CONSTRUCTION OF A *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*

REPORTER VECTOR FOR THE STUDY OF THE EXPRESSION

OF THE *rec*A GENE IN RESPONSE TO UV LIGHT

A Senior Honors Thesis

By

JULIE ANN COTTON

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of the

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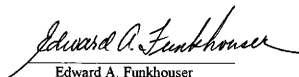
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ABSTRACT

Construction of a *Pseudomonas syringae* pv. *syringae* reporter vector

for the study of the expression of the *recA* gene

in response to UV light. (April 2000)

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Responsible for such diseases as brown spot of bean, *Pseudomonas syringae* pv. *syringae* is an inhabitant on the leaf surface (phyllosphere) of plants and must maintain large population numbers in order to infect host plants. The ability to repair genetic damage caused by UV-B light, that part of the solar spectrum from 290-320 nm, is essential to the survival and success of the bacterium. The *recA* gene is the initiating gene of the regulatory network, known as the SOS repair mechanism, which is responsible for repairing genetic damage blocking DNA replication in many species of bacteria.

We have cloned and characterized a putative promoter region of the *P.s. syringae recA* gene by DNA subcloning and sequencing techniques. We constructed a *recA::gfp* reporter vector using an improved green fluorescent protein cloning cassette shown to be effective in *P.s. syringae* (Miller and Lindow, 1997). We hope this vector will allow us to quantify the expression of the *recA* gene in response to UV-B light in two *gfp* fusion strains of *P.s. syringae*. The strains containing this plasmid may be used in several *in vitro* and environmental studies in order to understand the response of the *recA* gene and its role within the SOS repair mechanism, and to investigate the habitat of the bacteria within the phyllosphere.

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1. INTRODUCTION

Pseudomonas syringae pv. *syringae* is the causal agent of bacterial brown spot of snap beans, an economically significant disease for many producers (Hirano et al., 1995). A frequent problem for growers, *P.s. syringae* causes necrotic lesions on bean pods that reduce the quality of the product and can render entire fields unmarketable (Hirano et al., 1995). The bacteria can inhabit the leaves of the plant without causing disease until environmental factors, such as rain, favor bacterial population growth (Hirano et al., 1995). A threshold population size of 10^4 calls per gran of leaf tissue has been determined to be predictive of disease on snap bean (Hirano et al., 1995). The ability to tolerate high doses of UV radiation is critical for the maintenance of *P.s. syringae* populations in the phyllosphere; toleration of UV solar radiation may favor pathogen population expansion and lead to the occurrence of brown spot disease.

Solar radiation is the primary source of energy for life on earth. However, in addition to visible light, deleterious UV radiation, which induces DNA damage, is present in solar radiation. UV-B radiation, that part of the natural light spectrum from 290-320 nm, induces DNA damage in the form of pyrimidine dimers that can be deadly to microorganisms through the blockage of DNA replication (Friedberg et al., 1995). A widely researched bacterial survival mechanism following irradiation with UV wavelengths is the SOS inducible response. The *recA* gene has been characterized and determined to play an important role in cellular UV protection (Miller and Kokjohn, 1990). This is effected through the induction and the expression of the SOS regulatory network, a system comprised of at least 20 unlinked genes, many of which are involved in DNA repair (Miller and Kokjohn, 1990).

GENE: An international journal focusing on gene cloning and gene structure and function.

The SOS regulatory network, first suggested by Miroslav Radman in the early 1970's, allows DNA replication to continue, even following severe damage from UV radiation or other chemical mutagens (Friedberg et al., 1995). The LexA protein normally represses the expression of genes in the SOS regulatory network. The SOS inducing signal is generated by single-stranded regions produced when the cell attempts to replicate damaged DNA (Friedberg et al., 1995). The RecA protein recognizes this signal, and cleaves the repressor, LexA, causing SOS genes including *recA* to be expressed at increased levels (Friedberg et al., 1995). The expression of the DNA systems that are part of the SOS network is responsible for cellular survival and recovery following DNA damage. After the damage has been repaired, LexA again accumulates and represses the genes in the system (Friedberg et al., 1995).

