The effects of the rulAB operon of Pseudomonas syringae on mutagenic DNA repair and the transcriptional regulation of rulAB following irradiation with UV-B wavelengths were determined. For a rulB::Km insertion mutant constructed in P. syringae pv. syringae B86-17, sensitivity to UV-B irradiation increased and UV mutability decreased by 12- to 14-fold. rulAB-induced UV mutability was also tracked in phyllosphere populations of B86-17 for up to 5 days following plant innoculation. UV mutability to rifampin resistance (Rif\(^{\text{R}}\)) was detected at all sampling points at levels which were significantly greater than in nonirradiated controls. In P. aeruginosa PAO1, the cloned rulAB determinant on pJJK17 conferred a 30-fold increase in survival and a 200-fold increase in mutability following a UV-B dose of 1,900 J m\(^{-2}\). In comparative studies using defined genetic constructs, we determined that rulAB restored mutability to the Escherichia coli umuDC deletion mutant RW120 at a level between those of its homologs mucAB and umuDC. Analyses using a rulAB::inaZ transcriptional fusion in Pseudomonas fluorescens P15 showed that rulAB was rapidly induced after UV-B irradiation, with expression levels peaking at 4 h. At the highest UV-B dose administered, transcriptional activity of the rulAB promoter was elevated as much as 261-fold compared to that of a nonirradiated control. The importance of rulAB for survival of P. syringae in its phyllosphere habitat, coupled with its wide distribution among a broad range of P. syringae genotypes, suggests that this determinant would be appropriate for continued investigations into the ecological ramifications of mutagenic DNA repair.

The involvement of bacterial plasmids in increasing the survival of their hosts following irradiation with UV wavelengths was first reported in 1965 by Howarth (13), who was working with the ColIb-P9 plasmid in Salmonella enterica serovar Typhimurium LT2. Howarth also noted that the frequency of mutants in irradiated cultures of serovar Typhimurium LT2 (ColIb-P9) was increased (14). These two initial observations have been followed by the discovery that a large number of bacterial plasmids from many incompatibility groups confer phenotypes of increased UV survival and mutability (43, 52). Genes conferring the UV mutability phenotype are also chromosomally located in some cases, an important example being the umuDC operon of Escherichia coli.

The umuDC operon is one component of the SOS regulon of E. coli, a set of approximately 20 unlinked genes which are coordinately regulated in response to DNA damage (42). The umuDC operon is regulated by the recA and lexA gene products, with the UmuDC and RecA proteins alone required for UV mutability (42). LexA functions as a repressor through binding to a conserved DNA sequence (SOS box) located within the promoter region of SOS regulon genes (3, 25). The irradiation of E. coli cells with UV wavelengths results in the occurrence of DNA lesions of which the cyclobutane pyrimidine dimer and the pyrimidine(6-4)pyrimidinone photoproduct are the most typical (8); these lesions can result in a blockage of DNA polymerase activity, leading to a stalling of replication. Cellular perception of DNA damage is thought to occur through the binding of RecA to single-stranded DNA immediately downstream of a DNA lesion; during this process, RecA is converted to an activated form (RecA\(^{\text{A}}\)) (42). RecA\(^{\text{A}}\) then mediates a self-cleavage reaction of LexA, resulting in the removal of LexA and allowing the expression of the SOS response genes (24).

Following the expression of umuDC, a posttranslational self-modification of UmuD (removal of the first 24 amino acids of UmuD) mediated by RecA\(^{\text{A}}\) is required to generate the active UmuD\(^{\text{A}}\) protein (5, 40). UmuD\(^{\text{A}}\) forms a homodimer (UmuD\(^{\text{A}}\)\(_2\)) which complexes with UmuC, resulting in the mutagenically active UmuD\(^{\text{A}}\)UmuC complex (4). The function of the UmuD\(^{\text{A}}\)UmuC complex in mutagenic DNA repair (MDR) occurs as a result of translesion DNA synthesis (42, 50). The model for replicative lesion bypass includes DNA pol III holoenzyme complex, the UmuD\(^{\text{A}}\)UmuC complex, and RecA\(^{\text{A}}\) (48). However, recent evidence has shown that the UmuD\(^{\text{A}}\)UmuC complex itself may be sufficient in translesion synthesis with the possibility of either the UmuD\(^{\text{A}}\)UmuC complex or UmuC itself functioning as a DNA polymerase (36, 49).

