JOURNAL OF BACTERIOLOGY, Dec. 1999, p. 7176–7184 Vol. 181, No. 23
0021-9193/99/$04.00+0
Copyright © 1999, American Society for Microbiology. All Rights Reserved.

AlgT (σ^{22}) Controls Alginate Production and Tolerance to Environmental Stress in *Pseudomonas syringae*

LISA M. W. KEITH AND CAROL L. BENDER*

Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma 74078

Received 20 July 1999/Accepted 17 September 1999

***Pseudomonas aeruginosa* and the phytopathogen *P. syringae* produce the exopolysaccharide alginate, which is a copolymer of D-mannuronic and L-guluronic acids. One of the key regulatory genes controlling alginate biosynthesis in *P. aeruginosa* is *algT*, which encodes the alternate sigma factor, σ^{22}. In the present study, the *algT* gene product from *P. syringae* pv. syringae showed 90% amino acid identity with its *P. aeruginosa* counterpart, and sequence analysis of the region flanking *algT* in *P. syringae* revealed the presence of *nadB*, *mucA*, and *mucB* in an arrangement virtually identical to that of *P. aeruginosa*. An *algT* mutant of *P. syringae* was defective in alginate production but could be complemented with wild-type *algT* from *P. syringae* or *P. aeruginosa* when expressed in *trans*. The *algT* mutant also displayed increased sensitivity to heat, paraquat, and hydrogen peroxide (H_2O_2); the latter two compounds are known to generate reactive oxygen intermediates. Signals for activation of *algT* gene expression in *P. syringae* were investigated with an *algT*::*uidA* transcriptional fusion. Like that in *P. aeruginosa*, *algT* transcription in *P. syringae* was activated by heat shock. However, *algT* expression in *P. syringae* was also stimulated by osmotic stress and by exposure to paraquat, H_2O_2, and copper sulfate. The latter two compounds are frequently encountered during colonization of plant tissue and may be unique signals for *algT* activation in *P. syringae*.**

Many pseudomonads, including the phytopathogen *Pseudomonas syringae*, produce the exopolysaccharide alginate, a copolymer of *O*-acetylated β-1,4-linked D-mannuronic acid and its C-5 epimer, L-guluronic acid. *P. syringae* induces a wide variety of symptoms on plant hosts and can also exist as an epiphyte on plant surfaces without causing disease. Yu et al. (62) used a genetic approach to evaluate the role of alginate in the pathogenicity and epiphytic fitness of *P. syringae* pv. syringae 3525, which causes bacterial brown spot on beans. Alginate contributed significantly to both virulence and epiphytic survival of *P. syringae* pv. syringae 3525, perhaps by facilitating colonization and/or dissemination of the bacterium in planta (62).

Alginate has been extensively studied in *P. aeruginosa*, where it functions as a virulence factor in cystic fibrosis patients (47). An important feature of alginate production by *P. aeruginosa* is that the alginate biosynthetic genes are normally silent but are activated in the cystic fibrotic lung, which results in a mucoid phenotype. In *P. aeruginosa*, genes that encode the biosynthesis and regulation of alginate map to four chromosomal locations. With the exception of *algC*, which is located at 10 min, the structural genes are clustered within an 18-kb region located at 34 min (18, 48). The alginate biosynthetic gene cluster in *P. aeruginosa* is presumably organized as an operon with transcription initiating at the *algD* promoter (9).

Genes controlling the regulation of alginate production include *algR1* (*algR*), *algR2* (*algQ*), *algR3* (*algP*), and *algB* (20, 54). AlgR1 functions as a response regulator and binds to multiple sites upstream of *algC* and *algD* (25, 42, 64). The genes which mediate the conversion to constitutive alginate production are located at 68 min on the *P. aeruginosa* chromosome and include *algT* (*algU*), *mucA*, *mucB* (*algN*), *mucC* (*algM*), and *mucD* (*algY*). The alternative sigma factor encoded by *algT*, σ^{22}, is required for transcription of *algD*, *algR1*, and *algT* (21, 52, 60). Both the *algD* and *algR1* promoters show a consensus sequence at the −35/10 region, a finding which is consistent with recognition by σ^{22}, suggesting that an RNAP-σ^{22} complex binds to both promoters and positively regulates transcription (52). MucA is a negative regulator of *algT* transcription and encodes an anti-σ factor with affinity for σ^{22} (53, 61). MucB is also a negative regulator and is thought to interact with the periplasmic domain of MucA, thereby altering its conformation so that it binds σ^{22} and targets it for degradation (39). MucC and MucD also modulate the expression of *algT* and have been described elsewhere (6, 7).