In this report, we describe the subcloning process and sequencing results used to isolate a putative *recA* promoter region in *P.s. syringae*. A *recA-gfp* reporter vector was constructed using this region to facilitate the study the expression of the *recA* gene in *P.s. syringae* after exposure to UV light. The significant role that the *recA* gene may play in the survival of such pathogens is important to the understanding of phyllosphere habitat and may contribute to future beneficial biocontrol methods.

2. MATERIALS AND METHODS

2.1. Bacterial strains and plasmids

The bacterial strains and plasmids used in this study are listed in Table 1. *P.s. syringae* and *Escherichia coli* strains were grown in Luria-Bertani (LB) medium (Difco) at 28°C and 37°C, respectively. *Pseudomonas*-selective minimal media was obtained from Gibco-BRL. Ampicillin (Ap) was added to media at the concentration of 25 μg ml^{-1} for *P.s. syringae* and 75 μg ml^{-1} for *E. coli*. Kanamycin (Km) was added to media at 25 μg ml^{-1}, and tetracycline (Tc) was added to media at 25 μg ml^{-1}, according to resistance requirements. Competent *E. coli* cells were prepared from strain DH10B cultured in 2 x YT by treatment with Tris-HCl, calcium chloride and magnesium chloride for 30 minutes in ice, then transferred to a Tris-HCl, CaCl$_2$ and MgCl$_2$ with 20% glycerol solution for storage in -70°C. Sterile saline solution was made at the concentration of 8.5g L^{-1} NaCl in water.

2.2. DNA subcloning and sequencing

The *recA* gene and flanking sequences of *P.s. syringae* was obtained from Dr. Kyle Willis (University of Wisconsin), on the plasmid pKW11. The *recA* gene had been marked with the Tn*5* insert (5.7-kb), a commonly used DNA transposon encoding for kanamycin resistance. The *recA* gene, which the insert interrupts, is known to be about 1,040-bp (1.04 kb) according to the conservation of the gene sequence between species (Friedberg et al., 1995).

The promoter region was tentatively identified by subcloning and sequencing (see Table 1 for a list of bacterial strains and plasmids employed). Although the *recA* gene was known to be interrupted by Tn*5* marker, the direction of transcription of the gene was unknown. The pKW11

Table 1. Bacterial strains and plasmids used in this study and their relevant characteristics.

Strain or Plasmid	Relevant genotype or characteristic	Source or reference
Strains		
Escherichia coli		
DH5α	Δ(*lacZYA-argF*)$_{U169}$	Ausubel et al. (1987)
DH10B	Competent cells, contain no plasmids	Grant et al. (1990)
Pseudomonas syringae		
pv. *syringae*		
B86-17	one plasmid 61-70 kb	DE Legard
FF5	UV sensitive, contains no plasmids	Sundin and Bender (1993)
Plasmids		
pCR2.1	PCR cloning vector	Invitrogen
pRK2013	Km resistant, Mob⁺, Tra⁺	Figurski and Helinski (1979)
pProbe KT	Km resistant. 11 kb *gfp* cloning cassette for prokaryotic transcriptional transfusions	WG Miller
pBluescript SK+	Ap resistant, *lacZ*⁺. Cloning and transcription vector	Stratagene
pGem 3zf	Ap resistant, *lacZ*'. Cloning and transcription vector	Promega
pGem 7zf	Ap resistant, *lacZ*⁺. Cloning and transcription vector	Promega
pKW11	Tc, Km, Ap resistant. *P. syringae* (*recA4*::Tn*5*) mutation	DK Willis
pGWS166	8.8-kb *Sst*I from pKW11 in pBluescript (*recA*::Tn*5* insertion)	This study
pGWS168	Ap, Km resistant. 1.0-kb IS*50*-T7 PCR product from pGWS166 in pCR2.1	This study
pGWS169	Ap, Km resistant. 2.2-kb IS*50*-T7 PCR product from pGWS166 in pCR2.1	This study
pGWS171	1.2- kb *Sph*I-*Kpn*I from pGWS169 in pGem 3zf	This study
pJAC1	0.84-kb *Sph*I-*Eco*RV (Blunt cut and ligated to *Sma*I pGem 7zf) from pGWS171 in pGem7zf	This study
pJAC2	~0.5-kb *Sph*I to *Hinc*II (*Hinc*II-*Eco*ICRI removed) from pJAC1	This study
pJAC3	0.84-kb *Eco*RI (added restriction site)-*Hind*III segment from pJAC1 in pProbe-KT	This study