To date, five distinct plasmid-carried umuDC homologs have been characterized at the sequence level. These include imp-CAB, mucAB, rulAB, rumAB\(_{p391}\), and samAB (19, 26, 31, 33, 46). Each of these sequences contains a consensus LexA-binding site within the respective promoter regions and a conserved internal cleavage site within the umuD homolog. With the exception of rulAB, the other known MDR systems were isolated from enterobacteria and have not been well characterized in terms of their contribution to the ecological fitness of their hosts. The rulAB operon was originally cloned from...
pSR1, an indigenous plasmid from *Pseudomonas syringae* pv. *syringae* A2, and was initially characterized for its role in UV radiation (UVR) tolerance (46). Although not absolutely required for survival, the UVR tolerance phenotype conferred by *rulAB* was subsequently shown to increase *P. syringae* populations by 10- to 30-fold in its leaf surface (phyllosphere) habitat (47). *rulAB* is the most distantly related to *umuDC* of the other plasmid-carried *umuDC* homologs, as *rulA* and *rulB* share only 30.9% and 41.5% amino acid similarity to *umuD* and *umuC*, respectively (42). However, *rulAB* does share the important features of this group, including its function in UV tolerance and its lack of expression and activity in a *P. syringae recA* background (46). Recent evidence has also shown that *rulAB* is widely distributed among strains within and among pathovars of *P. syringae* (39, 47), suggesting the importance of this determinant to a wide range of genotypes.

Our laboratory is interested in further elucidating the role of the *rulAB* system in the population biology of *P. syringae*. To that end, we are fully characterizing the functional roles of *rulAB* (UVR tolerance and MDR), along with analyzing the regulation of this determinant so that we can ultimately address the biological significance of the *rulAB* system to *P. syringae* in its natural environment. Our studies reported here utilize UV-B (290 to 320 nm) wavelengths, which is in contrast to most analyses of DNA repair and UVR-induced mutagenesis in microorganisms, where higher-energy UV-C (254 nm) wavelengths are used. In nature, UV-C wavelengths are screened by the stratospheric ozone layer and do not reach the earth’s surface; thus, the use of UV-B wavelengths is relevant from an ecological standpoint. The most important types of UV-B-induced DNA lesions appear to be cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidone photoproducts (30). Ecological studies have shown that solar UV-B radiation has profound deleterious effects on microorganisms dwelling in locations with high sunlight exposure, including surface aquatic habitats and the phyllosphere (11, 15, 45).

In this study, we report an analysis of the MDR capacity of *rulAB* and an analysis of mutability in a *P. syringae* strain containing a stable insertional mutation within *rulAB*. We also examined the MDR potential of *rulAB*-containing *P. syringae* in planta and showed that UVR-induced mutability occurs in leaf surface populations at rates similar to those observed in vitro.

The instructions of the manufacturer. Hybridizations at 65°C followed by high-stringency washes were performed as described previously (44).

