibited increased survival of a UV light challenge dose. This response was inhibited in the presence of chloramphenicol. Frequencies of mutation to streptomycin, trimethoprim, and sulfanilamide resistance remained the same after the induction of this survival response and were not altered by treatment with mutagens, with the exception of mutation to streptomycin resistance after γ-irradiation or nitrosoguanidine or methyl methane sulfonate treatment. The results indicated that isolate 4 has a UV light-inducible UV light resistance mechanism which is not associated with increased mutagenesis. The characteristics of the radiation resistance response in this organism are similar to those of certain other common food contaminants. Therefore, considered as part of the total microflora of meat, isolate 4 and the other radiation-resistant *Moraxella-Acinetobacter* isolates should not pose unique problems in a proposed radappertization process.

Some members of the *Moraxella-Acinetobacter* group that are common contaminants of meat are highly radiation resistant (14, 15). Organisms of this group are also particularly prominent in the surviving microflora of beef which has been dehydrated (G. J. Silverman and M. K. Cohen, Bacteriol. Proc., p. 13, 1970), frozen (7), or refrigerated in defective vacuum packages for extended time periods (10). Because of their ability to withstand environmental stresses normally considered lethal to vegetative bacteria, further understanding of their survival characteristics and mechanisms is necessary for evaluating radappertization processes proposed for meats (8).

Although considerable work has been done on the mechanisms of radiation survival in both highly resistant and sensitive bacteria, no work has been reported on the possible relationships of known mechanisms to the mechanism operable in the radiation-resistant *Moraxella-Acinetobacter* group. The study of the resistant nature of the radiation response in the *Moraxella-Acinetobacter* group might be particularly fruitful because of opportunities for comparison with other gram-negative bacteria, for which the mechanisms have been studied extensively. The use of well-characterized organisms such as *Escherichia coli* and other closely related bacteria (4) as recipient strains for studies of the genetic basis of radiation resistance is a further possibility.

The γ-radiation resistance of the *Moraxella-Acinetobacter* group is comparable to that of *Micrococcus radiodurans*. Because of this similarity, the *Moraxella-Acinetobacter* group might also be similar to *M. radiodurans* in its response to killing and mutation when exposed to UV light and mutagenic chemicals (11).

This paper reports evidence for a UV light-inducible radiation resistance response in an isolate of the *Moraxella-Acinetobacter* group. Evidence is also presented to show that this response is similar to that of the error-free inducible repair system of *Haemophilus influenzae* (9) and dissimilar to the error-prone response of *E. coli* (16).

MATERIALS AND METHODS

Culture and propagation. Isolate 4, a highly radiation-resistant bacterium isolated from irradiated beef (15), was employed. It has characteristics of the M-5 group of unclassified clinical isolates of *Moraxella* described by Tatum et al. (12). Cultures were propagated in plate count agar (PCA; Difco Laboratories) at 32°C. Subcultures were made in m-Plate Count Broth (PCB; Difco) and incubated at 32°C in a shaker incubator.

Preexposure to UV light to obtain increased UV light

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resistance. An exponential-phase culture having 10^6 to 10^7 colony-forming units per ml was divided into two subsamples. Each subsample was centrifuged at 4,300 $\times$ *g* for 5 min and resuspended in the same volume of 0.2 mM phosphate buffer (pH 7.2) for UV irradiation. Irradiation was done at room temperature with a General Electric G15T8 germicidal lamp placed 33 cm above the bacterial suspension, which was agitated by a magnetic stirrer during irradiation. The incident dose rate was measured with a YSI-Kettering radiometer (model 65A) at 22 J $m^{-2}s^{-1}$.

One subsample was induced by a UV light dose of 660 J/m^2, suspended in PCB, incubated at 32°C for a length of time sufficient to allow induced UV light resistance to be expressed, and suspended in buffer for a challenge dose of 5,280 J/m^2, giving a total dose of 5,940 J/m^2. The other subsample of each pair served as the noninduced control and was irradiated with a dose of 5,940 J/m^2. Control and irradiated counts for each subsample were made by plating on PCA. The use of this method allowed the induced subsample ca. 20 to 25 min of incubation time in PCB beyond the time of plating of the noninduced subsample, becuase of the extra postinduction incubation and centrifugation steps involved.