plasmid DNA was isolated using the Qiagen plasmid purification kit according to manufacture's instructions. Restriction endonuclease digestion using the *SstI* restriction enzyme was carried out to cut the DNA encompassing the Tn*5* insert, excising an 8.8-kb fragment of DNA. After running the reaction on a 1-% agarose gel (Gibco-BRL), the fragment was extracted from the gel using the Qiagen gel extraction kit, according to manufacturer's protocol. The 8.8-kb fragment of DNA was ligated into the pBluescript SK vector, forming pGWS166, was transformed into *E. coli* DH10B competent cells using the protocol in the Promega Protocols and Applications Guide (Doyle 1996).

The transformed E. coli was plated to LB media amended with Ampicillin with X-gal, which selects for *lacZ* expression. Colonies that did not express the *lacZ* gene were selected from the media. The colonies were grown and cultured with Ap. pGWS166 DNA was isolated using the methods above. Two polymerase chain reactions (PCR) were carried out in order to identify the beginning of the *recA* gene. Known primer sites within the Tn*5* insert and on either side of the inserted DNA, in the pBluescript SK vector plasmid transcription initiation site, were utilized by PCR methods. This isolated 1-kb and 2.2-kb fragments, each including a section of the *recA* gene and possible flanking regions of DNA on either side of the insert. The synthetic oligonucleotide primers used for PCR runs and the *Taq* polymerase were supplied by New England Biolabs.

The PCR products were run on a 1-% agarose gel, and the 1-kb and 2.2-kb fragments were identified and cut from the gel. The two DNA fragments were extracted from the gel using the Qiagen gel extraction kit, according to manufacture's protocol. The two fragments were individually ligated into the pCR2.1 plasmid vector (Invitrogen), forming pGWS168 and pGWS169, respectively. PCR sequencing reactions were carried out using the ABI Sequencing

Big Dye kit, according to manufacturer's specifications. DNA sequences were run at the Texas A&M University Gene Technologies Laboratory. Homology analysis, using the raw DNA sequence were done using the National Center for Biotechnological Information Gene Bank Blast search online system.

The 2.2-kb subclone was found to include the beginning sequence of the *recA* gene, indicated with the ATG start codon and highly conserved *recA* DNA comparative sequences, as well as the end sequence of a preceding gene. Therefore, the promoter region was predicted to lie between the end of the preceding gene and the beginning of the *recA* gene. This region was furthur localized for sequencing using the following methods.

A 1.2-kb region was cut from pGWS169 using *Kpn*I and *Sph*I was ligated into the pGEM 3Zf vector forming pGWS171, and transformed into the *E. coli* strain DH10B as above. The plasmid was sequenced by methods similar to those listed above. The results from the *Kpn*I direction of the plasmid were readable, but the sequence from the *Sph*I side was unreliable. The results of the sequencing from the *Kpn*I side was adequate, as it included the *recA* start sequence and upstream sequence (see the solid arrow in Figure 1).