As in *P. aeruginosa*, the alginate biosynthetic genes in other pseudomonads are normally silent (19). Interestingly, an indigenous plasmid designated pPSR12 conferred constitutive alginate production to *P. syringae* pv. syringae FF5 (29). pPSR12 does not contain homologs of the biosynthetic or regulatory genes which control alginate production in *P. aeruginosa*; instead, this plasmid presumably contains regulatory genes which have not been characterized (29). Mutagenesis of FF5 (pPSR12) with Tn*5* resulted in the isolation of several alginate defective (Alg$^-$) mutants, including FF5.31 and FF5.32, which contain Tn*5* insertions in *algL* and *algR1*, respectively (15, 46). The arrangement of the alginate structural gene cluster and the genes flanking *algR1* were virtually identical in both *P. syringae* and *P. aeruginosa* (15, 46). However, complementation analyses indicated that the structural gene clusters in *P. aeruginosa* and *P. syringae* were not functionally interchangeable when expressed from their native promoters (46). Further experiments indicated that *P. syringae*, unlike *P. aeruginosa*, does not require a functional copy of *algR1* for activation of the *algD* promoter (15).

In the present study, an Alg$^-$ mutant of *P. syringae* pv. syringae FF5(pPSR12) was shown to contain a Tn*5* insertion upstream of the *algT-mucABCD* gene cluster. This region was cloned from *P. syringae*, and the role of *algT* in *P. syringae* was evaluated. An *algT* mutant was shown to be defective in algi-

* Corresponding author. Mailing address: 110 Noble Research Center, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078-3032. Phone: (405) 744-9945. Fax: (405) 744-7373. E-mail: cbender@okstate.edu.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
Strains		
Escherichia coli DH5α		50
Pseudomonas syringae pv. syringae		
FF5	No detectable plasmids, nonmucoid	29
FF5.36	Cu^r Km^r; contains pPSR12, slightly mucoid, *nadB*::Tn*5*	This study
FF5.LK1	Cm^r; contains pPSR12, nonmucoid, *algT*::Cm^r	This study
Plasmids		
pBluescript SK(+)	Ap^r; ColEI origin, cloning vehicle	Stratagene
pCR2.1	Ap^r Km^r; 3.9-kb cloning vector	Invitrogen
pRK415	Tc^r; RK2-derived cloning vector	27
pRK2013	Km^r; helper plasmid	17
pRK7813	Tc^r; cosmid vector	23
pBBR1MCS	Cm^r; 4.7-kb broad-host-range cloning vector	31
pBBR.Gus	Cm^r; 6.6-kb promoter probe vector containing *uidA* in pBBR1MCS	45
pPSR12	Cu^r Sm^r; 200-kb, confers constitutive alginate production to *P. syringae* pv. syringae FF5	29
pSL1	Ap^r Cm^r; 650-bp Cm^r cassette in pBluescript SK(+)	33
pMGm	Ap^r Gm^r; 2-kb Gm^r cassette	43
pJG309	Tc^r; contains *algT* from *P. aeruginosa*	19
pFF5.36	Tc^r Km^r; contains Tn*5*-inactivated alginate genes from FF5.36 in pRK7813	This study
pFF5.36B	Ap^r Km^r; contains a portion of Tn*5*, *nadB*, and *algT* as a 6-kb *Bam*HI fragment from pFF5.36 in pBluescript SK(+)	This study
pLKT5	Tc^r; cosmid clone from FF5(pPSR12) in pRK7813	This study
pBTB6.5	Ap^r; contains a 6.5-kb *Bam*HI fragment from pLKT5 in pBluescript SK(+)	This study
pBTB.Cm	Ap^r Cm^r; contains *algT*::Cm^r in pBluescript SK(+)	This study
pRTB6.5	Tc^r; contains a 6.5-kb *Bam*HI fragment from pLK5T5 in pRK415	This study
pRTB6.5.Cm	Tc^r Cm^r; contains *algT*::Cm^r in pRK415	This study
pCRalgTA	Ap^rKm^r; 1.2-kb PCR fragment in pCR2.1	This study
pCRalgTI	Ap^rKm^r; 1.2-kb PCR fragment in pCR2.1	This study
palgTA	Cm^r; 1-kb *Hin*dIII/*Pst*I fragment containing the *algT* promoter region in pBBR.Gus; transcriptionally active	This study
palgTA.1	Cm^r Gm^r; contains Gm^r cassette from pMGm in palgTA	This study
palgTI	Cm^r; 1-kb *Kpn*I/*Hin*dIII fragment containing the *algT* promoter region in pBBR.Gus; transcriptionally inactive	This study

nate production, indicating that *algT* is essential for alginate biosynthesis in *P. syringae*. The *algT* mutant was also more susceptible to killing by heat and superoxide-generating redox cycling compounds, indicating that AlgT (σ^{22}) regulates genes in *P. syringae* which respond to environmental stress.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Table 1 lists the bacterial strains and plasmids used in the present study. *P. syringae* was routinely maintained at 28°C on King's medium B (30), mannitol-glutamate (MG) (26), or MG supplemented with yeast extract at 0.25 g/liter (MGY). *Escherichia coli* strains were grown on Luria-Bertani medium (41) at 37°C. Antibiotics were added to media at the following concentrations (μg/ml): ampicillin, 100; tetracycline, 12.5; kanamycin, 25; spectinomycin, 25; chloramphenicol, 25; and gentamicin, 2.

