Diverse responses to UV light exposure in *Acinetobacter* include the capacity for DNA damage-induced mutagenesis in the opportunistic pathogens *Acinetobacter baumannii* and *Acinetobacter ursingii*

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Error-prone and error-free DNA damage repair responses that are induced in most bacteria after exposure to various chemicals, antibiotics or radiation sources were surveyed across the genus *Acinetobacter*. The error-prone SOS mutagenesis response occurs when DNA damage induces a cell's *umuDC*- or *dinP*-encoded error-prone polymerases. The model strain *Acinetobacter baylyi* ADP1 possesses an unusual, regulatory *umuD* allele (*umuDAb*) with an extended 5' region and only incomplete fragments of *umuC*. Diverse *Acinetobacter* species were investigated for the presence of *umuDC* and their ability to conduct UV-induced mutagenesis. Unlike ADP1, most *Acinetobacter* strains possessed multiple *umuDC* loci containing either *umuDAb* or a *umuD* allele resembling that of *Escherichia coli*. The nearly omnipresent *umuDAb* allele was the ancestral *umuD* in *Acinetobacter*, with horizontal gene transfer accounting for over half of the *umuDC* operons. Despite multiple *umuD(Ab)C* operons in many strains, only three species conducted UV-induced mutagenesis: *Acinetobacter baumannii*, *Acinetobacter ursingii* and *Acinetobacter beijerinckii*. The type of *umuDC* locus or mutagenesis phenotype a strain possessed was not correlated with its error-free response of survival after UV exposure, but similar diversity was apparent. The survival of 30 *Acinetobacter* strains after UV treatment ranged over five orders of magnitude, with the *Acinetobacter calcoaceticus*–*A. baumannii* (Acb) complex and haemolytic strains having lower survival than non-Acb or non-haemolytic strains. These observations demonstrate that a genus can possess a range of DNA damage response mechanisms, and suggest that DNA damage-induced mutation could be an important part of the evolution of the emerging pathogens *A. baumannii* and *A. ursingii*.

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INTRODUCTION

When bacteria are exposed to DNA-damaging agents, such as UV light or chemical mutagens, a wide variety of SOS response genes (genes that play critical roles in repairing damaged DNA) are specifically induced (Friedberg *et al.*, 1995; Walker, 1996). In the model developed by experimentation in *Escherichia coli*, SOS genes are negatively regulated by the LexA repressor binding to the SOS box in the promoter (Mount *et al.*, 1972). DNA damage causes the activation of RecA, which facilitates LexA self-cleavage and releases the repression of SOS genes such as *umuDC* (Little *et al.*, 1980; 1981). UmuD initially causes a pause in

cell division while DNA repair and replication occurs (Opperman *et al.*, 1999). However, within minutes, UmuD homodimerizes and carries out intermolecular self-cleavage, facilitated by activated RecA* interaction (Nohmi *et al.*, 1988). Two cleaved UmuD' molecules then associate with UmuC to form DNA polymerase V, which carries out error-prone, trans-lesion DNA replication (SOS mutagenesis) (Reuven *et al.*, 1999; Tang *et al.*, 1999). Another error-prone polymerase commonly involved in SOS mutagenesis as part of the DNA damage response is DinP (also called DinB), DNA polymerase IV, which can function either by itself (Kim *et al.*, 1997) or with UmuD (Foti *et al.*, 2010; Godoy *et al.*, 2007).

Variation on the standard *E. coli*-developed model of DNA damage responses is shown by differences in SOS-induced genes, the number or presence of LexA proteins, and specific SOS box sequences in genera such as *Bacillus* (Winterling *et al.*, 1998), *Xylella* (Campoy *et al.*, 2002) and

Abbreviation: Acb, *Acinetobacter calcoaceticus*–*Acinetobacter baumannii*.

The GenBank/EMBL/DDBJ accession numbers for the *umuD* gene sequences of *Acinetobacter baumannii* 19606^T, *Acinetobacter pittii* ATCC 19004^T and *Acinetobacter* sp. ATCC 49137 are FJ172295, EF583611 and EF583614, respectively.

Mycobacterium (Davis *et al.*, 2002). We study DNA damage responses of the Gram-negative *Acinetobacter baylyi* ADP1. Members of the genus *Acinetobacter* [order *Pseudomonadales* (Towner *et al.*, 1991)] are abundant in most environments and are classified into at least 32 genomic species, many of which have not been assigned formal species names (Dijkshoorn & Nemec, 2008). The taxonomy of the genus is currently defined by DNA–DNA hybridization techniques, and identification of species based on biochemical or phenotypic differences is very difficult (Bernards *et al.*, 1996), often requiring DNA fingerprinting, amplified fragment length polymorphism and/or amplified rDNA restriction analysis (Chang *et al.*, 2005). Most *Acinetobacter* species are non-pathogenic or opportunistic, with significant research efforts focused on the frequently antibiotic-resistant, opportunistic pathogen *Acinetobacter baumannii*. However, opportunistic infections are also caused by *Acinetobacter lwoffii*, *Acinetobacter ursingii* (Turton *et al.*, 2010) or strains in the *Acinetobacter calcoaceticus*–*A. baumannii* (Acb) complex (Gerner-Smidt *et al.*, 1991), most commonly by genomic groups 13TU and 3 (Chuang *et al.*, 2011; Karah *et al.*, 2011) which correspond to the species *Acinetobacter nosocomialis* and *Acinetobacter pittii*, respectively (Nemec *et al.*, 2011). The genome of the non-pathogenic, naturally competent *A. baylyi* ADP1 has been sequenced (Barbe *et al.*, 2004), and strain ADP1 serves as an excellent model organism for genetic and metabolic engineering (de Berardinis *et al.*, 2009; Metzgar *et al.*, 2004).