Subsequent plasmids (see diamond-ended lines on Figure 1) were constructed and sequenced using the same methods as described for pGWS171. pJAC1 was constructed by subcloning the 0.84-kb *Sph*I-*Eco*RV fragment from pGWS169 to reduce excess *recA* gene sequence and to place the DNA in the appropriate 5' to 3' direction. The pJAC2 plasmid was formed by extracting the *Hinc*II - *Eco*ICRI region of pJAC1 in order to confirm the sequencing results of pJAC1. A novel oligonucleotide Md1 (5' GGGGCAAGGATGATGCGC 3') was created (Gene Technologies Laboratory, Texas A&M University) to recognize a region before the

*Hinc*II site of the plasmid pJAC1, and compared with results from the 3' (*Eco*RV) end of the

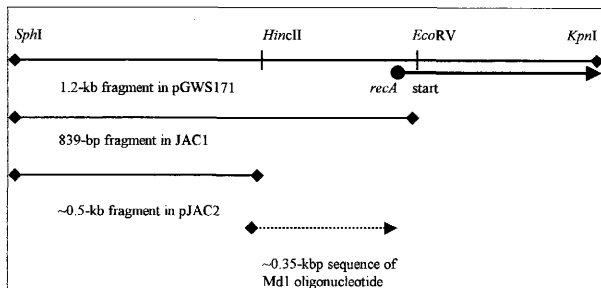pJAC1 plasmid to confirm the remaining sequence of the pJAC1 fragment.

Figure 1. Diagram of sequenced subclones. Diamond-end lines represent the subclones pGWS171, pJAC1, and pJAC2. The circle of the solid arrow denotes the *recA* gene start and the arrow points in the direction of transcription. The binding site of the Md1 oligonucleotide is denoted with a diamond and the dashed arrow points in the direction of the sequence. Major restriction enzyme sites are shown above pGWS171.

2.3 Construction of a recA::gfp transcriptional fusion

The pProbe-KT vector was supplied by W.G. Miller in *E. coli* strain DH5α. The strain

was cultured in a LB amended with Km. A large scale plasmid preparatio of pProbe-KT cut with

*Hind*III and *Eco*RI. The vector pJAC1 did not have matching cloning sites to allow for the direct

cloning of the 0.84-kb fragment of DNA. Therefore, the fragment of DNA was replicated by

PCR using the SP6 primer and a novel oligonucleotide T7-*Eco*RI (5' GCGAATTCTAATACGA-

CTCACTATAGGCG 3') consisting of the T7 primer with an *Eco*RI restriction site added to the

end. Originally obtained from the Texas A&M University Gene Technologies Laboratory, the oligonucleotide concentration could not be determined and several PCR reactions using different primer concentration were ineffective. After reordering the oligonucleotide from New England Biolabs, with concentration specified, the reaction worked properly and the fragment was replicated. This allowed the PCR-generated DNA to be cut with *Eco*RI and *Hind*III and ligated by the method above into the pProbe-KT vector, forming the pJAC3 plasmid (see Figure 2). pJAC3 was transformed into DH10B *E. coli* as described in section 2.2, and Km resistant colonies were selected.

Tri-parental matings of the *E. coli* host strain containing pJAC3 and the recipient *P.s. syringae* strains B86-17 or FF5 were performed. This method includes a conjugation proficient donor *E. coli* strain containing pRK2013 that assists in transferring the plasmid from the host strain to the recipient bacteria of interest (Figurski and Helinski, 1979). Each strain of bacteria was cultured in LB with selective antibiotics when required. The cultures were spun in a centrifuge to collect the bacteria, and resuspended in sterile saline solution. The host, donor, and recipient, strains were mixed in microfuge tubes in different proportions. The mixture was spot plated on plain LB media, dried briefly in a laminar flow hood, and incubated overnight at 28°C. Transconjugants were selected by resuspending the mating mix in sterile saline solution and plating to minimal medium amended with Ap (25 μg ml⁻¹), which selects for *P. syringae*, and Km which selects for the Km marker of pProbe-KT. These plates were incubated for several days. The mating was most successful when the recipient strain of *P. syringae* was three times or more that of the donor *E. coli* strain. The resulting larger colonies were streaked to LB medium amended with Km. The isolates were then cultured and the plasmid DNA isolated as specified above. Gel electrophoresis of the plasmids cut with *Eco*RI suggested that the pJAC3 vector had