To reduce elapsed time due to centrifugation, we used an alternative procedure for some experiments. The original subsamples in PCB were diluted 1:10 in buffer (PCB:buffer) before irradiation. Each diluted sample was then diluted 1:10 in PCB for postinduction incubation, followed by a 1:10 dilution in buffer for irradiation with the challenging dose. With this method, the challenge doses could be begun simultaneously for both the induced and noninduced subsamples, thereby eliminating the 20- to 25-min difference in postirradiation elapsed time in buffer described above. Both methods gave similar results, and replications with either method were therefore averaged together.

Because of the tendency of isolate 4 to form clumps and adhere to culture vessels during growth in PCB, a Vortex blender was employed for a length of time sufficient to prevent inaccuracies in viable counts for UV light induction experiments.

Treatment with mutagens. Exponential-phase cultures (10^7 to 10^8 colony-forming units per ml) for exposure to mutagens were divided into two subsamples. One subsample was induced with UV light at 660 J/m^2, followed by 15 min of incubation in PCB at 32°C. Both subsamples were simultaneously treated with a mutagen and then inoculated into fresh PCB to a density of 10^6 to 10^7 colony-forming units per ml. Exposure to mutagens was as follows. (i) For UV irradiation, subsamples were suspended in buffer and irradiated with a dose of 3,960 J/m^2. (ii) For γ-irradiation, subsamples in PCB were suspended in buffer and irradiated with 200 krad at a dosage rate of approximately 5 krad/min by γ-rays from a ^{60}Co source. (iii) Subsamples were exposed to 250 μg of filter-sterilized *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) per ml in PCB for 1 h at 32°C and subsequently washed once in sterile buffer. (iv) Subsamples were exposed to 250 μg of filter-sterilized hydroxylamine (HA) per ml for 1 h at 32°C and washed in sterile buffer. (v) Subsamples were exposed to 10 μl of ethyl methane sulfonate (EMS) per ml in PCB for 30 min at 32°C and washed in sterile buffer. (vi) Subsamples were exposed to 10 μl of methyl methane sulfonate (MMS) per ml in PCB for 20 min at 32°C and washed in sterile buffer.

With the exception of γ-irradiation, each of the above treatments caused a 50 to 70% reduction in viable counts. During γ-irradiation, the viable count increased slightly. Mutagen-exposed cells were incubated at 32°C until approximate maximum cell density was reached (36 h), and the cultures were concentrated 40-fold by centrifugation and plated on PCA and selective media for mutation analysis.

Determining mutation frequencies. Frequencies of mutation to antibiotic resistance were determined with streptomycin, trimethoprim, and sulfanilamide. Streptomycin was made in a stock solution of 10 mg/ml with sterile distilled water and allowed to stand overnight at 5°C. This solution was added to PCA at 45°C to a final concentration of 100 μg/ml immediately before use. Trimethoprim (75 μg/ml) and sulfanilamide (1 mg/ml) were sterilized with PCA by autoclaving just before use and cooled immediately to 45°C. Plates containing antibiotics were incubated 10 to 12 days before counting to allow for slow colony growth. Only colonies larger than 0.5 mm were counted in trimethoprim and sulfanilamide plates. Mutation frequencies for each treatment were calculated as the ratio of mutation counts on antibiotic media to total viable counts on PCA.

RESULTS

Effect of preexposure to UV light on UV light resistance of isolate 4. The possibility that an inducible mechanism contributes to the extreme UV light resistance of isolate 4 was investigated by irradiating cells with a UV light dose of 660 J/m^2, followed by a 15-min incubation period in PCB (32°C) and a challenge dose of 5,280 J/m^2. This preexposure induced a significant increase in survival rate over that of cells which received the same equivalent total dose (5,940 J/m^2) without preexposure (Table 1). Variability in the differences observed between survival rates of induced and noninduced cultures was high, but the pattern of the induced response was consistent. The results given in Table 1 are the average of 12 replications.