Molecular genetic techniques. Plasmids were isolated from *P. syringae* as described by Kado and Liu (24). Restriction enzyme digests, agarose gel electrophoresis, Southern transfers, and isolation of DNA fragments from agarose gels were performed by using standard protocols (50). Genomic DNA was isolated from *P. syringae* by using established procedures (56), and a genomic library of FF5.36 was constructed in pRK7813 as described previously (2). Clones were mobilized into recipient strains by using a triparental mating procedure and the mobilizer plasmid pRK2013 (4).

DNA fragments were labeled with digoxigenin (Genius Labeling and Detection Kit; Boehringer Mannheim, Indianapolis, Ind.) or with [α-^{32}P]dCTP (Rad Prime DNA Labeling System; Gibco BRL, Gaithersburg, Md.). Hybridizations and posthybridization washes were conducted under high-stringency conditions (57).

Isolation and quantitation of alginate. Selected strains were inoculated by dilution streaking to MGY agar (three plates per strain) and incubated at 28°C for 96 h. Each plate was handled separately for quantitation of alginate. Cells were washed from each plate and resuspended in 0.9% NaCl. Removal of cellular material from the mucoid growth and estimation of total cellular protein were performed as described previously (40). Alginate production was assessed by the carbazole method, an assay which quantifies the total amount of uronic acid polymers (40). In addition to alginate, other uronic acid polymers are detected by this assay, but we previously demonstrated that these are very minor components of the mucoid material isolated from FF5(pPSR12) (29). Alginic acid from seaweed (*Macrocystis pyrifera*; Sigma Chemical Co., St. Louis, Mo.) was used as a standard in these experiments. Mean values of three replicates were expressed as micrograms of alginate per milligrams of protein.

DNA sequencing and analysis. Nucleotide sequencing reactions were performed by the dideoxynucleotide method (50) with Ampli*Taq* DNA polymerase (Perkin-Elmer, Foster City, Calif.). Automated DNA sequencing was accomplished by using an ABI 373A apparatus and the ABI PRISM Dye Primer Cycle Sequencing Kit (Perkin-Elmer). Automated sequencing was provided by the Oklahoma State University Recombinant DNA-Protein Resource Facility. The Tn*5* insertion in FF5.36 was localized by sequencing the DNA flanking the transposon by using the oligonucleotide 5′-GGTTCCGTTCAGGACGCTAC, which is derived from the border region of IS*50* (49). Sequence data were aligned and homology searches were executed by using the University of Wisconsin Genetics Computer Group Sequence Analysis Package (version 9.0) or the National Center for Biotechnology Information BLAST network server.

Construction of an *algT* mutant of *P. syringae*. The chloramphenicol resistance (Cm^r) gene in pSL1 was used to construct a nonpolar mutation in *algT*. pBTB6.5, which contains *algT* in a 6.5-kb *Bam*HI fragment in pBluescript SK(+), was linearized with *Nru*I, which generates a unique site within *algT* (Fig. 1A). The Cm^r cassette in pSL1 was excised as a 0.65-kb *Sma*I fragment and ligated into linearized pBTB6.5, resulting in pBTB.Cm. The 7.15-kb *Bam*HI fragment in pBTB.Cm was then excised and ligated into *Bam*HI-digested pRK415. pRTB.Cm, the construct containing *algT*::Cm^r in pRK415, was then introduced into *P. syringae* pv. syringae FF5(pPSR12) by triparental mating, and selection pressure for the vector (Tc^r) was removed to facilitate homologous recombination (4).

Heat killing assays. Bacterial cultures were grown to an A_{600} of 0.45 at 28°C and then incubated at 43°C for 0, 15, 30, 45, and 60 min; three replicate cultures were sampled at each time point. Cell dilutions were plated onto MGY agar in triplicate, and viable cells were scored as CFU. Survival was expressed as the percentage of input cells which retained viability.

Susceptibility to killing with ROI. Sensitivity to paraquat or hydrogen peroxide (H_2O_2) was determined by measuring the diameter of the inhibition zone surrounding filters impregnated with reactive oxygen intermediates (ROI)-generating agents. Filter disks (6 mm) were soaked with 5 μl of 1.9% paraquat or 3% H_2O_2 and placed on a layer of soft agar (2 ml of 0.6% agar) containing 100 μl of an overnight culture of *P. syringae*; this was allowed to gel on 25 ml of MGY