**Construction of* P. syringae* pv. *syringae* GWS242 (B86-17 *rulAB*).** The random disruption on the *rulAB* plasmid pgB8617A strain B86-17 was created via gene replacement using a homologous recombination strategy. The intact *rulAB* sequence on pgB8617A was initially cloned into plUSB1 vector IlS (43) as a 6.4-kb BamHI fragment (pJ3K1) and subcloned as a partial 3.8-kb EcoRI fragment into pGEM 7z, creating pJ3K12. This plasmid was then digested with *Bst*II, resulting in the removal of a 0.6-kb fragment from within *rulB*. Following *Bst*II digestion, the ends were made blunt by incubation with the Klenow fragment of DNA polymerase (Life Technologies), and the 1.2-kb kanamycin resistant (Kmr) gene cassette (released from pSB56) was ligated in. The entire DNA region was excised as a 4.4-kb XbaI-BamHI fragment and ligated into the suicide gene replacement vector pQ2005K, creating pJ3K16. pJ3K16 contained approximately 1.0 kb of Banking DNA sequences on each side of the *rulAB* (*rulB*) sequence. Following transfer of pJ3K16 into *P. syringae* pv. *syringae* B86-17, those *P. syringae* cells in which a plasmid integration event had occurred were selected on GM supplemented with Gm. Several isolated Gm* colonies were then cultured in LB broth containing Km. After 2 days of incubation, 0.1-ml aliquots were plated onto LB containing Km and 5% sucrose to counterselect against the suc* gene encoded on pQ2005K. The sucrose-resistant (Suc*) colonies recovered were subsequently tested for sensitivity to Gm as a phenotypic assay for loss of the vector sequences. Since there are no *Bam*HI sites within the *rulAB* or the Km* cassette, the increased size of pgB8617A *rulB*-Km was visualized by comparing the sizes of the *Bam*HI fragment from pgB8617A and pgB8617A *rulB*-Km, which hybridized to an internal *rulAB* gene probe consisting of the 0.7-kb HindIII-Porl fragment from pgWS140. Further Southern hybridization of the plasmid DNA were done using the Km* gene cassette released from pSB56 as a probe and identified in. The entire DNA region was excised as a 4.4-kb XbaI-BamHI fragment and ligated into the suicide gene replacement vector pQ2005K, creating pJ3K16. *pJ3K16* contained approximately 1.0 kb of Banking DNA sequences on each side of the *rulAB* (*rulB*) sequence. Following transfer of pJ3K16 into

**Materials and methods.** Bacterial strains, plasmids, and culture conditions. The bacterial strains, plasmids, and specific oligonucleotides utilized which were relevant to this study are listed in Table 1. All bacterial strains were grown in Luria-Bertani (LB) medium (Difco) or King’s medium B (KB) (17). *E. coli* strains and *Pseudomonas aeruginosa* PAO1 were grown at 37°C, and *Pseudomonas fluorescens* PS5 and *P. syringae* pv. *syringae* B86-17 were grown at 28°C. Plasmid transfer from *E. coli* to *Pseudomonas syringae* was accomplished by tripolar mating using the helper plasmid pRK2013. The exconjugants were selected on MG medium (16) or KM medium amended with cycloheximide alone (KBc) and KBc containing rifampin. Hybridizations at 65°C followed by high-stringency washes were performed as described previously (44).
This study

other genetic constructs were made by first ligating the

of pET-5a, allowing optimal spacing from a Shine-Dalgarno sequence present on

of the MDR coding sequences was the ATG sequence within the

amplified coding sequence into pJJK20. In each case, the translational start site

umu

firmly as having the correct size on an agarose gel, digested with the appropriate

umuDC

9

..3

9

P.

at

P. aera

3

UV, no detectable plasmids

A. M. Chakrabarty

P. syringae pv. syringae

UV, inaZ reporter vector

27

GWS242 As B86-17 but also rulB::Km

This study

Plasmids

pBluescript SK(+) Ap', cloning vector

Stratagene

pBSI86 Source of Km' cassette

1

pCR2.1 Ap' Km', direct cloning vector for PCR products

Invitrogen

pET-5a Ap', source of Shine-Dalgarno sequence

Promega

pGem7zf Bacterial strains

5

Plasmids

pET-5a Arr, source of Shine-Dalgarno sequence

Promega

pCR2.1 Ap r Kmr, direct cloning vector for PCR products

Invitrogen

pJJK16 4.4-kb rulA

This study

pJJK5 1.7-kb mucAB as BamHI in pGB2

This study

pJJK20 0.75-kb umuDC' promoter as SphI-XbaI in pET-5a

This study

pJJK21 1.7-kb rulAB as NdeI-BamHI from pJJK12 in pJJK20

This study

pJJK22 0.75-kb umuDC' promoter as SphI-XbaI in pJJK5

This study

pJJK23 1.7-kb umuDC as NdeI-EcoRI from pRW154 in pET-5a

This study

pJJK24 0.75-kb umuDC' promoter as SphI-XbaI in pJJK23

This study

pJJK25 2.45-kb umuDC' promoter + rulAB as SalI-BamHI in pJJK23

This study

pJJK26 2.45-kb umuDC' promoter + mucAB as SphI-BamHI in pJJK23

This study

pJJK27 2.45-kb umuDC' promoter + umuDC as SphI-EcoRI in pJJK23

This study

pJJK40 1.1-kb rulAB promoter region in pCR2.1

This study

pJJK41 0.9-kb rulAB promoter as HindIII-EcoRI from pJJK40 in pPROBE KI'

