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Role of *Acinetobacter baumannii* UmuD Homologs in Antibiotic Resistance Acquired through DNA Damage-Induced Mutagenesis

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The role of *Acinetobacter baumannii* ATCC 17978 UmuDC homologs A1S_0636-A1S_0637, A1S_1174-A1S_1173, and A1S_1389 ($UmuD_{Ab}$) in antibiotic resistance acquired through UV-induced mutagenesis was evaluated. Neither the growth rate nor the UV-related survival of any of the three mutants was significantly different from that of the wild-type parental strain. However, all mutants, and especially the $umuD_{Ab}$ mutant, were less able to acquire resistance to rifampin and streptomycin through the activities of their error-prone DNA polymerases. Furthermore, in the *A. baumannii* mutant defective in the $umuD_{Ab}$ gene, the spectrum of mutations included a dramatic reduction in the frequency of transition mutations, the mutagenic signature of the DNA polymerase V encoded by *umuDC*.

The exposure of some strains of the nosocomial pathogen *Acinetobacter baumannii* (e.g., strain ATCC 17978) to DNA-damaging agents results in a much higher frequency of DNA damage-induced rifampin resistance (Rif^r) mutations than spontaneous Rif^r mutations (1). Rifampin resistance can be acquired through the *recA*-dependent DNA damage response, most commonly following DNA base-pair substitutions in the *rpoB* gene, which encodes the β subunit of RNA polymerase (1, 2). In *Escherichia coli*, Y-family error-prone DNA polymerases Pol IV and Pol V (encoded by *dinB* and *umuDC*, respectively) are induced as part of the SOS system, a coordinated cellular response to environmental stress (3).

The DNA damage-induced mutation frequency in a *recA* mutant of *A. baumannii* 17978 is significantly lower than in its isogenic parental strain (1). However, in a *dinB* mutant, a previous study showed the same frequency of DNA damage-induced Rif^r mutants as in the parental ATCC 17978 strain (1). It is worth noting that in *A. baumannii* ATCC 17978, two complete *umuDC* operons (*A1S_0636-A1S_0637* and *A1S_1174-A1S_1173*) and one unlinked *umuD* homolog (*A1S_1389*, referred to here as $umuD_{Ab}$) have been identified, and all of them, rather than only the *dinB* gene, are induced by DNA damage (1). The mutation signatures of DNA Pol V and DinB are transition mutations and −1 frameshifts, respectively (4–6). Interestingly, the most frequent mutations determined in Rif^r mutants of the ATCC 17978 strain after UV-induced mutagenesis involve a C→T transition (T. Witkowski, A. Grice, and J. Hare, presented at the Ninth International Symposium on Biology of *Acinetobacter*, Cologne, Germany, 19 to 21 June 2013). Together, these findings suggest that the multiple DNA Pol V components encoded by the ATCC 17978 genome have an important function in DNA damage-induced mutagenesis. To investigate the role of the two putative *umuDC* operons, *A1S_0636-A1S_0637* and *A1S_1174-A1S_1173*, and the unlinked $umuD_{Ab}$ gene in the DNA damage-induced mutagenesis phenotype of strain ATCC 17978, we inactivated both putative operons and the $umuD_{Ab}$ gene and then evaluated their abilities to generate DNA damage-induced mutations. The $umuD_{Ab}$ mutant was described elsewhere (7). The *A1S_0636-A1S_0637* and *A1S_1174-A1S_1173* mutants were obtained as reported previously (8), using the oligonucleotides listed in Table 1. The growth rate of the three isogenic mutants (*A1S_0636-A1S_0637*, *A1S_1174-A1S_1173*, and $umuD_{Ab}$) was not significantly different from that of the wild-type (WT) ATCC 17978 parental strain (data not shown), as reported for *E. coli* and other bacteria (9). However, although the difference was not statistically significant, the UV survival rate of strains *0636-7* and *1174-3* was slightly lower than that of the WT parental strain whereas this was not the case for the $umuD_{Ab}$ mutant (Fig. 1).