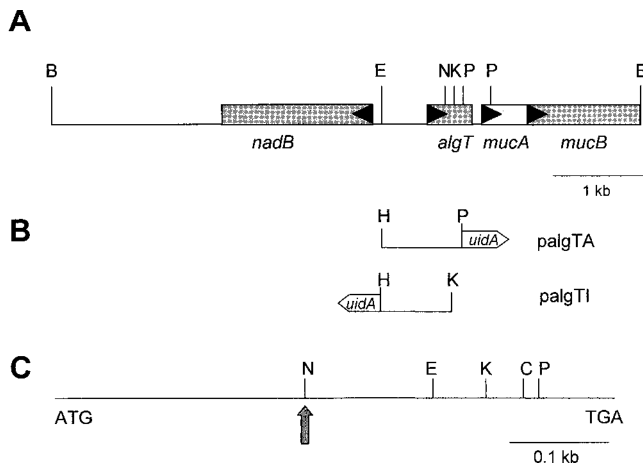

FIG. 1. (A) Physical and functional map of the 6.5-kb *Bam*HI fragment in pBTB6.5 and pRTB6.5. The arrows within each open reading frame indicate the direction of translation for each gene. (B) Location and orientation of the *algT*::*uidA* transcriptional fusions in palgTA and palgTI. The *Hin*dIII site was added during PCR amplification. (C) Expanded view of the 582-bp *algT* gene from *P. syringae* FF5(pPSR12). The arrow indicates the location used for insertion of the antibiotic resistance cassette (Cm^r). Abbreviations: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; K, *Kpn*I; N, *Nru*I; P, *Pst*I.

containing 1.5% agar. Cells were incubated at 28°C, and inhibition zones were measured 12 to 16 h after inoculation.

Construction of transcriptional fusions. pBBR.Gus, which contains a promoterless glucuronidase gene (*uidA*) downstream of the polylinker in pBBR1MCS, was used to create *algT*::*uidA* transcriptional fusions. To obtain the *algT* promoter region in transcriptionally active and inactive orientations, a 1-kb PCR product was cloned into the *Hin*dIII-*Pst*I or *Kpn*I-*Hin*dIII sites of pBBR.Gus, respectively. The promoter region was amplified from pBTB6.5 by using the forward primer 5′-CTGAAGCTTCTGCCCTTGGCGACCAC (the *Hin*dIII site is underscored and is followed by nucleotides corresponding to −488 to −472 in Fig. 2) and the reverse primer 5′-CTCTTGGGCTATCGCCGCTGTCTC (the complement of nucleotides 580 to 604 in Fig. 2). After amplification of the 1-kb PCR product, ligation in pCR2.1, and transformation into *E. coli* DH5α, plasmids pCRalgTA and pCRalgT1 were recovered. These were digested with *Hin*dIII and *Pst*I (pCRalgTA) or *Hin*dIII and *Kpn*I (pCRalgTI) and ligated into pBBR.Gus, resulting in palgTA and palgTI, respectively (Fig. 1B).

GUS assays. Transcriptional activity was initially screened by spotting bacterial suspensions (A_{600} = 0.1) onto MG agar medium amended with chloramphenicol and 20 μg of X-Gluc (5-bromo-4-chloro-3-indolyl glucuronide) per ml; plates were then incubated at 28°C for 48 h. Prior to quantitative glucuronidase (GUS) assays, all strains were grown overnight in MGY broth containing chloramphenicol. Bacterial concentrations were then adjusted to an A_{600} of 0.1 in MGY broth and incubated at 28°C at 250 rpm. For temporal studies, 1-ml aliquots (three replicates per time point) were removed at 0, 1, 2, 5, 8, 12, 24, and 30 h and analyzed for GUS activity as described previously (44). GUS activity was expressed in units per milligram of protein with 1 U equivalent to 1 nmol of methylumbelliferone formed per min. The effect of salt, sorbitol, and copper sulfate on *algT* expression was evaluated by adjusting the bacterial concentration to an A_{600} of 0.1 and incubating the cells for 10 h in MGY broth amended with NaCl (0.15, 0.3, or 0.4 M), sorbitol (0.3, 0.6, or 0.8 M), or $CuSO_4$ (50, 100, or 200 μM). *algT* transcription was also investigated by preparing bacterial suspensions as described above, growing them to an A_{600} of 0.5, and incubating them at elevated temperature (50°C) or in media amended with H_2O_2 or paraquat (0.001 or 0.01%). Bacterial cells (1 ml) were removed at 0, 15, 30, 60, and 120 min for temperature studies and at 0, 15, 30, and 60 min for assays with H_2O_2 and paraquat.

Nucleotide sequence accession number. The nucleotide sequence for *algT* in *P. syringae* pv. syringae has been deposited in the GenBank database under accession no. AF190580.

RESULTS

Location of Tn*5* insertion in FF5.36. The Tn*5* mutant FF5.36 exhibited a leaky phenotype for alginate and produced low amounts of the exopolysaccharide in vitro; this mutant was previously isolated by mutagenesis of FF5(pPSR12), which produces alginate constitutively at high levels (29). To locate the Tn*5* insertion in FF5.36, a genomic library of this mutant was constructed in pRK7813, and a cosmid clone containing the Tn*5* insertion was recovered and designated pFF5.36. The internal *Bam*HI site in Tn*5* and 3 kb of flanking DNA from FF5.36 were cloned from pFF5.36 into pBluescript SK(+), resulting in a clone named pFF5.36B. A primer specific for the border region of IS*50* indicated that the Tn*5* insertion was located within *nadB* at nucleotide 61 of the corresponding *P. aeruginosa* sequence (12). The *nadB* gene encodes L-aspartate oxidase and is located approximately 400 bp upstream of *algT* in *P. aeruginosa* (12). A 600-bp region downstream of the Tn*5* insertion was sequenced in pFF5.36B; this region showed 73% nucleotide sequence identity to the first 100 bp of *nadB* and 65% nucleotide sequence identity to the *nadB-algT* intergenic region and the 5′ end of *algT* from *P. aeruginosa*. These results indicated that the location of *nadB* and *algT* was conserved in *P. syringae* and *P. aeruginosa*.