This study

TABLE 1. Bacterial strains, plasmids, and oligonucleotide primers utilized in this study and their relevant characteristics

Strain, plasmid, or oligonucleotide primer

Relevant characteristics or nucleotide sequence

Source or reference

Bacterial strains

E. coli DH10B Plasmid-free strain used for cloning

9

P. aeruginosa RW120 lexA' recA' Δ(umuDC)595::cat

12

P. fluorescens PA01 UV', no detectable plasmids

A. M. Chakrabarty

P. syringae pv. syringae B86-17 UV', contains rulAB on pB8617A

21

GWS242 As B86-17 but also rulB::Km

This study

Plasmids

pBluescript SK(+) Ap', cloning vector

Stratagene

pBSI86 Source of Km' cassette

1

pCR2.1 Ap' Km', direct cloning vector for PCR products

Invitrogen

pET-5a Ap', source of Shine-Dalgarno sequence

Promega

pGem7zf Ap', cloning vector

Promega

pJJK31 Ch', broad-host-range cloning vector

S. E. Lindow

pJQ200SK Gm' sacB, suicide gene replacement vector

34

pPROBE KI' Km', broad-host-range, inaZ reporter vector

pB8617A Spr, 2.8-kb HinHI fragment

This study

pJJK2013 Helper plasmid for triparental matings

7

pRW144 Sp', 2.4-kb mucAB as BamHI in pGB2

12

pRW154 Sp', 2.8-kb umuDC as EcoRI in pGB2

12

pB8617A rulAB', native plasmid from P. syringae pv. syringae B86-17

This study

pGWS140 0.7-kb HindIII-PstI from pSM1 in pBluescript SK(+)

47

pJJK1 6.4-kb rulAB as BamHI from pB8617A in pBluescript SK(+)

This study

pJJK5 1.7-kb mucAB as NdeI-BamHI from pRW144 in pET-5a

This study

pJJK12 3.8-kb rulAB as partial EcoRI from pJJK1 in pGem7zf

This study

pJJK15 1.2-kb Km' cassette as HindIII from pBSL86 in pJJK12 at blunt-ended BssHII

This study

pJJK16 4.4-kb rulA, rulB::Km as XbaI-BamHI in pJQ200SK

This study

pJJK17 3.8-kb rulAB as XbaI-BamHI from pJJK12 in pJJB21

This study

pJJK20 0.75-kb umuDC' promoter as SphI-XbaI in pET-5a

This study

pJJK21 1.7-kb rulAB as NdeI-BamHI from pJJK12 in pJJK20

This study

pJJK22 0.75-kb umuDC' promoter as SphI-XbaI in pJJK5

This study

pJJK23 1.7-kb umuDC as NdeI-EcoRI from pRW154 in pET-5a

This study

pJJK24 0.75-kb umuDC' promoter as SphI-XbaI in pJJK23

This study

pJJK25 2.45-kb umuDC' promoter + rulAB as SalI-BamHI in pJJK23

This study

pJJK26 2.45-kb umuDC' promoter + mucAB as SphI-BamHI in pJJK23

This study

pJJK27 2.45-kb umuDC' promoter + umuDC as SphI-EcoRI in pJJK23

This study

pJJK40 1.1-kb rulAB promoter region in pCR2.1

This study

pJJK41 0.9-kb rulAB promoter as HindIII-EcoRI from pJJK40 in pPROBE KI'

This study

Oligonucleotide primers

mucAB Nde 5' 5'-GGAATTCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

mucAB Bam 3' 5'-GATCGGATCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

rulAB Nde 5' 5'-GGAATTCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

3' rulAB TAA BamHI 5'-GATCGGATCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

rul PX 5'-GGAATTCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

umuDC Nde 5' 5'-GATCGGATCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

umuDC Eco 3' 5'-GATCGGATCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

umu Pro 5' SpII 5'-GATCGGATCCATATGCGATATTTTGTGTCGATTATTTTG-3' This study

umu Pro 3' TCTAGAGCTCGTGCAAGTTAATTAGTACTG-3' This study

a Abbreviations: Ap, ampicillin; Cb, carbenicillin; Gm, gentamicin; Km, kanamycin; Sp, spectinomycin.