ADP1 responds to DNA damage differently than *E. coli*, with induction of its *recA* gene not dependent on RecA function (Rauch *et al.*, 1996). Many other changes are associated with the *umuDC* operon. The *umuC* gene of ADP1 is broken into two fragments that comprise only 43 % of the ORF, and *umuDab* encodes an extra 59 aa N-terminal region that is not encoded by other *umuD* genes (Hare *et al.*, 2006). Furthermore, *umuDab* is transcribed at high levels in both the presence and the absence of RecA, perhaps due to the lack of LexA in ADP1 (Hare *et al.*, 2006). Finally, *umuDab* regulates the induction of *ddrR*, a DNA damage-inducible locus in ADP1 that is adjacent to (but transcribed divergently from) *umuDab* (Hare *et al.*, 2006). These results suggest that ADP1 may respond to DNA damage differently than in the typical SOS response, although it is unclear whether its *umuC* mutation or unusual *umuDab* allele are anomalies or represent novel mechanisms present throughout this genus.

Our goal was to test whether the unusual *umuDab* allele observed in ADP1 was shared by *A. baumannii* or other acinetobacters, and whether this allele was correlated with an ability of UV exposure to induce mutagenesis. Antibiotic exposure can induce the SOS response in bacteria (Kelley, 2006), which could lead to expression of the UmuD'2–UmuC polymerase V and subsequent mutation through SOS mutagenesis, if present in *A. baumannii*. A recent review reported that *A. baumannii* strains appear to contain an *umuDab*-like gene in an operon with *umuC*

(Robinson *et al.*, 2010), which suggests that they may conduct SOS mutagenesis with this unusual *umuD* allele. To investigate this possibility, we examined whether *Acinetobacter* species possess *umuD* and *umuC* genes similar to those in ADP1 or *E. coli*, and if the capacity of these species to survive DNA damage and undergo UV-induced mutagenesis are similar across the genus.

METHODS

Strains used. The strains used in UV-induced mutagenesis and UV survival experiments are given in Table 1. The type strains of recognized species and the reference strains of other (unrecognized) *Acinetobacter* species were purchased from the American Type Culture Collection (ATCC), with the following exceptions: wild-type and $\Delta umuD::nptII$ *E. coli* AB1157 strains (Penny Beuning, Northeastern University, Boston, MA, USA), *A. baylyi* ADP1 (Leslie Gregg-Jolly, Grinnell College, Grinnell, IA, USA), *A. baumannii* AB0057 (Robert Bonomo, Case Western Reserve University, Cleveland, OH, USA), and *Acinetobacter beijerinckii* NIPH 838^T, *Acinetobacter guillouiae* LMG 988^T, *Acinetobacter gyllenbergii* NIPH 2150^T, *Acinetobacter parvus* NIPH 384^T, *Acinetobacter tjernbergiae* CCM 7200^T, *Acinetobacter venetianus* RAG-1^T and all *A. ursingii* strains except ATCC BAA-617^T (Alexandr Nemec, National Institute of Public Health, Czech Republic).

Cloning *umuD* from *Acinetobacter* strains. Touchdown PCR (Don *et al.*, 1991) was used to amplify *umuDC* alleles from genomic DNA of *A. pittii* ATCC 19004^T, *Acinetobacter* sp. ATCC 49137 and *A. baumannii* 19606^T. Primers used to amplify the extended *umuDab* allele were derived from the *umuDab* sequence of ADP1: CL0, 5'-AGCCAACCTAAAGTCATTTCG (forward), and CL5, 5'-AGATCACGAGTTCTTGACC (reverse). Primers 16SForward and 16SReverse (5'-GCACCTGTATGTAGATTCC and 5'-TACTCGCAGAATAAGCACC, respectively) were used to confirm the quality of genomic DNA for PCR analyses, and were derived from the ADP1 16S rRNA gene (GenBank accession no. AY289925) (Barbe *et al.*, 2004). The primers 3'AbumuD (5'-GTTATAGGTCACTACTCCCC) and 3'AbddrR (5'-CCCTTTTACAATATCTGTG) were used to amplify a 1.1 kb fragment containing the *ddrR*–*umuDab* region from *A. baumannii* 19606^T, and were derived from the sequence of *A. baumannii* 17978 (GenBank accession no. CP000521; Smith *et al.*, 2007).

Sources and analyses of genome sequence information. Unpublished sources of genome sequences were Genoscope (www.genoscope.cns.fr) for all sequences except *A. ursingii* DSM 16037^T (AciBASE; <http://acinetobacter.bham.ac.uk/>) and GenBank sequences obtained in this study: *A. pittii* ATCC 19004^T (EF583611), *Acinetobacter* sp. ATCC 49137 (EF583614) and *A. baumannii* ATCC 19606^T (FJ172295). Published genome sequences represented in Genoscope include *A. baylyi* ADP1 (Barbe *et al.*, 2004), '*Acinetobacter oleivorans*' DR1 (Jung *et al.*, 2010), *A. baumannii* AB0057, AB900 and AB307-0294 (Adams *et al.*, 2008), ACICU (Iacono *et al.*, 2008), 17978 (Smith *et al.*, 2007), and SDF and AYE (Vallenet *et al.*, 2008). Table 1 provides specific strain descriptions. Identification and analysis of the various allele homologies were conducted using keyword searches (terms: polymerase V, *umuD*, *umuC*, *dinP*, *lexA*) and BLAST analyses on the MaGe interface on the MicroScope platform of Genoscope (Vallenet *et al.*, 2006, 2009). The CLUSTAL W2 Multiple Sequence Alignment program at EMBL-EBI (www.ebi.ac.uk) and BLAST programs of NCBI (www.ncbi.nlm.nih.gov/BLAST) were also used. The program PAUP* 4.1b was used for bootstrap analysis.