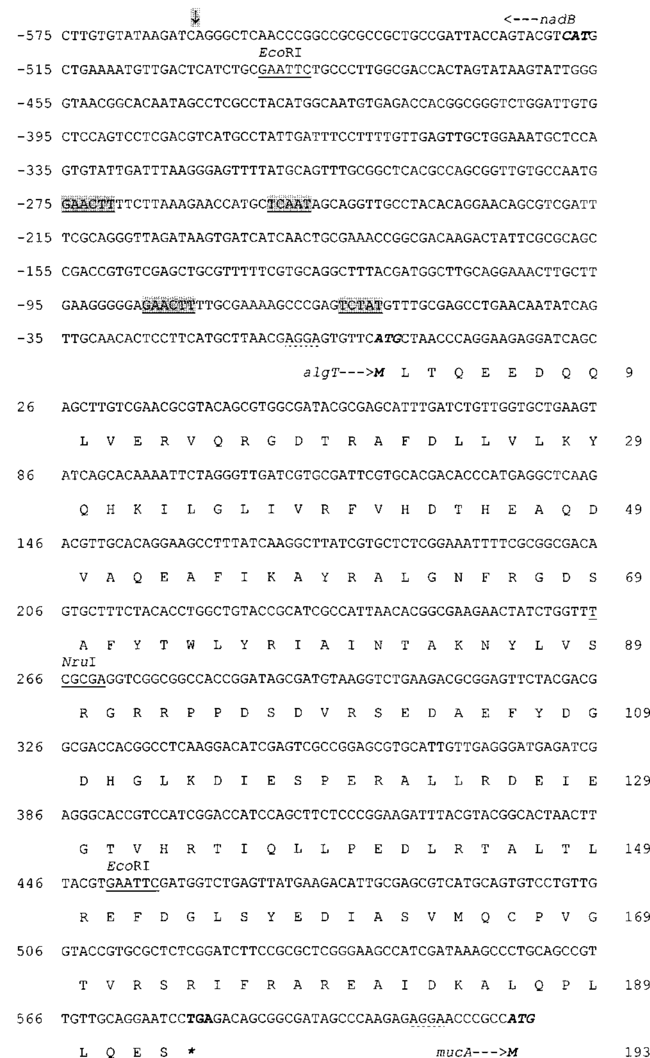

FIG. 2. Nucleotide sequence of *algT* from *P. syringae* pv. syringae FF5 (pPSR12) containing the 5′ end of *nadB* and the *nadB-algT* intergenic region. The vertical arrow shows the Tn*5* insertion site in mutant FF5.36. Potential recognition sequences for σ^{22} are shaded and underlined, and the putative ribosome binding site for *algT* is underlined. Nucleotides are numbered with respect to the *algT* translational start site and are indicated on the left; amino acid residues for AlgT are indicated on the right. Translational start sequences are shown in bold italics, and the translational stop codon for *algT* is indicated by a bold asterisk.

FIG. 3. Alignment of the *algT* promoter sequences from *P. syringae* pv. syringae FF5(pPSR12) (Ps algT) and *P. aeruginosa* (Pa algT). The nucleotides for the *P. aeruginosa* sequence are shown on the left with +1 (see asterisk) corresponding to the transcriptional start site. Nucleotides for the *P. syringae* pv. syringae *algT* promoter are shown on the right with +1 corresponding to the translational start site. Gaps (--) were used to maximize the alignment, and identical bases are shaded. The σ^{22} recognition sequences in both species are indicated in boldface type and are double-underscored. The *nadB* and *algT* translational start sites are in boldface, and the direction of translation is indicated with an arrow.

Cloning of *algT* from *P. syringae.* A genomic library of *P. syringae* FF5(pPSR12) was previously constructed in pRK7813 (46). In the current study, the 6-kb *Bam*HI fragment from pFF5.36B, which contains a portion of *algT*, was used to screen the library for clones containing the complete *algT* coding region. One clone designated pLKT5 was chosen for further study and contained a 6.5-kb *Bam*HI fragment which hybridized with the probe. This fragment was subcloned in pBluescript SK(+), resulting in pBTB6.5, and partially sequenced by using the T7 and T3 primers. Sequence analysis indicated that the right border of this fragment contained DNA homologous to *mucB* (Fig. 1A). Since *algT* is generally associated with the *mucABCD* gene cluster (20, 38), we suspected that pBTB6.5 contained a functional copy of *algT*.