b Restriction sites incorporated in primers are underlined. GGATCC, B; GCGATC, b; GATC, a.

rulB TAA BamHI, and pJJK12 for rulAB; and (iii) umuDC Nde 5', umuDC Eco 3', and pRW154 for umuDC. The amplified product from pRW144 was confirmed as having the correct size on an agarose gel, digested with the appropriate restriction enzymes, and ligated into pET-5a, creating pJJK5. A 0.75-kb region upstream of umuDC on the E. coli chromosome was then amplified from pRW154 using the primers inaZ Pro SpII 5' and umu Pro 3', digested, and ligated upstream of the mucAB coding sequence in pJJK5, creating pJJK22. The other genetic constructs were made by first ligating the umuDC promoter into pET-5a, creating pJJK20, and then ligating the respective rulAB or umuDC amplified coding sequence into pJJK20. In each case, the translational start site of the MDR coding sequences was the ATG sequence within the NdeI sequence of pET-5a, allowing optimal spacing from a Shine-Dalgarno sequence present on the peT-5a vector. The final constructs, including the umuDC promoter sequences, Shine-Dalgaro sequence from pET-5a, and the respective MDR coding sequences were ligated into pB321, creating pJJK25, pJJK26, and pJJK27. Each of these plasmids was transferred into E. coli RW120 and P. aeruginosa PA01 and utilized in MDR assays as described above.

Determination of rulAB promoter activity using the inaZ reporter gene. The intergenic sequences between rulAB and repA on pB8617A were fused to the promoterless ice nucleation gene (inaZ) in pPROBE KI' in order to quantify rulAB expression following UV-B irradiation. The primers T7 (Promega) and rul PX were used to amplify a 1.1-kb fragment from pJJK12; the PCR product was directly ligated into pCR2.1, creating pJJK40. A 0.9-kb HindIII-EcoRI fragment was subsequently excised from pJJK40 and ligated into pPROBE KI', resulting...
in a transcriptional fusion of the *rulAB* promoter region with *inaZ* in the final construct, pJKK41. Ice nucleation activity (INA) was chosen as a reporter because of the sensitivity with which INA can be detected and the large range of INA which can be quantified (26). INA is also easily quantified in situ, and our future experiments would be geared toward *rulAB* expression analysis in the phyllosphere. Unfortunately, most strains of *P. syringae* produce the ice nucleation protein naturally; however, we felt that the sensitivity of the reporter would enable us to detect differences in expression resulting after small differences in the UV-B dose and that it justified the use of *inaZ* even though a different *Pseudomonas* species would be used in the assay.

The *rulAB::inaZ* expression analyses were performed in *P. fluorescens* PV5, an ice nucleation-negative strain, following the confirmation of *rulAB* activity (increased UV-B survival and MDR) in strain PV5. Cells of *P. fluorescens* PV5 (pJKK4) were grown overnight in KB broth containing Km, pelleted by centrifugation, resuspended in 0.01 M potassium phosphate buffer (pH 7.0), and exposed to various UV-B doses using the methods described above. Twenty-five ml of UV-B-irradiated or nonirradiated cells was then cultured in 25 ml of KB broth under dark conditions. Aliquots were withdrawn immediately upon culture and at designated intervals up to 18 h following irradiation and then resuspended in a small volume of 0.01 M potassium phosphate buffer for assessment of INA. The number of ice nuclei per cell was estimated by the droplet-freezing assay as described by Lindow (23), with the number of ice nuclei calculated as the fraction of droplets that froze within 5 min. Dilution plating on KB was used to count the number of viable cells so that the data could be normalized on the basis of numbers of ice nuclei per cell.

**RESULTS**

**Construction and analysis of a *rulB* insertional mutant of *P. syringae* pv. *syringae* B86-17.** The *rulAB* determinant was initially isolated from the indigenous plasmid pB8617A from *P. syringae* pv. *syringae* A2, a pathogen of ornamental pear trees (46). In this and subsequent studies, it was of interest to work with the *rulAB* determinant of a *P. syringae* strain which was pathogenic on bean, a plant host which is more easily utilized in growth chamber studies. A bean-pathogenic strain, *P. syringae* pv. *syringae* B86-17, was chosen for use; this strain has a phenotype of UV tolerance and encodes *rulAB* on its large indigenous plasmid (47). For our experimental analyses, the *rulAB* determinant from pB8617A, the indigenous plasmid harbored by *P. syringae* pv. *syringae* B86-17, was subcloned as a 3.8-kb *XbaI-BamHI* fragment in the broad-host-range low-copy-number vector pJBJ321, creating the plasmid pJKK17.