UV-induced mutagenesis. The frequency of rifampicin-resistant mutants arising due to UV-C exposure was measured as an indication

Table 1. Strains used in wet laboratory experiments versus *in silico* analyses

BHI, Brain heart infusion.

Species	Strains used in UV mutagenesis and survival experiments		Strains used in <i>in silico</i> analyses	
	Strain	Medium, temperature (°C)*	Strain	GenBank accession no.
<i>A. baumannii</i>	ATCC 19606 ^T	Nutrient, 37	ATCC 19606 ^T	ACQB00000000, FJ172295 (this study)
	ATCC 17978	Nutrient, 37	ATCC 17978	CP000521
	AB0057	Nutrient, 37	AB0057	ACYR000000000
	–	–	ACICU	ACYQ000000000
	–	–	AYE	ACYS000000000
	–	–	SDF	CP000863–CP000865
	–	–	AB056	CU459141
	–	–	AB058	CU468230
	–	–	AB059	CP001182
	–	–	AB900	ADGZ000000000
	–	–	AB307-0294	ADHA000000000
	–	–	6013113	ADHB000000000
	–	–	6013150	ABXK000000000
<i>A. baylyi</i>	ADP1	Nutrient, 37	ADP1	CR543861
	NIPH 838 ^T	Blood, 30	–	–
	ATCC 17924 ^T	Nutrient, 26	–	–
	ATCC 23055 ^T	Nutrient, 30	RUH 2202	ACPK000000000
	LMG 988 ^T	Nutrient, 30	–	–
	ATCC 51551	Blood, 30	–	–
	NIPH 2150 ^T	Nutrient, 30	–	–
	ATCC 17906 ^T	Nutrient, 26	ATCC 19194	ADMT000000000
	ATCC 17909 ^T	Nutrient, 26	SH 046	ACPL000000000
	ATCC 17908 ^T	Nutrient, 26	SH 205	ACPM000000000
	ATCC 15309 ^T	BHI, 37	SH 145	ACPN000000000
	ATCC 17903	Nutrient, 37	RUH 2624	ACQF000000000
	–	–	DR1	CP002080
<i>A. parvus</i>	NIPH 384 ^T	Blood agar, 30	–	–
	ATCC 19004 ^T	Nutrient, 37	ATCC 19004 ^T	EF583611 (this study)
	–	–	SH 024	ADCH000000000
	ATCC 43998 ^T	Nutrient, 30	SH 164	ACPO000000000
	–	–	SK 82	ACVR000000000
	ATCC BAA-618 ^T	Nutrient, 30	–	–
	CCM 7200 ^T	Nutrient, 30	–	–
	ATCC BAA-617 ^T	Nutrient, 30	DSM 16037 ^T	Not available†
	NIPH 177	Nutrient, 30	–	–
	NIPH 375	Nutrient, 30	–	–
	NIPH 706	Nutrient, 30	–	–
	AK001	Nutrient, 30	–	–
	RAG-1 ^T	Nutrient, 30	–	–
<i>A. venetianus</i>	ATCC 17979	BHI, 37	–	–
	ATCC 9957	Nutrient, 37	–	–
	ATCC 17905	Nutrient, 26	–	–
	ATCC 51552	Nutrient, 30	–	–
	ATCC 17988	Nutrient, 37	–	–
	ATCC 49137	Nutrient, 30	ATCC 49137	EF583614 (this study)
	–	–	ATCC 27244	ABYN000000000
	–	–	–	–
	–	–	–	–
	–	–	–	–
	–	–	–	–
	–	–	–	–
	–	–	–	–

*Temperatures reflect ATCC guidelines for all ATCC source strains. Temperatures for all non-ATCC strains are from the journal articles defining each species.

†AciBASE sequence; no GenBank accession no. available.

of UV-induced mutagenesis induced by UV light (Eisenstadt *et al.*, 1994). Late-exponential phase cells grown as indicated in Table 1 in broth medium were harvested, resuspended in PBS and exposed to doses of UV-C light from a Stratagene UV Stratalinker 1800 that resulted in 0.1–1 % survival (doses of 90–375 J m⁻² UV-C, depending on the strain), or a control treatment of no UV exposure. [The frequency of rifampicin-resistant mutants (if present) was dose-dependent (data not shown).] Immediately after UV exposure or a mock exposure, cells were diluted approximately 1:20 in fresh medium and grown, with shaking, at the appropriate temperature (Table 1) for 24 h. All steps after UV exposure until handling the next day were performed in the dark to minimize possible photoreactivation, which has been observed for a few *Acinetobacter* species (Fernández Zenoff *et al.*, 2006). Overnight cultures were plated onto agar media containing 100 µg rifampicin ml⁻¹ or no rifampicin to calculate frequencies of rifampicin-resistant mutants. Although rifampicin resistance occurs due to a limited number of specific mutations, the frequency of rifampicin resistance is often used as an indication of general mutation frequencies in UV-induced mutagenesis experiments (Eisenstadt *et al.*, 1994; Taddei *et al.*, 1995).