been transferred to the *P. syringae* strains. Similar procedures were carried out to generate *P. syringae* strains with only the pProbe-KT vector for use as controls.
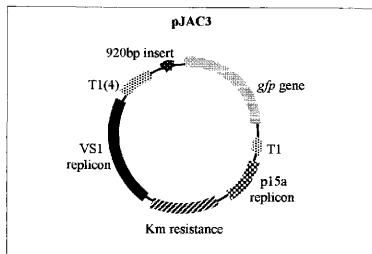

Figure 2. Map of the *recA-gfp* reporter vector pJAC3. T1 represents transcription-blocking regions. Note that the vector has multiple regions before the *recA* promoter insert site and reporter gene to reduce the production of the green fluorescent protein when the promoter sequence has not been induced.

3. RESULTS

3.1 Sequencing results

The sequencing of the *Kpn*I end of the fragment yielded a 557-bp sequence was retrieved and entered into the National Center for Biotechnological Information Blast search system for comparison with other identified gene sequences. Regions of *recA* promoter and *recA* gene similarity were ranked. The last 408 bases in the sequence encode for the first 136 amino acids in the RecA protein. The Blast system found this gene sequence to be 100% identical to the *recA* gene of *P. flourescens*, a closely related Pseudomonad.

A putative promoter region beginning 51-bp downstream of the search-identified *recA* start codon was determined by visual comparison of this DNA sequence to the consensus sequence LexA protein binding sites (Walker 1984). When *recA* is in the uninduced state, the LexA protein binds to the promoter region characterized by the CTG and CAG sequences. The putative LexA binding region is located at the base pair 70-85 (see Figure 3). The *recA* start codon, ATG, are the 136-138th nucleotides in the sequence.
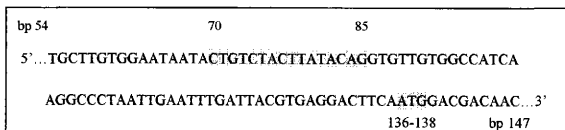
bp 54   70   85

5'...TGCTTGTGGAATAATACTGTCTACTTATACAGGTGTTGTTGGCCATCA

    AGGCCCTAATTGAATTTGATTACGTGAGGACTTCAATGGACGACAAC...3'

                                   136-138   bp 147

Figure 3. Results of the *Kpn*I end sequence of pGWS171.

The pJAC1 sequencing results were combined with those obtained from the sequencing of

pJAC2 and Md1 sequencing reaction (see Figure 4). This allowed for a review and confirmation

of the 0.84-kb sequence of pJAC1 that was ligated into the pProbe-KT vector to form the pJAC3

reporter vector construct.