Sequence analysis of *algT*. A physical map of pBTB6.5 was constructed to further localize the alginate regulatory genes on this fragment (Fig. 1A). Sequence data for the *P. syringae algT* gene were initially derived by using a primer based on the nucleotide sequence downstream of the Tn*5* insertion located in FF5.36 (see vertical arrow, Fig. 2). Additional sequence data was obtained by primer walking, and both DNA strands were sequenced for verification. The *P. syringae algT* homologue was 582 bp and was highly related to *algT* from *P. aeruginosa* (81 and 90% nucleotide and amino acid identities, respectively) (13). The deduced translational product of *algT* is a protein consisting of 193 amino acids with a predicted mass of 22.3 kDa. A potential ribosome binding site was identified 7 bp upstream of the predicted translational start site. Two putative AlgT (σ^{22}) recognition sites were located 60 and 248 bp upstream of the *algT* translational start site (Fig. 2). The location and sequence of the first σ^{22} recognition site (60 bp upstream of the initiation codon) was conserved in both *P. syringae* and *P. aeruginosa* (Fig. 3). The *nadB* gene in *P. syringae* was located 516 bp upstream of the *algT* translational start site and was divergently transcribed with respect to *algT* (Fig. 1 and 2). Interestingly, nucleotide identity in the 516-bp intergenic region between *nadB* and *algT* was only 46% when the *P. syringae* and *P. aeruginosa* regions were compared (Fig. 3). Additional sequencing downstream of *algT* revealed *mucA* and *mucB* homologues (Fig. 1) which showed 68 and 70% nucleotide identities, respectively, to the genes previously sequenced in *P. aeruginosa* (19, 35). In summary, the arrangement of *nadB*, *algT*, *mucA*, and *mucB* is conserved in *P. syringae*, *P. aeruginosa*, and *A. vinelandii* (12, 35, 38).

Construction of an *algT* mutant. FF5.36, which contains a Tn*5* insertion in *nadB*, was unstable with respect to alginate production. To avoid potential polar effects on adjacent genes, we constructed an *algT* mutant with a Cm^r cassette which lacks transcriptional terminators. Recombination of the Cm^r cassette into *algT* was verified by PCR and sequence analysis. FF5.LK1, the *algT* mutant resulting from this experiment, produced 61 μg of uronic acid polymers/mg of protein, a level approximately 43-fold lower and significantly less ($P = 0.01$) than FF5(pPSR12), which synthesized 2,652 μg of uronic acid polymers/mg of protein. Previous work indicated that most of the uronic acid polymers synthesized by FF5(pPSR12) were alginate (29). Furthermore, alginate-defective strains of FF5 (pPSR12) still synthesized low amounts of uronic acid polymers in the carbazole assay (15, 29, 46); therefore, we concluded that the *algT* mutant, FF5.LK1, was defective in alginate production.

Complementation experiments. pRTB6.5, which contains *algT*, *mucA*, and *mucB* in pRK415, was evaluated for its ability

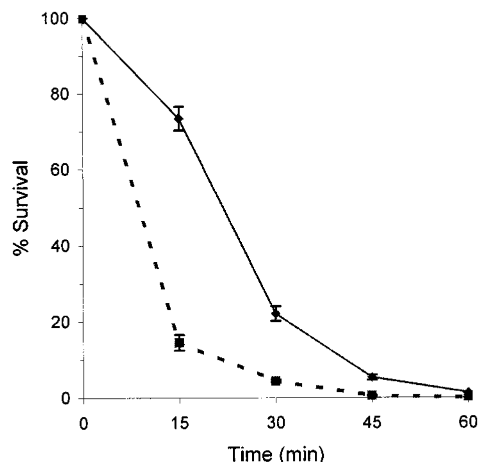

FIG. 4. Heat-killing curves for *P. syringae* pv. syringae FF5(pPSR12) (wild-type [◆]) and FF5.LK1 (*algT* mutant [■]). The strains were incubated at 43°C for 15, 30, 45, and 60 min, and surviving cells were counted as CFU. Bars indicate standard errors of the means, and survival is expressed as the percentage of input CFU at time zero. The experiment was repeated with similar results.