UV survival. Cultures grown for ~18 h in nutrient broth were diluted in PBS, plated onto agar medium, left unexposed or exposed to UV-C light at 150 J m⁻² in a Stratagene UV Stratalinker 1800 in the dark. Plates were incubated overnight in the dark to minimize possible photoreactivation. Colonies were counted and c.f.u. ml⁻¹ was calculated. Survival was calculated by dividing c.f.u. ml⁻¹ in the presence of UV exposure by the value in the absence of UV exposure. Statistical tests to assess whether haemolytic or the Acb complex strains had different survival were conducted with the GraphPad InStat program. The Mann–Whitney U test was used due to the non-normality of the sample sets.

RESULTS AND DISCUSSION

In this study, 56 unique strains of *Acinetobacter* were used, representing 20 recognized species, seven unrecognized species or as yet unclassified strains. Table 1 lists the 32 strains used in the UV-induced mutagenesis and UV sensitivity experiments, which are the type strains of a wide variety of species within the genus, as shown, for example, in 16S rRNA gene-based dendrograms (Vanechoutte *et al.*, 2006). However, most of the 27 strains for which genome sequence data are available online are not type strains. Thus most of the *in silico* analysis was performed on the genome sequences of different strains of the same species as used for the UV-induced mutagenesis and UV survival experiments. Table 1 shows the relationship between strains used in these experiments versus the *in silico* analyses.

Both *umuDAB* and *umuD* alleles are abundant in *Acinetobacter* species

In strain ADP1, the two unique characteristics of the ADP1 *umuD* gene relative to *E. coli*, namely its extra 5' region and the presence of five additional amino acids after the putative catalytic lysine residue in its C terminus, distinguish it from typical *umuD* alleles found in *E. coli* or *Legionella*, and warrant referring to the UmuD-like protein encoded by ADP1 as UmuDAB (Hare *et al.*, 2006). The fragmented and incomplete *umuC* allele in ADP1

further differentiates the ADP1 *umuDC* operon from other *umuDC* operons.

Conventional PCR using primers CL0 and CL5 to amplify the *umuDAB* allele from genomic DNA of 16 non-ADP1 *Acinetobacter* strains was unsuccessful, although 16S rRNA gene sequences were successfully amplified from their DNA. However, a touchdown PCR method amplified a 504 bp region of the 612 bp *umuDAB* gene from three of these *Acinetobacter* strains (*A. baumannii* 19606^T, *A. pittii* 19004^T and *Acinetobacter* sp. ATCC 49137). The PCR products from these strains were cloned, sequenced and showed 75–77 % nucleotide identity to *umuDAB*, including the extra 5' region of the gene.

In this study, we used these cloned PCR products, the 25 sequenced *Acinetobacter* genomes contained in the Genoscope database and the sequence of *A. ursingii* DSM 16037^T in the Acibase database (Table 1). We analysed these sequences to determine whether the *umuDAB* allele present in *A. baylyi* ADP1 was present throughout the genus, whether intact *umuDC* operons existed in this genus and whether the DNA damage-regulated *ddrR* gene was linked to this *umuDAB*, as in ADP1. A surprising number and variety of combinations of these genes (*ddrR*, *umuDAB*, *umuD* and *umuC*) was present throughout the genus. Most strains contained at least two *umuD*–*umuC* loci of some type (Table 2), all located on the bacterial chromosome, unlike in other species where *umuDC* operons may be also found on plasmids (McNally *et al.*, 1990; Permina *et al.*, 2002). Four major locus types were observed and classified based on their size and encoded motifs described below: (A) *umuDAB* encoding 198–203 aa, linked to *ddrR* and *umuC*; (B) *umuDAB* encoding 198–203 aa, linked only to *ddrR*; (C) *umuD* encoding 161–166 aa, linked only to *umuC*; and (D) *umuD* encoding a protein of intermediate or unusually large size (188–192, or 208–211 aa), linked only to *umuC* (Table 2).

Other forms of *umuD* alleles were also found: *A. ursingii* DSM 16037^T possessed a *umuDAB*–*dinP* operon, and *A. lwoffii* SH 145 possessed only a chimeric *umuD* allele with features of both ADP1 and *E. coli* UmuD proteins. This chimeric *umuD* encoded a 191 aa protein of intermediate size (between type A/B and D sizes) with an extra N-terminal region homologous to UmuDAB, but lacked the extra C terminus amino acids characteristic of all other UmuDAB forms (types A, B and D). Type D *umuD* alleles also constitute chimeric *umuD* alleles, but of the opposite configuration as the *umuD* of *A. lwoffii*: any N-terminal region they possessed was not homologous to UmuDAB, but they did possess an extra C-terminal 19 amino acids where ADP1 possessed five.