AGCCCCGTTG	CGCGGCAGGT	GCGCTGCGTC	AGGATTCAAC	TTTACAGGCT	TTCCAGCATG
AACGATTACA	CCGAGGATGA	GATAACCGGG	CTTGCCGATA	CGCTTGGCAG	GCTTCTGGGT
GCAATGGGTG	CGCAAGTGAC	CACTGCCGAA	TCCTGTACGG	GGGGCGGAAT	AGCCGAAGCG
ATCACGCGCA	TTGCCGGCAG	CTCGGCGTGG	TTCGAAGCGG	GCTACGTGAC	CTATTCGAAT
GCCCAGAAGA	CCCGCCAGTT	AGGGGTGCCC	GAGGTGTTGT	TCGCACAGGT	CGGGGCGGTC
AGTCAGCCGG	TTGTCGAGGC	CATGGTGCGA	GGCGCGCAGC	GTGAAAGCGG	TGCACGATTC
GCCGTCGCGC	TCAGCGGTGT	CGCAGGGCCT	GGCGGCGGTT	CGCCGGACAA	ACCGGTCGGT
ACTGTGTGGC	TGTCGTGGGG	CAAGGATGAT	GCGCTG¹TGAT	CGCCCGGCGT	TGCCACTTCG
ATGGCGACCG	TGATCAGGTC	CGTCGAC²GGA	CGGTCGAAGC	CGCGTTGCAC	GGGCTGATAC
AGCTTGCGCG	CGCGTTGCAC	GGGCTGATAC	AGCTTGCGCG	CGGAGAAATG	CCAAAACAGG
GGTAGGCGCG	CGCTCACTGC	TGTGGAATAA	TACTGTCTAC	TTATACAGGT	GTTGTGGCCA
TCAAGGCCCT	AATTGAATTT	GATTACGTGA	GGACTTCAAT	GGACGACAAC	AAGAAGAAAG
CCTTGGCTGC	GGCCTTGGGT	CAGATCGAGC	GTCAGTTCGG	TAAAGGCGCC	GTGATGCGCA
TGGGCGATCA	TGATCGCCAG	GCGATCCCTG	CCATCTCCAC	CGGTTCGCTG	GGTCTGGAT

1 = Md1 primer site
2 = *Hinc*II restriction site

Figure 4. Sequence of pJAC1 (5'-3'). The putative LexA binding is highlighted. The ATG start codon appears in bold letter. Other sites of importance are underlined and identified by number. The beginning amino acid sequence is shown above the *recA* gene region.

3.2 Confirming the recA-gfp vector

The *P.s. syringae* bacterial strains B86-17/p JAC3, FF5/pJAC3, B86-17/pProbe KT and FF5/pProbe KT were grown overnight in LB broth amended with Ap. The culture was diluted to 10^{-6} and 50μl aliquots were spot plated to Ap amended LB media. The plates were incubated at 28°C for 24 hours. The plates were then opened and exposed to 500 UV-B light provided by an XX-15L lamp (UVP Products). Duplicate plates were not exposed. The plates were incubated for one hour, and then exposed to 490 nm light to excite GFP.

Unfortunately, the equipment available did not allow for definite confirmation of GFP production. I will soon utilize equipment better suited to evaluate GFP fluorescence emissions.

4. CONCLUSIONS

4.1 Use of the green fluorescent protein as a prokaryotic reporter gene

The green fluorescent protein (*gfp*) isolated from the jellyfish *Aequirea victoria* is able to produce fluorescence without additional cofactors or damage to the organism in which it is used (Plautz 1996). For these reasons, it is being used increasingly as a reporter gene to indicate gene expression and regulation.

The cloning cassette designed for prokaryotic transcriptional fusions provided by W. G. Miller (1997) was used in the formation of the vector. In our plasmid construct, the GFP will be produced when the *recA* promoter sequence is induced after DNA damage has occurred. The GFP cloning vector contains a 'red shift' mutation that allows the excitation maximum to shift to 490 nm, a wavelength near common laser excitation sources, which allows the reporter vector to be viewed with less specialized equipment (Miller and Lindow, 1997). Another mutation causes increased protein solubility, a characteristic that reduces erroneous long-term expression results (Miller and Lindow, 1997). *gfp* is expressed at increased levels comparative to the wild-type *gfp*, possibly allowing weakly transcribed genes to be measured (Miller and Lindow, 1997), which may be of benefit if low UV doses (such as solar radiation) are used to test expression levels.

I hope to be able to utilize the above features in future testing such as the possible studies listed below.

4.2 Future research possibilities

(1) Further subcloning of the unidentified region of the pJAC1 sequence may be done to determine the minimum sequence needed for *recA* promoter function.