TABLE 2. Sensitivity to killing by paraquat and H_2O_2 in the wild-type and *algT* mutant of *P. syringae* pv. syringae

Strain	Characteristics	Growth inhibition zone (mean diam [mm] ± SE)[a]	
		Paraquat (1.9%)	H_2O_2 (3%)
FF5(pPSR12)	Mucoid, wild-type	24.4 ± 0.2	13.3 ± 0.1
FF5.LK1	Nonmucoid, *algT*::Cm^r	32.2 ± 0.2	16.5 ± 0.1

[a] Sensitivities to paraquat and H_2O_2 are expressed as diameters of growth inhibition zones surrounding filter disks impregnated with 5 μl of the indicated solutions. The experiment was repeated with similar results.

to complement *P. syringae* pv. syringae FF5.LK1 for alginate production. pRTB6.5 did not restore alginate production to FF5.LK1, possibly because this plasmid also contains the negative regulators, *mucA* and *mucB*, which could suppress the conversion to mucoidy (19, 36). Consequently, we examined whether palgTA.1, which contains *algT* but lacks extraneous flanking DNA, could restore alginate production to FF5.LK1. Transconjugants of FF5.LK1 containing palgTA.1 were visibly mucoid and produced 1,086 μg of alginate/mg of protein; this amount was significantly higher ($P = 0.01$) than the level synthesized by FF5.LK1, indicating that palgTA.1 could partially complement the *algT* mutant. We also investigated whether pJG309, which contains *algT* from *P. aeruginosa*, could complement FF5.LK1 for alginate production. FF5.LK1(pJG309) transconjugants produced 1,081 μg of alginate per mg of protein, a level equivalent to that obtained with FF5.LK1 (palgTA.1), which suggests that the two genes may be functionally interchangeable.

Effects of *algT* on susceptibility to heat and ROI. Previous reports indicate that *algT* functions as an alternative sigma factor in *P. aeruginosa* and is involved in the transcriptional activation of heat shock genes (37, 52). Therefore, we evaluated whether the *algT* mutation in FF5.LK1 resulted in an increased sensitivity to heat killing when compared to the wild-type FF5(pPSR12). Survival after exposure to 43°C was significantly reduced in the *algT* mutant compared to the wild-type strain (Fig. 4). Within 15 min there was an 85% reduction in the viability of the mutant compared with only 27% in the wild-type (Fig. 4). No difference in viability between the wild-type and mutant strains was apparent after a 60-min incubation at 43°C (Fig. 4).

To determine whether *algT* is involved in tolerance to compounds that generate ROIs, the wild-type FF5(pPSR12) and *algT* mutant FF5.LK1 were exposed to H_2O_2 and paraquat, a superoxide-generating redox cycling compound (16). FF5.LK1 was significantly more sensitive to paraquat and H_2O_2 than FF5 (pPSR12) (Table 2), indicating that *algT* has a role in mediating resistance to ROIs in *P. syringae*. Both FF5(pPSR12) and FF5.LK1 grew at identical rates in vitro (data not shown), indicating that the *algT* mutation did not significantly affect growth.

Kinetics of *algT* transcription. palgTA, palgTI, and pBBR.Gus were mobilized into *P. syringae* pv. syringae FF5 and assayed for GUS activity. Colonies of FF5(palgTA) turned blue on media containing X-Gluc, indicating that *algT* was transcribed at physiological temperatures (28°C). FF5 transconjugants containing palgTI (*algT* in the transcriptionally inactive orientation) or pBBR.Gus (vector control) remained colorless on X-Gluc media. When FF5 transconjugants containing palgTA, palgTI, or pBBR.Gus were grown in MGY broth at 28°C, growth curves were similar, indicating that the transcriptional fusions had no significant effect on growth (data not shown). A time course experiment at 28°C indicated that *algT* transcriptional activity increased steadily over time with 960 U of GUS/mg of protein at 30 h; this gradual increase in expression is similar to observations made for *algT* in *P. aeruginosa* (13). GUS activity in FF5(palgTI) and FF5(pBBR.Gus) remained low (1 to 14 U) throughout the sampling period and was not significantly different between the two transconjugants; consequently, FF5 (pBBR.Gus) was used as a negative control in all subsequent experiments.