TABLE 1 Oligonucleotides used in this work

Name	Sequence[a]	Application
MUT0636Y7F	5′-CTGGAATCGATATCAACG	Mutant construction
MUT0636Y7R	5′-AATGTCTTGAGCGCATTG	Mutant construction
0636Y7EXTF	5′-TGATGGATATGAATGAGC	Mutant verification
0636Y7EXTR	5′-CGACCTATACCAACACAG	Mutant verification
MUT1174Y3F	5′-ACAGGTAAGCCTAACCAC	Mutant construction
MUT1174Y3	5′-ACTATCAATGCTCAATGC	Mutant construction
EXT1174Y3F	5′-CGTCGATAAGCGAGAAGT	Mutant verification
EXT1174Y3R	5′-GATAAAGCTCTCGACATG	Mutant verification
M13FpU	5′-GTTTTCCCAGTCACGAC	Mutant verification
M13RpUC	5′-CAGGAAACAGCTATGAC	Mutant verification
UmuDAb-XbaI	5′-ATCGTCTAGAATGCCAAA GAAGAAAGAA	$umuD_{Ab}$ complementation
UmuDAb-NcoI	5′-ATCGCCATGGTTATCTCA TTCGTTTGAG	$umuD_{Ab}$ complementation

[a] Restriction endonuclease recognition sites are underlined.

UV-induced mutagenesis was carried out using a modification of the protocol described by Norton et al. (1). Similarly to rifampin, chromosomally acquired streptomycin resistance (Str^r) is fre-

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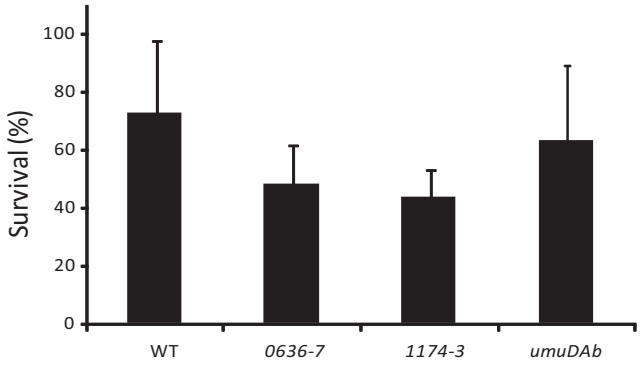

FIG 1 Survival of strain ATCC 17978 (WT) and mutants *A1S_0636-A1S_0637* (*0636-7*), *A1S_1174-A1S_1173* (*1174-3*), and *A1S_1389* (*umuD*$_{Ab}$) after exposure to UV light (100 J/m^2). The percentage of surviving cells in UV-irradiated cultures was determined by comparison with nonirradiated cells. Error bars represent the standard errors of the means of the results of three independent experiments. Data were analyzed by a two-tailed, one-way analysis of variance (ANOVA). In all cases, the difference from the WT parental strain (ATCC 17978) was not significant ($P > 0.05$).