(2) *In vitro* UV-B exposure studies using the *P.s. syringae* strains containing pJAC3 are planned to quantify the time frame of expression of the *recA* gene, as well as the level of expression of *recA* in response to varying exposure to UV light.

(3) Plant studies may be carried out to determine habitat of the *P.s. syringae* bacteria on leaves. Research suggests that solar radiation has great significance to the microbial ecology of the phyllosphere (Sundin and Jacobs, 1999). It has been suggested that the ability to access protected sites of leaves contributes to the survival of *P.s. syringae* in the phyllosphere and may increase the opportunity for pathogenicity (Wilson et al., 1999). Plants can be set under emitting lamps and GFP may be viewed without destroying the physical habitat of the bacteria. This experiment may reveal the specific leaf structures *P.s. syringae* prefer.

(4) Long-term research plans involve an examination of the regulation of the *recA* gene of *P. syringae* and of the *rulAB* locus (Sundin and Murillo, 1999), which encodes tolerance to UV radiation. *rulAB* is part of the cellular SOS system in *P. syringae* (Sundin et al., 1996), activated after exposure to UV radiation. To our knowledge, the role of solar radiation in the regulation of *recA* and downstream components of a SOS system has not been examined previously.

(4) Increased expression of the *recA* and *rulAB* genes has been confirmed *in vitro* by exposure to a UV radiation (Sundin and Murillo 1999), but the rate of expression when exposed to incident solar radiation outdoors has not yet been determined. Future plans also include

outdoor solar studies using specially designed enclosed boxes which will allow us to see the response of the bacteria to actual solar radiation. A special Mylar film (Kodak) which blocks UV-B light can be added to provide an UV-B⁻ control. The use of the film would provide useful insight in comparison to the full spectrum exposure. The boxes must be kept on ice to prevent heat effects and constantly agitated on a platform in order to expose all of the bacteria in suspension within the box.

The investigation of *rulAB* and *recA* expression is significant to others working with bacterial repair mechanisms, as many of the genetic traits of these repair mechanisms are conserved throughout many bacterial strains. A broad-host-range plasmid investigation using the *E. coli recA*⁺ gene showed that at least 20 different genera of gram-negative bacteria were able to repress the expression of the *E. coli recA*⁺ gene to some extent in the absence of DNA damage and to induce it after DNA damage, suggesting that the bacteria possess an SOS regulatory system and that the LexA binding site is significantly conserved in these various systems (Friedburg and others 1995). Homologous *rulAB* genes have also been found, such as *umuDC* in *E. coli*, *mucAB* in naturally occurring plasmids, and *samAB* and in *Salmonella typhimuirium* (Miller and Kokjohn 1990).

Such observations as habitat on the leaf phyllosphere and response to solar radiation are critical to an understanding of the ecology this plant pathogen. Microorganisms that inhabit UV-stressed environments such as the leaf phyllosphere but do not cause disease may be a biocontrol possibility in the future. Understanding the survival process such as the SOS repair mechanism of these competing bacteria may contribute to the development of future biocontrol strategies.

REFERENCES

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Siedman, J.G., Smith, J.A. and Struhl, K. (1987) Current Protocols in Molecular Biology. Green Publishing, NY.

Cox, M.M. (1991) The RecA protein as a recombinational repair system. Mol Microbiol 5, 1295-1299.

Doyle, K. and Miles, J., editors. (1996) Cloning in Plasmid Vectors. In: Promega Protocols and Applications Guide. 3rd ed. USA: Promega Corporation. p. 50-51.

Elasri, M.O. and Miller, R.V. (1998) A *Pseudomonas aeruginosa* biosensor responds to exposure to ultraviolet radiation. Appl Microbiol Biotechnol 50, 455-458.

Figurski, D.H. and Helinski, D.R. (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided *in trans*. Proc. Natl. Acad. Sci. USA 79, 1648-1652.

Friedberg, E.C., Walker, G.C. and Siede, W. (1995) SOS Responses and DNA Damage Tolerance in Prokaryotes. In: DNA Repair and Mutagenesis. Washington, DC: AMC Press. p. 407-464.