The function of *rulAB* in elevating UV survival and MDR was evaluated by using *P. syringae* pv. *syringae* GWS242 (B86-17 *rulB::Km*), a strain that was constructed by gene replacement using a sucrose-mediated counterselection system. Confirmation of the insertion of a kanamycin cassette within the *rulAB* determinant from pB8617A, the indigenous plasmid of strain PAO1, a naturally UV-sensitive strain which, according to previous studies, does not contain a functional MDR system within its chromosome (29, 41). We transferred either pJKK17 or the vector pJBJ321 into strain PAO1 by triparental mating and subsequently determined that *rulAB* conferred an increase in UV-B survival to this strain of as much as 30-fold at the highest UV-B dose (Fig. 2A). UV-B mutability was also significant (up to a 200-fold increase in Rif* mutants following irradiation at 1,900 J m⁻²) and clearly inducible by UV-B irradiation (Fig. 2B).

In planta UV-B mutability of *P. syringae* pv. *syringae* B86-17. The role of the *rulAB* determinant in enabling *P. syringae* strains to maintain and increase population size in their natural leaf surface (phyllosphere) habitat has been previously established (47). However, we have not previously assessed UV-B mutability in planta and, to our knowledge, the occurrence of MDR has not been previously shown using any bacteria in their natural habitat. Our studies were done by inoculating populations of *P. syringae* pv. *syringae* B86-17 onto bean leaves, excising the leaves at various sampling times after inoculation, irradiating the leaves with UV-B at 500 J m⁻², and subsequently incubating the excised leaves for 12 h in a sterile,
FIG. 2. (A) Survival of P. aeruginosa PAO1(pJB321) (○) and PAO1(pJJK17) (●) after UV-B irradiation. Each data point represents the mean (± the standard error of the mean) from three replicate UV sensitivity experiments. (B) Analysis of rulAB-mediated MDR in PAO1. Rifr strains were irradiated with different doses of UV-B, samples were removed to initiate cultures that were incubated in LB for 18 h, and the number of Rifr colonies was determined. The number of spontaneous mutations conferring Rifr in the absence of UV-B irradiation has been subtracted. Each data point represents the mean (± the standard error of the mean) from three replicate experiments. Symbols: ○, PAO1(pJB321); ●, PAO1(pJJK17).

TABLE 2. Analysis of rulAB-mediated MDR in P. syringae pv. syringae B86-17 at each sampling point following inoculation to the phylosphere of bean

<table>
<thead>
<tr>
<th>Category of leaf</th>
<th>No. of Rifr mutants per 10^8 cells recovered at each time point (h) after inoculation^a</th>
<th>0 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonirradiated control</td>
<td>3.8* 3.3</td>
<td>3.6</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irradiated at 500 J m⁻²</td>
<td>18.3*** 16.3**</td>
<td>13.0*</td>
<td>13.0*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Values reported are the means from six independent experiments. Significant differences between means at each sampling time are indicated (*, P = 0.05; **, P = 0.01; *** P = 0.001).
The irradiation of Pf5 containing the rulAB promoter-inaZ fusion construct pJK41 with UV-B at 75, 150, and 300 J m$^{-2}$, cultures were initiated using the irradiated cells and samples were removed at designated times for INA analysis. An increase in expression was observed within 30 min at the two higher UV-B doses and within 2 h at the 75-J m$^{-2}$ dose (Fig. 4). The increase in rulAB promoter activity continued until 4 h after irradiation with the observed levels of INA ranging as high as $10^5$ greater than those for the nonirradiated control (Fig. 4). There is an exponential relationship between INA and the abundance of ice nuclei, with INA increasing with the square of InaZ concentration until INA reaches $10^{-3}$ nuclei per cell (22). Between $10^{-1}$ and $10^0$ ice nuclei per cell, INA increases with the third power (cube) of InaZ concentration (28). Thus, quantification of InaZ levels in our experiments reflects the transcriptional activity of the rulAB promoter and, when compared to InaZ levels detected in the control strain containing the pPROBE KI vector, indicates that rulAB transcription was increased up to 261-fold at 4 h after irradiation with the 300-J m$^{-2}$ dose (Fig. 4). Because of the exponential relationship between INA and the abundance of ice nuclei, calculations of fold increases in promoter activity are sensitive to the INA measurements in the nonirradiated control treatment. Thus, the decrease in INA between 4 and 6 h after irradiation in the control treatment was large enough to result in an elevated increase in InaZ levels (Fig. 4, 150- and 300-J m$^{-2}$ treatments) even though the INA observed at these treatments had actually decreased. Overall, a stair-step effect was consistently observed, with the lower UV-B doses resulting in lower promoter activity at all time points. INA levels expressed from the rulAB promoter were still elevated through 18 h after irradiation, although this observation could also be due to the stability of the InaZ protein (Fig. 4).