quently due to DNA base-pair substitutions but in genes encoding either ribosomal proteins or rRNAs (10). Overnight cultures of the corresponding strain (1 ml) were diluted in 19 ml of NaCl (0.9% [wt/vol]) and then thinly spread on a sterile petri plate. The samples were irradiated with UV-C light (254 nm) at a dose of 100 J/m^2. After treatment, 1 ml of each sample was diluted in 9 ml of Luria Bertani (LB) broth and grown for 24 h at 37°C. The appropriate cell dilutions were then deposited on LB plates with and without streptomycin (100 μg/ml) to assess, respectively, the number of Str^r mutants and the total number of CFU. The plates were incubated at 37°C for 24 h. Mutation frequency was calculated by dividing the number of Str^r mutants by the total number of CFU. All experiments with UV-irradiated cells were performed in the dark to prevent the photoreactivation of pyrimidine dimers. Spontaneous Str^r mutants from untreated cultures were determined as described above but without UV irradiation. The spontaneous Str^r mutation frequency was approximately 10^{-10} in both WT and mutant strains. In contrast, the UV-induced Str^r mutation frequency was at least 100-fold higher in the irradiated WT strain but significantly lower ($P < 0.01$) in all of the irradiated mutant strains (Fig. 2A). The most important UmuD homolog contributing to the UV-induced Str^r mutation frequency was UmuD$_{Ab}$, followed by a DNA polymerase V encoded by the *A1S_1174-A1S_1173* operon (Fig. 2A) and by the A1S_0636-A1S_0637 DNA polymerase V, which showed modest (but statistically significant) UV-induced Str^r mutation acquisition compared to the WT parental strain (Fig. 2A). The spontaneous and UV-induced Rif^r mutation frequencies were also studied as described above but using LB plates with and without rifampin (50 μg/ml). Under these conditions, the spontaneous Rif^r mutation frequency was approximately 10^{-8} in both the WT and the mutant strains whereas the UV-induced Rif^r mutation frequency was about 100-fold higher in the WT strain and significantly lower ($P < 0.01$) in all the mutant strains (Fig. 2B). Thus, the contributions of A1S_0636-A1S_0637, A1S_1174-A1S_1173, and UmuD$_{Ab}$ to the acquisition of Rif^r after UV treatment were similar to their contributions to Str^r acquisition under the same conditions.

To analyze the spectra of mutations produced by each of the *umu* mutants after UV treatment, we used an established Rif^r assay for *A. baumannii* (1). Colony PCR was performed on 10 individual *A. baumannii* Rif^r mutants obtained from 10 independent experiments for each strain (Table 2). Oligonucleotides *rpoB*-1441F and *rpoB*-2095R amplify a 654-bp region of the gene *rpoB* (*A1S_0287* in *A. baumannii* ATCC 17978), encoding the β subunit of the RNA polymerase, the site of frequent Rif^r-inducing base-pair substitutions in this species (1). Sequencing (Macrogen) was carried out using the same oligonucleotide set, and the data were analyzed with SeqManII (DNAstar). Interestingly, most of the mutations detected in the WT and in the mutant strains *A1S_0636-A1S_0637* and *A1S_1174-A1S_1173* were C→T transitions (Table 2). For the WT strain, these results agree with those reported very recently (T. Witkowski, A. Grice, and J. Hare, presented at the Ninth International Symposium on Biology of *Acinetobacter*, Cologne, Germany, 19 to 21 June 2013). However, in the *umuD*$_{Ab}$ mutant, only 1 of 10 (10%) mutations was a transition (Table 2). It is worth noting that the mutagenic signature for DNA polymerase V is its incorporation of guanine opposite the 3′ thymine of a thymine-thymine pyrimidine (6-4) pyrimidone photoproduct, i.e., a transition mutation (6). These data agree with the finding that in *A. baumannii* ATCC 17978 the most important UmuD homolog contributing to the UV-induced mutation frequency is UmuD$_{Ab}$ (Fig. 2).

To complement the *umuD*$_{Ab}$ mutant, this gene was PCR amplified using the oligonucleotides UmuDAb-XbaI and UmuDAb-

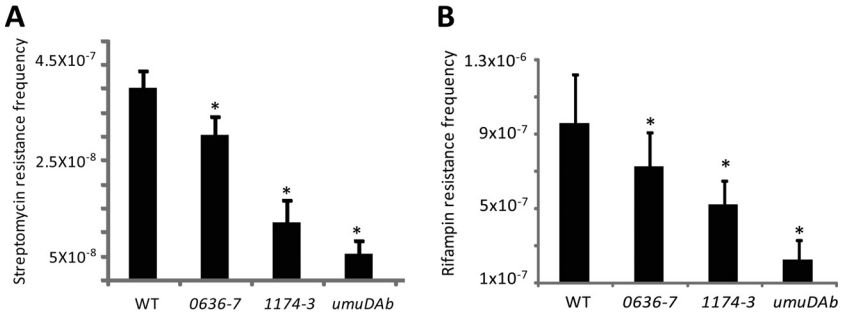

FIG 2 Frequency of streptomycin (A) and rifampin (B) resistance after UV treatment of strain ATCC 17978 (WT) and mutants *A1S_0636-A1S_0637* (*0636-7*), *A1S_1174-A1S_1173* (*1174-3*), and *A1S_1389* (*umuD*$_{Ab}$). Error bars represent the standard deviations of the means of the results for at least three independently tested cultures. Data were analyzed by a two-tailed, one-way analysis of variance (ANOVA), followed by the Tukey test for *post hoc* multiple group comparisons. *, $P < 0.01$ compared to the WT parental strain (ATCC 17978).