The most common combination of these allele types in the 12 non-*baumannii* strains was an *E. coli*-like *umuDC* operon (type C) combined with either a *ddrR*–*umuDAB*–*umuC* (type A) or *ddrR*–*umuDAB* (type B) locus. In the 14 *A. baumannii* strains, which all possessed the *ddrR*–*umuDAB* (type B) locus except SDF, half of these strains

Table 2. Combinations of *ddrR*, *umuDAb*, *umuD* and *umuC* genes present in various *Acinetobacter* strains

Locus type designation	Locus containing:	Non- <i>baumannii</i> strains	<i>A. baumannii</i> strains
A	<i>ddrR-umuDAb-umuC</i>	<i>A. haemolyticus</i> , <i>A. sp.</i> 27244, <i>A. junii</i> , <i>A. baylyi</i> *	
B	<i>ddrR-umuDAb</i>	<i>A. calcoaceticus</i> , <i>A. pittii</i> SH024, 'A. <i>oleivorans</i> ', <i>A. nosocomialis</i>	19606, 17978, 6013113, 6013150, 6014059, ACICU‡, AB0057‡, AYE‡, AB056, AB058, AB059, AB900, AB307–0294
C	<i>umuD-umuC</i> (<i>E. coli</i> -like <i>umuD</i>)	<i>A. calcoaceticus</i> †, <i>Acinetobacter sp.</i> 27244†, <i>A. pittii</i> SH024†, <i>A. nosocomialis</i> , 'A. <i>oleivorans</i> ' (2 loci§)	19606†, 17978†, AB0057† ‡, 6013113†, 6013150, AB056, AB059
D	<i>umuD-umuC</i> (chimeric <i>umuD</i>)	<i>A. haemolyticus</i> , <i>A. johnsonii</i> (2 loci), <i>A. ursingii</i> (2 loci)	

*The *A. baylyi umuC* gene is split into two incomplete fragments.

†Evidence suggests acquisition through horizontal gene transfer.

‡Multi-drug resistant strains (Adams *et al.*, 2008).

§One is horizontally acquired, one is not.

also possessed an *E. coli*-like *umuDC* (type C) locus. None possessed a *umuDAb-umuC* allele, as suggested by an earlier report (Robinson *et al.*, 2010). Neither the type nor the number of *umuD* loci was associated with the status of these *A. baumannii* strains as international clones status 1, 2 or 3, their of drug resistance (see Table 2) or their invasion ability. This variation is consistent with the significant variation found by multi-locus sequence typing within this species (Sahl *et al.*, 2011). The absence of *umuDAb*, *umuD* and *lexA* genes in *A. baumannii* SDF is consistent with the findings of Vallenet *et al.* (2008), who observed numerous insertion sequences causing gene loss in this strain.

In spite of the encoded variation among allele types (amino acid identity to UmuDAb was 78–82 % in type A and B loci, 49–53 % in type C and only 27–31 % in type D), there was significant homology between the *Acinetobacter* proteins and the *E. coli* UmuD (26–43 % amino acid identity across types A–D). The catalytic serine and lysine amino acids required for UmuD self-cleavage in *E. coli*, as well as the Ala/Gly RecA*-mediated predicted self-cleavage site (Cys–Gly in *E. coli*), were encoded by all of the *umuDAb* and *umuD* alleles. This conservation of protein motifs suggests that self-cleavage may be possible for both the UmuDAb and the UmuD proteins of *Acinetobacter*. Experiments are under way to determine whether UmuDAb can self-cleave, and if so, whether this cleavage is a part of the *Acinetobacter* DNA damage response or required for UmuDAb regulatory action on *ddrR*.

In examining other DNA damage-related properties, the observation that strain ADP1 does not contain any *lexA* gene (Hare *et al.*, 2006) was also extended throughout the genus. Searches via BLASTP of these 26 strains with the LexA sequence of *E. coli* as well as the more closely related *Pseudomonas aeruginosa* yielded hits only to *umuDAb*- and *umuD*-encoded proteins, in spite of genes annotated as

lexA in six *Acinetobacter* species. The absence of LexA, while not common, has been observed in diverse bacteria such as *Mycoplasma*, *Chlamydia*, *Borrelia*, *Helicobacter* and *Coxiella* (Campoy *et al.*, 2002; Mertens *et al.*, 2008), and shows that alternative mechanisms of regulating responses to DNA damage exist in bacteria.

The *umuDAb* allele is ancestral in *Acinetobacter*, while *umuDC* was probably acquired through horizontal gene transfer

Initial analysis of nucleotide similarity between alleles in these different strains suggested that members of the Acb group shared a form of the *umuDAb* gene slightly different from that of the ADP1 *umuDAb*. Upon further multiple alignment analysis of all individual *umuDAb* and *umuD* sequences with CLUSTAL W (Larkin *et al.*, 2007), a genealogy based on homology between type A–D *umuD* alleles was constructed (Fig. 1). This phylogram indicated that the clustering of these alleles corresponded not only to nucleotide homology between *umuD* and *umuDAb* alleles, but also to their local gene environment. Type A loci, containing *ddrR-umuDAb-umuC*, clustered in a separate clade from type B loci, containing only *ddrR-umuDAb*. Furthermore, all Acb strains tested (*A. calcoaceticus*, *A. pittii*, *A. nosocomialis* and *A. baumannii*) possessed a type B locus but not a type A locus, indicating that the type B locus defines this subgeneric clade. These results were supported strongly by bootstrap analyses, and are consistent with multilocus sequence typing and *rpoB* gene sequence analyses that also place Acb group members in a subgeneric clade (Nemec *et al.*, 2011).