Grant, S.G., Jessee, J., Bloom, F.R, and Hanahan. (1990) Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants. Proc Natl Acad Sci USA 87, 4645-4649.

Hirano, S.S., Rouse, D.I., Cayton, M.K. and Upper, C.D. (1995). *Pseudomonas syringae* pv. syringae and bacterial brown spot of snap bean: a study of epiphytic phytopathogenic bacteria and associated disease. Plant Dis 79, 1085-1093.

Little, J.W. and Mount, D.W. (1982) The SOS regulatory system of *Escherichia coli*. Cell 29, 11-22.

Miller, R.V. and Kokjohn, T.A. (1990) General Microbiology of *recA*: Environmental and Evolutionary Significance. Annu Rev Microbiol 44, 365-394.

Miller, W.G. and Lindow, S.E. (1997) An improved GFP cloning cassette designed for prokaryotic transcriptional fusions. Gene 191, 149-153.

National Center for Biotechnological Information. (1999) Ungapped Blast Search. <http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-blast?Jform=1>.

Plautz, J.D., Day, R.N., Daily, G.M., Welsh, S.B., Hall, J.C., Halpain, S. and Kay, S.A. (1996) Green fluorescent protein and its derivatives as versatile markers for gene expression in living Drosophila melanogaster, plant and animal cells. Gene 173, 83-87.

Sundin, G.W. and Bender, C.L. (1996) Molecular analysis of closely-related copper- and
streptomycin-resistance plasmids in *Pseudomonas syringae* pv. syringae. Plasmid 35, 98-107.

Sundin, G.W. and Jacobs, J.L. (1999) Ultraviolet radiation (UVR) sensitivity analysis and UVR
survival strategies of a bacterial community from the phyllosphere of field-grown peanut
(*Arachis hypogeae* L.). Microb Ecol 38, 27-38.

Sundin, G.W. and Murillo J. (1999) Functional analysis of the *Pseudomonas syringae rulAB*
determinant in tolerance to ultraviolet B (290-320 nm) radiation and distribution of *rulAB* among
P. syringae pathovars. Environ Microbiol 1, 75-88.

Walker, G.C. (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in
Escherichia coli. Microbiol Rev 48, 60-93.

Wills, D.K., Hrabak, E.M., Lindlow, S.E. and Panopoulos, N.J. (1988) Construction and
characterization of *Pseudomonas syringae recA* mutant strains. Mol Plant-Microbe Interactions
1, 80-86.

Wilson, M., Hirano, S.S. and Lindow, S.E. (1999) Location and survival of leaf-associated
bacteria in relation to pathogenicity and potential of growth within the leaf. Appl Environ
Microbiol 65, 1435-1443.

Yang, M.K. and Wu, P.I. (1999) Identification of the promoter region of the *Xanthomonas*

camperstris pv. *citri recA* gene responsible for induction by DNA-damaging agents. FEMS

Microbiol Letters 176, 57-65.

VITA

After graduating from John Marshall High school in San Antonio, Texas, in 1996, I began my higher education at Texas A&M University, College Station. I look forward to receiving a bachelor of science in Bioenvironmental Science and a minor in English upon graduation in December of 2000. I plan to graduate with University and Foundation Honors.

In addition to the Undergraduate Research Fellows Program, I served in the Agricultural and Natural Resource Policy Congressional Internship Program in the office of Congressman Ruben Hinojosa, and became a member of the Golden Key National Honors Society. I have earned several University scholarships, including the Academic Excellence Award and the Aggie Spirit Collegiate Award, and am grateful to the Texas Seed Trade Association for their support through the Ed Hefley Memorial Scholarship Fund.

I gained much experience as an assistant in the laboratory of Dr. George Sundin, where I have been employed for almost two years. I look forward to graduate school in the fall of 2001.

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