DISCUSSION

The rulAB operon, which is widely distributed among diverse strains of P. syringae isolated from many plant hosts, plays an important functional role in protecting strains from the DNA-damaging effects of UV-B radiation. Exposure of P. syringae strains to UV-B wavelengths present in solar radiation would be predicted to occur with regularity in the phyllosphere habitat of this organism. Analysis of the UV-B mutability of the rulAB operon in E. coli RW120 indicated that rulAB is functionally similar to umuDC and other previously characterized MDR operons, such as mucAB and rumAB (29, 31). Indeed, rulAB shares many other features with the known MDR systems, including the presence of a binding site for the LexA repressor within its putative promoter region, possession of a conserved internal cleavage site within the rulA sequence, and lack of function in a recA background (46). Each of the known umu-like systems varies in its ability to promote mutagenesis in different cellular backgrounds. For example, the approximately threefold increase in our experiments of mutagenesis of mucAB in E. coli RW120 as compared to that of umuDC has been observed by others as well and is attributed to a more efficient processing of MucA to the truncated MucA$^*$ form in an E. coli host (10). It is interesting to note that the difference in mutation frequency between rulAB and mucAB was reduced in the P. aeruginosa PAO1 background (Fig. 3B) due to a larger decrease in MucAB-mediated MDR in P. aeruginosa than in E. coli. This is probably due to a decreased affinity of the MucAB system with P. aeruginosa RecA or DNA polymerase.

In the Pseudomonas backgrounds, rulAB has a large effect on UV-B survival for P. syringae as noted previously (46, 47) and for P. aeruginosa in the current study. Although the rates of survival of P. aeruginosa PAO1(pJK17) and P. syringae pv. syringae B86-17 (rulAB$^+$) were similar for UV-B doses up to 1,900 J m$^{-2}$, the frequency of mutation to Rif$^+$ was approximately sevenfold higher in P. aeruginosa. The increased mutation frequency in P. aeruginosa could be due to the relative contribution of other non-MDR systems and rulAB to the overall repair effort. At the 1,900-J m$^{-2}$ UV-B dose, the survival rate of P. aeruginosa PAO1(pJBJ321) is approximately 20-fold lower than that of P. syringae pv. syringae GWS242(pJBJ321), suggesting that P. syringae is more efficient in non-MDR than P. aeruginosa.

Through analysis of UV-B mutability in the rulAB::Km strain GWS242, we demonstrated a small (10- to 20-fold) increase in mutability to Rif$^+$, an observation that we have also made when examining other natural P. syringae strains which do not contain rulAB (Kim and Sundin, unpublished). These observations are consistent with the occurrence of another source of UV mutability within the P. syringae genome. Hybridizations of BamHI-digested genomic DNA of the rulAB-negative strain P. syringae pv. syringae FF5 with a rulAB probe under low-stringency conditions resulted in the observation of a single hybridizing band (Kim and Sundin, unpublished). We are currently attempting to clone this chromosomal sequence and determine its role in UV survival and mutability. Other examples of MDR systems cooccurring on plasmids and chromosomes in a single strain have been examined in serovar Typhimurium (31) and more recently in Shigella flexneri (37). In serovar Typhimurium, the chromosomally encoded umuDC determinant is active in UV-inducible mutagenesis, while the plasmid-encoded samAB determinant does not contribute to UV mutability (18). The situation is reversed in S. flexneri, as the plasmid-encoded impCAB determinant is required for UV mutability while the chromosomally encoded umuDC operon is not expressed following UV irradiation and therefore is apparently unable to promote UV-induced mutagenesis (37).