Effect of preexposure dosage level on subsequent UV light resistance. The effect of dosage level on the magnitude of inducible UV light resistance was studied by varying the induction dose while keeping the total dose (preexposure plus challenge) constant (Table 2). For induction doses ranging from 220 to 1,980 J/m^2, the level of induced survival was fairly constant, and it is reasonable to assume that the observed differences were small enough to be attributed to normal variation. These preexposure doses lay within the shoulder of the UV light death curve of isolate 4, before the onset of exponential death rate (data not given).

Effect of postinduction incubation time. The data for the magnitude of inducible resistance after each of several intervals of postinduction incubation in PCB are shown in Table 3. As

TABLE 1. Effect of preexposure to UV irradiation on UV light resistance of isolate 4, with and without resistance-inhibiting treatments

Treatment	Surviving fraction
Exponential-phase culture, no inhibitors[a]	
660 J/m^2	0.87
660 J/m^2 + PCB incubation[b] + 5,280 J/m^2	0.87
5,940 J/m^2	0.35
660 J/m^2 + PCB incubation[b] + 5,280 J/m^2 − correction factor (see text)	0.66
Quick freezing in dry ice-acetone[c,d]	
660 J/m^2 + freezing for 15 min and thawing + 5,280 J/m^2	0.36
CAP exposure before induction dose[e]	
660 J/m^2	0.63
660 J/m^2 + PCB incubation[b] + 5,280 J/m^2	0.06
5,940 J/m^2	0.03
CAP exposure immediately after induction dose[c]	
660 J/m^2	0.87
660 J/m^2 + PCB incubation[b] + 5,280 J/m^2	0.60
5,940 J/m^2	0.48

[a] Average of 12 replications.
[b] 15 min at 30°C.
[c] Average of two replications.
[d] Corrected for 6% reduction in viable counts due to freezing and thawing.
[e] Average of three replications.

incubation time was increased from 10 to 100 min, the effect of induction was confounded by the normal increase in cell population. When the amount of increase in the surviving fraction due to population increase (assuming an exponential rate of growth) was then subtracted from each surviving fraction value, it was apparent that an incubation period of ca. 30 min provided the maximum inducible resistance response. After 10 min, a significant increase in the level of resistance was apparent. Therefore, a postinduction incubation period of 10 to 15 min was chosen for most experiments to provide a significant increase in observable inducible resistance with minimal confounding of induction effects due to population increase.

TABLE 2. Effect of preexposure dosage level on inducible resistance response of isolate 4[a]

Dose (J/m^2)		Log fraction of survivors[c]
Preexposure	Challenge[b]	
0	0	6.61
0	5,940	6.35
220	5,720	6.56
660	5,280	6.61
1,320	4,620	6.40
1,980	3,960	6.41

[a] Total dose delivered to each sample was 5,940 J/m^2.
[b] A challenge dose was delivered after 15 min. PCB incubation (32°C) followed the preexposure dose.
[c] Average of three replications.

Effect of CAP treatment on response to UV irradiation. A growth-inhibiting nonlethal level of chloramphenicol (CAP) (10 μg/ml) was added to cultures of isolate 4 to test whether the inducible survival response would be affected. Cultures exposed for 16 h to CAP before UV light induction experiments were less resistant to UV light killing, and induced cultures were not significantly more resistant than noninduced cultures (Table 1). The latter observation suggests that active protein synthesis may be necessary for the inducible survival response to be expressed.

When CAP was added immediately after the induction dose, a decrease in the magnitude of the inducible survival response occurred (Table 1). However, the resistance system was still partially operative; otherwise, survival rates of the induced and noninduced cultures would have been nearly equal, as occurred when CAP exposure took place before induction.