Interestingly, keyword searches and BLAST analyses of the sequenced genomes of *Psychrobacter* and *Moraxella*, members of the family *Moraxellaceae* to which *Acinetobacter* belongs, revealed no *umuD* or *umuDAb* genes to use as

Fig. 1. Gene phylogeny of the *umuD* alleles present in *Acinetobacter* species, showing distinct groupings that correlate to their local genetic environment (adjacent to either *ddrR*, *umuC* and/or *dinP*). The tree was constructed based on the nucleotide sequences of the various *umuD* alleles, using neighbour-joining methods within the CLUSTAL W program of EMBL-EBI to generate the tree. Numbers at each node are levels of bootstrap support (percentages of 1000 samples) determined using PAUP*. Outgroups used were *MucA* and *UmuD* sequences from *P. aeruginosa*, *Shewanella baltica* OS223, *E. coli* and *Vibrio cholerae* B33. Asterisks indicate loci that display evidence of having been acquired through horizontal gene transfer. Brackets on the right of the diagram indicate the type of *umuD* locus (denoted A–D) found in each clade. The first four letters of each abbreviation in the phylogram refer to the genus and first three letters of each species name, followed by the strain designation, and (where present) the abbreviation ‘sh’ at the end of a strain refers to the shortest, type C, form of the *umuD* allele. Strains of each species correspond to strain names given in Table 1, e.g. Apit=*A. pittii* 19004 or SH024.

outgroups. Consequently, outgroup *mucA* and *umuD* gene sequences from a species (*P. aeruginosa*) in the same order as well as in other orders (*Alteromonadales*, *Enterobacteriales* and *Vibrionales*) were used and grouped outside the *umuD* alleles of *Acinetobacter* species, with the *Pseudomonas mucA* and *umuD* gene sequences grouping closest to *Acinetobacter*.

In eight of 13 strains possessing a type C locus, the *umuDC* operon displayed evidence of acquisition through horizontal gene transfer (located adjacent to transposases or bacteriophage integrases). In *A. pittii* SH 024 and *A. baumannii* 19606^T, 17978 and AB0057, the *umuD*–*samB*/*rumB* gene cluster was adjacent or very near to an alanyl-tRNA synthase/phage integrase gene pair, which is often a sign of bacteriophage-mediated gene insertion (Hacker & Kaper, 2000). Similar placements of *umuDC* near integrase, bacteriophage and/or transposase genes were observed in *A. calcoaceticus*, *A. baumannii* 6013113, *Acinetobacter* sp. A27244 and ‘*A. oleivorans*’ DR-1. This association of genes encoding polymerase V components with mobile genetic elements such as transposons and bacteriophages has been

previously observed in both Gram-negative and Gram-positive bacteria (Permina *et al.*, 2002). These data suggest that the *E. coli*-like, shorter *umuD* allele is not the ancestral form in this genus, with the *umuD* gene having been acquired more recently through horizontal gene transfer. Supporting this conclusion is the observation that all *Acinetobacter* strains except for *A. baumannii* SDF and *Acinetobacter radioresistens* possessed the unusual *umuDAb* allele, whereas only half of all strains possessed the type C *umuD*. Furthermore, no *Acinetobacter* strain possessed a type C locus as its only *umuD* gene. Finally, in no *Acinetobacter* strain was a type C *umuD* found adjacent to *ddrR*, further suggesting the shared evolution of both *umuDAb* and *ddrR*.

An additional characteristic indicating the likely shared origin of the *umuDAb* allele was the encoding of additional amino acids in the C terminus of the protein, immediately after the Lys–Arg motif required for cleavage in *E. coli* (Sutton *et al.*, 2001). All *umuDAb* alleles examined in this study, whether type A or B, also encoded five additional

amino acids of varying identity at this location, type D *umuD* alleles encoded an additional 19 amino acids, but none of the type C alleles encoded additional amino acids. The features constituting the consensus *umuDAB* gene in *Acinetobacter* that emerges from this study (types A, B and D) are shown in Fig. 2.

The possession of both *ddrR-umuDab* and *umuD-umuC* (and the horizontal gene transfer of the latter), and the homology to *umuDAB* of Acb group species, suggests that *Acinetobacter* sp. DR1, recently proposed to be the type strain of '*A. oleivorans*' (Kang *et al.*, 2011), also belongs to the Acb group. Similar results observed in *Acinetobacter* sp. 27244 imply that this strain may also be an Acb group member, although the evidence is weaker.

Species conducting DNA damage-induced mutagenesis are the emerging pathogens *A. baumannii* and *A. ursingii*

The only strain of *Acinetobacter* in which UV-inducible DNA repair has been previously investigated is *Acinetobacter* sp. Ac.78, which lacks both phage induction and UV-induced mutagenesis (Berenstein, 1987). Both this strain and the frequently studied ADP1 strain of *A. baylyi* were named *A. calcoaceticus* when the genus only contained one species. However, strain ADP1 was recently placed in *A. baylyi* (Vanechoutte *et al.*, 2006), creating a need to determine whether *A. baylyi* ADP1, like *Acinetobacter* sp. Ac.78, also lacks the capacity for mutagenesis in response to DNA damage. The previously observed defective *umuC* allele of ADP1 (Barbe *et al.*, 2004; Hare *et al.*, 2006) suggests that ADP1 would also lack UV-induced mutagenesis. The presence of intact *umuDC* or *umuDAB-umuC* operons typically associated with UV-induced mutagenesis (Nohmi, 2006) in half of all sequenced *A. baumannii* strains, and nine of 11 other *Acinetobacter* strains, however, suggests that many *Acinetobacter* species may be mutagenic upon UV exposure. As *A. baumannii*, other Acb group members and even non-Acb group species are associated with nosocomial infections (Turton *et al.*, 2010), it is critical to understand whether this genus has the capacity to mutate in response to

DNA-damaging events that the bacteria are likely to encounter, such as UV light or antibiotic exposure.