Thus, in each of these situations, only one of the MDR operons plays the important role in UV mutability, which leads to the obvious question—why are two operons present? Recently, Opperman et al. (32) showed that the E. coli umuDC gene products play an additional role in cell cycle control following DNA damage. These authors demonstrated individ-
ual roles for the UmuD and UmuD' proteins and proposed a model in which the UmuD and UmuC proteins were involved in a delay in the recovery of DNA replication following DNA damage (32). RecA-mediated cleavage of UmuD and the formation of the UmuD'-C complex would then be required for MDR mediated by translesion synthesis; this would be followed by the resumption of DNA replication (32, 35). Cell cycle control and a delay in replication restart following DNA damage are presumably important because they allow for non-MDR processes such as excision repair to occur, ultimately lowering the cellular mutational load. Organisms which encode two MDR systems in which one is functionally dominant in terms of actual repair may utilize the other system in cell cycle control. Alternatively, the formation of chimeric MDR complexes may also be involved in the posttranslational regulation of these systems. We are interested in examining the chromosomal source of UV mutability in P. syringae in order to understand the contribution of this system and rulAB to the overall repair process and UV survival.

The regulation analyses performed in this study indicate that rulAB is expressed in a UV-inducible manner with increased promoter activity in response to increasing UV-B dosage. The rapid induction of expression, maintenance of transcriptional activity at high levels for 4 to 6 h following irradiation, and overall importance of the rulAB determinant to UV-B survival suggest that MDR could play a major role in P. syringae survival in the environment. As more studies show the significance of DNA repair processes to organismal survival in habitats with high solar radiation exposure (e.g., reference 15), it would be important to determine the occurrence and role of MDR in organisms in these habitats.

It was previously shown that rulAB-containing P. syringae strains maintained significantly larger phyloplankton populations following UV-B irradiation (47). Our current results from assays involving phyloplankton populations of P. syringae pv. syringae B86-17 indicate that MDR occurs and is detectable during the period when inoculated cells are establishing an infection on their host. Populations of P. syringae pv. syringae inoculated to bean leaf surfaces typically initiate disease, which is manifested by the occurrence of leaf spots, within 4 to 6 days. In our experiments, we observed leaf spot symptoms at the 5-day sampling time. Our data indicate that UV-B mutability occurs at levels similar to those recorded in vitro when UV-B irradiation is administered immediately after inoculation. Over time, as strain B86-17 established an infection on its bean host, the frequency of UV-B mutability to Rifr was slightly reduced (from 4.8- to 2.9-fold) but still significantly greater than in a nonirradiated control. As P. syringae strains become established in the phyloplankton, it is thought that a proportion of the cell population colonizes sites on leaves which are protected from external stresses (51). However, our data on UV-B mutability in the phyloplankton and previous data on rulAB-mediated UV-B survival in the phyloplankton (47) also clearly indicate the important contribution of the rulAB determinant to relative fitness during colonization and establishment of infection in plants.

The importance of rulAB to UV tolerance and the potential recurring necessity of this determinant for survival in the phyloplankton seem to distinguish rulAB from the other umu-like operons from an ecological standpoint. Each of the other umu-like operons characterized to date has been isolated from enteric organisms, whose exposure to UV radiation or chemical mutagens would probably be sporadic. One example of the consequence of a limited necessity for MDR in enteric organisms may be an attenuation of activity of the corresponding MDR determinant. For example, when located on its natural plasmid R46, the activity of mucAB is repressed by another gene located approximately 2 kb away (20). The role of some of the enteric-organism umu-like operons in UV protection is also unclear, as inactivation of either or both umudDCST and samAB in serovar Typhimurium has no effect on UV survival (18). Thus, the distinguishing features of the rulAB system in terms of activity when present on its native plasmid, function in UV tolerance, and potentially daily expression in response to solar UV damage make this system appropriate for continued analysis of the ecological and evolutionary ramifications of UV-induced mutagenesis.

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