Mutation frequencies of isolate 4 before and after induction. To test whether the inducible survival response of isolate 4 was associated with increased frequency of mutations, we exposed paired subcultures, of which one subculture each was induced by preexposure to UV light, to various mutagens. The resulting frequencies of mutation to streptomycin, trimethoprim, and sulfanilamide resistance both before

TABLE 3. Effect of postinduction incubation time in PCB (32°C) on survival of isolate 4 after UV irradiation

Postinduction incubation (min)	Log fraction of survivors after challenge dose[a]	
	Observed	Corrected[b]
0	5.62	5.62
10	6.14	6.10
30	6.42	6.30
60	6.40	6.18
100	6.42	6.10

[a] The preexposure dose was 660 J/m^2; the challenge dose was 5,280 J/m^2; average of two replications.

[b] After correction for postinduction population increase. Doubling time during maximum exponential growth was 100 min.

and after induction are shown in Table 4. No significant difference ($P < 0.10$) was found between the mutation frequencies of induced and noninduced cultures after each mutagenic treatment. NG and MMS each caused a detectable increase in the frequency of streptomycin-resistant colonies above the spontaneous frequency. γ-irradiation caused a large (ca. 100-fold) increase in the frequency of mutation to streptomycin resistance. However, the observed rates of increase in streptomycin resistance were the same in noninduced and induced cultures. NG, MMS, and γ-irradiation did not affect the rates of mutation to trimethoprim or sulfanilamide resistance.

DISCUSSION

Increased survival after preexposure to UV light. When isolate 4 was preexposed to UV light, a significant increase in survival was observed after susequent UV light challenge. Although this increase can be attributed primarily to an inducible resistance response, contributions from two other sources must be considered. One is the contribution due to repair of UV light damage during postinduction incubation, and the other is the contribution due to the 20 to 25 min of postinduction growth in PCB.

The contribution of postinduction repair processes to the apparent increase in survival of cultures can be estimated from the results of experiments with postinduction exposure to CAP. Assuming that postinduction exposure to CAP halts the operation of the inducible survival response, the difference between the surviving fraction (0.12) of induced and noninduced cultures can be attributed to the operation of a constitutive, or noninducible, repair system (Table 1). This estimate is liberal, as no contribution due to inducible resistance is allowed by the assumption that CAP inhibits such a response immediately and completely, which may not be the case. The contribution to the surviving fraction by population increase during postinduction incubation of induced cultures is 0.09, assuming that population increase occurs at the maximum exponential growth rate during this period.

When the increased population contribution and repair system contribution estimates (0.09 + 0.12) were both subtracted from the induced survival rate, the difference between induced and noninduced surviving fractions was lower, but still significant (see Table 1). When all repair and growth processes in induced cultures were halted by quick freezing in dry ice-acetone during the postinduction period, the surviving fraction after thawing and irradiating to a total dose of 5,940 J/m^2 was the same as in noninduced cultures (Table 1).

The difference between induced and noninduced surviving fractions after a total dose of 5,940 J/m^2 was greater than can be accounted for by repair and growth during postinduction incubation and is therefore attributable to an inducible resistance system.

Role of protein synthesis in induction of UV light resistance and possible regulatory mechanisms. Exposure to 10 μg of CAP per ml during

TABLE 4. Frequencies of mutation of isolate 4 to streptomycin, trimethoprim, and sulfanilamide resistance after exposure to various mutagenic agents before and after UV light induction[a]

Mutagen[b]	Mutation frequency		
	Str^r (×10^{-10})	Tmp^r (×10^{-4})	Su^r (×10^{-5})
Control (no mutagen exposure)	6.5	7.9	6.2
UV light	2.0	3.1	3.0
UV light$_i$	6.6	2.6	3.6
γ irradiation	487.6	3.7	1.4
γ irradiation$_i$	782.6	5.2	2.3
NG[c]	42.6	6.5	3.4
NG$_i$[c]	38.0	4.5	7.0
MMS	39.4	4.3	3.6
MMS$_i$[c]	16.5	4.4	11.3
EMS	3.0	4.5	1.2
EMS$_i$[c]	8.7	2.9	8.9
HA[c]	1.4	4.4	1.8
HA$_i$[c]	1.4	1.1	1.7

[a] Induction was achieved by preexposure to 1,140 ergs of UV irradiation per mm^2 followed by PCB incubation (15 min, 32°C).