Acinetobacter strains belonging to different recognized phenotypic clusters (Acb group, haemolytic species) were assayed for UV-induced mutagenesis activity by measuring the increase in the frequency of resistance to rifampicin after exposure to UV-C light and subsequent overnight growth. Additionally, all of the *umuD* or *umuDAB* alleles (types A–D) shown in Table 2 were represented in the strains tested. Unlike wild-type *E. coli* AB1157, which showed an approximately 370-fold increase of mutation frequency to rifampicin resistance in UV-exposed cells versus unexposed cells (which was dependent on the presence of *umuD*), there was no significant difference between the mutation frequency of UV-exposed cells versus unexposed cells in most *Acinetobacter* strains: ADP1, Acb complex members *A. baumannii* 19606^T, *A. pittii* and *A. nosocomialis*, haemolytic species *A. gyllenbergii*, *A. haemolyticus* and genomic group 14TU, and *A. johnsonii*, *A. bereziniae*, *A. radioresistens* and *A. schindleri* (Fig. 3). The ratio of rifampicin resistance between UV-exposed and control cells in these strains was below 1.5-fold. This lack of error-prone polymerase activity is surprising, given the presence of *dinP* and intact *umuDC* and/or *umuDABC* operons in all of these strains except ADP1 and *A. radioresistens*, but is consistent with the lack of *lexA* in *Acinetobacter* that may signify variation from the typical SOS response.

Like the diversity of *umuDC* genes in this genus, however, there was diversity in this error-prone response phenotype. Of the seven *A. baumannii* strains that possessed an intact *umuDC* locus, three were tested for UV-induced mutagenesis activity (AB0057, 17978 and 19606^T), and two (AB0057 and 17978) possessed this phenotype (Fig. 3). Additionally, all *A. ursingii* strains tested (BAA-617^T, NIPH 177, NIPH 375, NIPH 706; Nemec *et al.*, 2000, 2001), AK001 (Kilic *et al.*, 2008; Nemec *et al.*, 2001, 2008) and *A. beijerinckii* also consistently displayed UV-induced mutagenesis. The mutations were only observed after overnight growth, as no rifampicin-resistant mutants were present immediately after UV-C exposure (data not shown). The increase in rifampicin resistance after UV exposure was variable, ranging from 18-fold in *A. baumannii* AB0057 to 330-fold in *A. baumannii* 17978, 15- to 427-fold in the five *A. ursingii* strains, and 25-fold in *A. beijerinckii* (Fig. 3). There was no association of UV-induced mutagenesis with the drug-resistance status of the *A. baumannii* strains (Table 2), nor were there significant homology differences among the *umuDC* loci of these three strains. However, the observation that the first and third most common pathogens in this genus (*A. baumannii* and *A. ursingii*, respectively; Turton *et al.*, 2010) can undergo mutagenesis after DNA damage is crucial to understanding the evolution of these emerging pathogens.

A. ursingii is notable for having three *umuD* genes: *umuDAB-dinP* and two type D *umuDC* loci. Although all *Acinetobacter* strains possess a *dinP* gene, only *A. ursingii*

Fig. 2. Features observed in *umuD*-encoded proteins of *Acinetobacter* type A–D loci. Amino acid identities or features are identified within the boxes. 'AG' refers to the site of *E. coli* UmuD self-cleavage (in *E. coli*, the UmuD cleavage site is CG, as opposed to AG in most gammaproteobacteria), and 'S' and 'K' are the catalytic residues required for self-cleavage.

Fig. 3. UV-induced mutagenesis capability as indicated by frequency of rifampicin-resistant mutants after exposure to UV-C light (black bars) or no exposure (white bars). Ec, *E. coli* AB1157; Ec uD, *E. coli* AB1157 $\Delta umuD::kanR$; Abay, *A. baylyi* ADP1; Ab19, *A. baumannii* 19606^T; AbAB, *A. baumannii* AB0057; Ab17, *A. baumannii* 17978; Anos, *A. nosocomialis*; Apit, *A. pittii* ATCC 19004; Ahae, *A. haemolyticus*; 14TU, genomic group 14TU; Ajoh, *A. johnsonii*; Asch, *A. schindleri*; Aber, *A. bereziniae*; Arad, *A. radioresistens*; Aurs, *A. ursingii* BAA-617^T; Abei, *A. beijerinckii*. The bars show mean $\pm$ SD from at least three experiments per strain.

possesses a *umuD*–*dinP* locus, raising the possibility that this unique locus might be associated with its capacity to conduct UV-induced mutagenesis. The UmuD₂ homodimer can interact with DinP (DNA pol IV), and alter its mutagenic activity in *E. coli* (Foti *et al.*, 2010; Godoy *et al.*, 2007). Supporting this possibility is the distinctiveness of this *dinP*–linked *umuD* allele, as strongly supported by bootstrap analysis (Fig. 1), and the lack of UV-induced mutagenesis seen in other strains possessing type D loci, which is the only other type of locus that *A. ursingii* possesses. However, a *umuD*–*dinP* arrangement is not present in either *A. baumannii* 17978 or AB0057, suggesting a lack of consensus of DNA damage responses within this genus. Our data suggest that the type A and B *umuDAB* loci may not support UV-induced mutagenesis, but because this phenotype is rare in *Acinetobacter*, the role of UmuDAB and UmuD in the DNA damage response of *Acinetobacter* remains unknown. This UV-induced mutation may be part of an SOS response, but crucial aspects of the canonically defined SOS response, such as LexA involvement in gene regulation, have not been yet demonstrated in these species.