TABLE 2 Spectrum of mutations produced by the indicated strain after UV treatment

Strain and mutation category	Mutation type and *rpoB* nucleotide change[a]	RpoB amino acid substitution	Mutation frequency (%)
ATCC 17978 WT	1564 **C**AG→**A**AG	522 Gln→Leu	30
transversions	1619 T**C**T→T**A**T	540 Ser→Tyr	10
ATCC 17978 WT	1562 T**C**T→T**T**T	521 Ser→Phe	10
transitions	1613 C**G**T→C**A**T	537 Arg→His	10
	1619 T**C**T→T**T**T	540 Ser→Phe	30
	1718 C**C**T→C**T**T	573 Pro→Leu	10
0636_7 mutant	1565 C**A**G→C**T**G	522 Gln→Leu	10
transversions	1741 **A**TC→**T**TC	581 Ile→Phe	10
0636_7 mutant	1562 T**C**T→T**T**T	521 Ser→Phe	30
transitions	1603 **C**AT→**T**AT	535 His →Tyr	20
	1619 T**C**T→T**T**T	540 Ser→Phe	30
1174_3 mutant transversions	1604 C**A**T→C**T**T	535 His→Leu	10
1174_3 mutant	1562 T**C**T→T**T**T	521 Ser→Phe	40
transitions	1619 T**C**T→T**T**T	540 Ser→Phe	40
	1718 C**C**T→C**T**T	573 Pro→Leu	10
1389 (*umuD*$_{Ab}$)	1565 C**A**G→C**T**G	522 Gln→Leu	20
mutant	1574 G**A**C→G**T**C	525 Asp→Val	10
transversions	1619 T**C**T→T**A**T	540 Ser→Tyr	40
	1741 **A**TC→**T**TC	581 Ile→Phe	20
1389 (*umuD*$_{Ab}$) mutant transitions	1718 C**C**T→C**T**T	573 Pro→Leu	10

[a] Boldface and underlining indicates nucleotide changes.

NcoI (Table 1). The resulting product was cloned into the XbaI-NcoI restriction sites of the pET-RA plasmid (11), yielding pETRA-UmuD$_{Ab}$, which was introduced into the *A. baumannii* *umuD*$_{Ab}$ mutant by electroporation, as reported previously (11). Since this plasmid contains a gene encoding Rif^r, we compared the abilities of the complemented *umuD*$_{Ab}$ mutant and the same strain carrying the empty pET-RA plasmid to generate DNA damage-induced Str^r mutants, as described above. The acquisition of Str^r was approximately 100-fold higher in the UV-treated complemented *umuD*$_{Ab}$ mutant than in the UV-treated *umuD*$_{Ab}$ mutant carrying the empty pET-RA vector ($P < 0.01$). This result indicates that UmuD$_{Ab}$ is an important DNA polymerase V component that probably binds to either of the products of the two unlinked UmuC homologs identified in the ATCC 17978 genome (A1S_2008 and/or A1S_2015) and/or to any of the *umuC* homologs belonging to the operons mentioned above, as all these genes are induced after DNA damage (1, 7).

To our knowledge, this is the first experimental demonstration that in *A. baumannii* ATCC 17978 a *umuD* homolog that acts as a LexA regulator analog (7) is also the most active error-prone DNA polymerase V component conferring resistance to both rifampin and streptomycin. Thus, in conclusion, the data presented in this work suggest that the two complete *umuDC* operons and a *umuD*$_{Ab}$ gene, together acting as DNA polymerase V components, provide an adaptation mechanism for this nosocomial pathogen.

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We declare that we have no conflicts of interest.

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