[b] Subscript indicates induced cultures. Average of four replications except where noted.

[c] Average of two replications.

postinduction incubation lowered the difference in survival rates between induced and noninduced samples, indicating the necessity for new protein synthesis for the heightened survival response to be expressed. Cultures which were preincubated with CAP for 16 h before induction were much more sensitive to UV irradiation than control cultures and showed no evidence of an inducible resistance response (Table 1). This would be expected due to the inability of the cultures to maintain the normal level of constitutive repair enzymes in the absence of protein synthesis as well as to respond normally to signals initiating the inducible response after irradiation.

CAP is lethal to isolate 4 at concentrations higher than 10 μg/ml, precluding the use of higher concentrations in these inhibition studies. The toxicity of CAP for isolate 4 is similar to that noted by DeLeys and Juni (2), who reported that exposure of *Moraxella osloensis* to 50 μg of CAP per ml caused immediate cessation of protein synthesis but also was bactericidal. It is reasonable to assume that a toxic effect per se was not a factor in the loss of inducible resistance in isolate 4 cells exposed to CAP, because the concentration used was nonlethal.

The occurrence of maximum resistance induction after approximately 30 min of postirradiation incubation (Table 3) is similar to the induction period reported by Defais et al. (1) in kinetics studies of inducible error-prone repair in *E. coli*. Further study of the induction mechanism of isolate 4 might determine whether control is by genetic regulation, as suggested by the abolition of an induced response when isolate 4 is preincubated with CAP, by allosteric regulation, or by a combination of genetic and allosteric regulation, as suggested by the reduced level of induced response after postinduction exposure to CAP. Tessman and Peterson (13) have raised the possibility of the operation of allosteric regulation in *tif-1* mutants of *E. coli* in which the degree of induction was influenced by the ratio of adenine to cytidine, modulators of the *recA* protein, in the culture medium.

Mutation frequency in induced cultures of isolate 4. Assuming that increased UV light resistance of induced cultures is due to repair of DNA damage, the absence of an increased incidence of mutation after induction is evidence that DNA repair in isolate 4 is highly accurate. This contrasts with the error-prone characteristic associated with inducible repair in *E. coli* (16). In this respect, the inducible response of isolate 4 is similar to that of *H. influenzae*, as reported by Notani and Setlow (9). Other similarities to *H. influenzae* are found in the increased incidence of streptomycin resistance after γ-irradiation (N. K. Notani and V. R. Joshi, Radiat. Res. 59:76–77, 1970) and in mutability after exposure to NG (5, 6). It is not apparent why γ-irradiation, NG, and MMS each affect the frequency of mutation to streptomycin resistance in isolate 4 but not the frequency of mutation to trimethoprim or sulfanilamide resistance.

The inability to detect changes in the rate of mutation after induction may also be due to the high accuracy of repair manifest upon exposure to mutagens, such as NG, HA, and EMS, that are considered to be able to produce mutations without the presence of a recombination repair system (3). The results indicate that isolate 4 is not only highly resistant to killing by agents which damage DNA, but also to mutation by agents. These results are similar to the findings of Sweet and Moseley (11) which led them to suggest that *M. radiodurans* does not possess an inducible error-promoting factor; therefore, repair of DNA damage is not only highly efficient in this organism, but also highly accurate.

The possibility exists that the mechanisms of resistance to mutation and killing by radiation might be connected with the observed resistance of the *Moraxella-Acinetobacter* group to lethal effects of drying and freezing or extended cold storage. If true, further knowledge of the mechanism(s) of radiation resistance would provide additional understanding of the survival of vegetative bacteria exposed to stresses involved in the processing of foods.

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