***Acinetobacter* strains are highly variable in their UV survival rate**

To understand whether the diversity of gene loci and phenotypes related to error-prone DNA damage repair in *Acinetobacter* was also present in their error-free responses, we compared the survival of strains after exposure to levels of UV-C light sufficient to induce UV mutagenesis. We hypothesized that this variance in survival would be independent of the presence and type of *umuDC* locus, and of the ability to conduct UV-induced mutagenesis, as *umuDAB* and *umuD* mutants show no obvious defect in UV survivorship (Hare *et al.*, 2006). A UV dose was chosen based on its ability to yield suitable survival for the UV-induced mutagenesis experiment in roughly half of the

strains (150 J m⁻²). Percentage survival varied over five orders of magnitude, showing that although *Acinetobacter* species share an unusual *umuDAB* allele, present in various configurations, they vary tremendously in their ability to survive UV exposure (Fig. 4). Whereas *A. radioresistens*, a generally radiation-resistant species (Nishimura *et al.*, 1994), had the second highest survivorship after 150 J m⁻² of UV exposure (66%), strain ADP1 showed the fifth highest survival (42%). By comparison, *E. coli* AB1157 displayed a low (0.01%) survivorship at this level of UV exposure, with only three *Acinetobacter* strains having poorer survival.

To test whether this difference in survival was idiosyncratic to each strain, we tested five *A. ursingii* strains for their homogeneity of UV survival, and observed very consistent survival phenotypes in four of the five strains (range 10–15%), with the additional strain having a survival rate of 28% (Fig. 4). Similarly, close survival percentages were observed between the two strains of *A. guillouiae* tested (see Fig. 4). Given the tremendous range of variation across the genus, this suggests that while UV survival mechanisms are highly variable between species, they may be consistent within each species. However, as hypothesized, there was no association between the type of *umuDC* locus and strain survival, although two of the top five survivors lacked any of the type A–D *umuD* alleles.

Survival after UV exposure was, however, associated with belonging to either the Acb group or a haemolytic species [*A. glylbergii* and genomic groups 6, 13, 14 and 16 (Tjernberg & Ursing, 1989)]. Each of these groups had a statistically lower survivorship than non-Acb or non-haemolytic species (each group tested independently with $P < 0.05$ in a Mann–Whitney U test). As UV-C-emitting appliances are being tested for use in hospital rooms to decontaminate them from pathogens such as multi-drug resistant *A. baumannii* (Rutala *et al.*, 2010), developing an understanding of these pathogens' relative resistance to UV light is important.

Fig. 4. Comparison of the survival of *Acinetobacter* strains after UV exposure (150 J cm^{-2}). Acal, *A. calcoaceticus*; Ajoh, *A. johnsonii*; Abau, *A. baumannii* 19606^T; Aven, *A. venetianus*; Aber, *A. bereziniae*; Ahae, *A. haemolyticus*; Anos, *A. nosocomialis*; Ajun, *A. junii*; Agu1, *A. guillouiae* LMG 988^T; Atje, *A. tjernbergiae*; Aani, *Acinetobacter* sp. ATCC 49137; Agu2, *A. guillouiae* ATCC 51551; Agyl, *A. gyllenbergii*; Abei, *A. beijeirickii*; Au1–Au5, *A. ursingii* BAA-617^T, AK001, 177, 375 and 706, respectively; Apit, *A. pittii*; Abay, *A. baylyi*; Apar, *A. parvus*; Asch, *A. schindleri*; Arad, *A. radioresistens*; Alwo, *A. lwoffii*. 'gs-' or '-TU' indicate genomic species. Grey bars indicate *A. guillouiae* strains; white bars indicate *A. ursingii* strains. The bars show mean $\pm$ SD from at least three experiments per strain.

To our knowledge, this is the first survey of intra-genus differences in responses to UV radiation in bacteria. Other studies investigating differences in UV survivorship have focused on bacteria isolated from one particular type of environment, such as high-altitude lakes or other extreme environmental conditions (Fernández Zenoff *et al.*, 2006). In one such study, a novel *A. johnsonii* strain designated A2 was isolated from Andean wetlands and was found to be significantly more resistant to UV-B than *A. johnsonii* ATCC 17909^T (Di Capua *et al.*, 2011), which we also observed to be very sensitive to UV-C radiation. Our observation that *A. radioresistens* is highly UV-C resistant compared with other *Acinetobacter* strains is consistent with previous findings that *A. radioresistens* is four- to eightfold more resistant to gamma radiation (Saha & Chopade, 2009) and ~28-fold more resistant to ionizing radiation (Nishimura *et al.*, 1994) than a limited set of other *Acinetobacter* strains, although the range of difference in survival was significantly greater (~300 000 fold) in this study than in these previous works.

This study indicates that DNA damage responses within a genus can vary tremendously, both in their error-prone (genetic variation in *umuDC* operons and UV-induced mutagenesis phenotypes) and in their error-free (UV survival) phenotypes. However, members of the Acb complex form a distinct group within the genus *Acinetobacter*, based on *umuDab* homologies, possession of *ddrR–umuDab* and their low UV survival rate.

Both the structure of the *ddrR–umuDab* gene cluster and the lack of DNA damage-induced mutagenesis across most of this genus indicate that the distinctive DNA damage-related

features observed in ADP1 probably represent novel (albeit not completely conserved) DNA mechanisms throughout the genus, rather than an isolated example of a mutation in ADP1 alone. Work to uncover the mechanisms of action of the novel UmuDab regulatory phenotype will enhance and broaden our understanding of cellular responses to DNA damage.

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