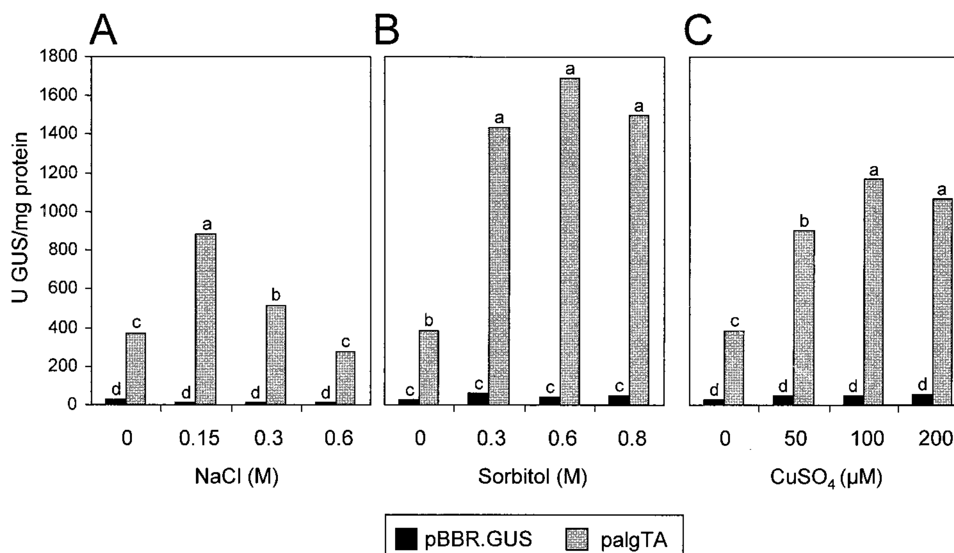

FIG. 5. GUS activity in *P. syringae* pv. syringae FF5 derivatives grown in MGY broth containing sodium chloride (A), sorbitol (B), or copper sulfate (C). Prior to the GUS assays, all strains were grown for 20 h in MGY broth containing chloramphenicol. The bacterial concentration was adjusted to an A_{600} of 0.1, and the cells were incubated at 250 rpm for 10 h at 28°C in MGY broth amended with NaCl, sorbitol, or copper sulfate. palgTA contains the *algT* promoter in the transcriptionally active orientation, and pBBR.Gus contains a promoterless glucuronidase gene. Values are the mean from one experiment containing three replicates, and the experiment was repeated with similar results. Treatments accompanied by the same lowercase letter were not significantly different at a P of 0.01 as shown by Duncan's multiple-range test.

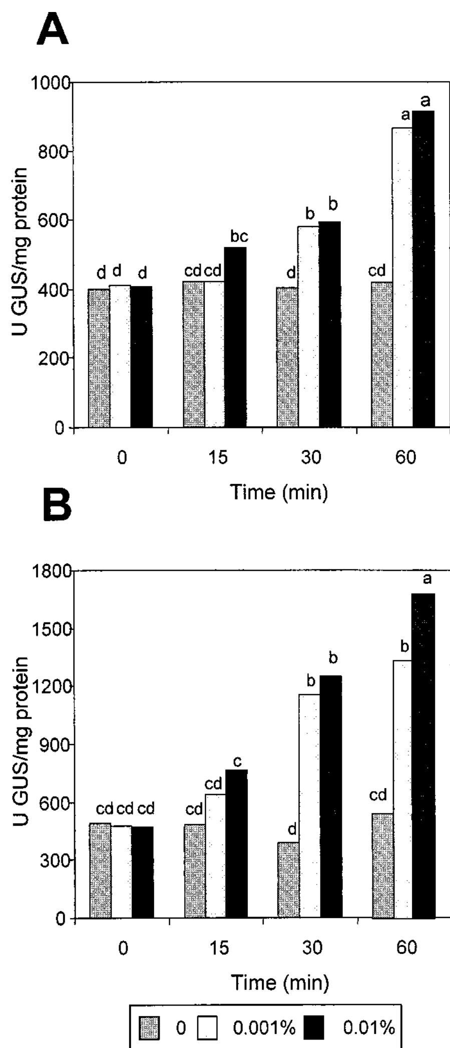

FIG. 6. GUS activity in *P. syringae* pv. syringae FF5 derivatives grown in MGY broth containing H_2O_2 (A) or paraquat (B). Prior to the GUS assays, all strains were initially grown as described in Fig. 5; bacterial concentrations were then adjusted to an A_{600} of 0.5 and incubated in MGY broth amended with H_2O_2 or paraquat. palgTA is described in Fig. 5. Values represent the mean from one experiment containing three replicates; the experiment was repeated with similar results. Treatments accompanied by the same lowercase letter were not significantly different at a *P* of 0.01 as determined by Duncan's multiple-range test.

***algT* expression in response to selected factors.** GUS activity in FF5(palgTA) was significantly higher ($P = 0.01$) when the growth medium was amended with 0.15 or 0.3 M NaCl; in contrast, FF5(pBBR.Gus) showed no response to the addition of NaCl (Fig. 5A). To determine whether the effect of NaCl was ionic or osmotic, sorbitol (a nonionic, nonmetabolizable solute) was examined for its effect on *algT* expression. Sorbitol was added to MGY broth at 0.3, 0.6, and 0.8 M, concentrations which are osmotically equivalent to 0.15, 0.3, and 0.4 M NaCl, respectively. The transcriptional activity of *algT* was significantly higher ($P = 0.01$) than the nonsupplemented control when sorbitol was added at all concentrations tested (Fig. 5B). Therefore, the stimulation of *algT* gene expression by NaCl is due to increased osmolarity rather than an ionic effect.

We previously demonstrated that the addition of copper sulfate to the growth medium increased both alginate production and *algD* transcriptional activity in *P. syringae* pv. syringae FF5 (29, 46). In the present study, we found that *algT* gene expression was significantly higher ($P = 0.01$) than the nonamended control when copper sulfate was added at all concentrations tested (Fig. 5C). Since the *algT* gene product, σ^{22}, binds to the *algD* promoter and activates transcription, these results suggest that copper sulfate may stimulate alginate production via the *algT* signal transduction pathway.

The addition of H_2O_2 or paraquat to actively growing cultures of FF5(palgTA) at 0.001 and 0.01% stimulated *algT* expression 30 min after each compound was added (Fig. 6). When FF5(palgTA) was incubated for 60 min in H_2O_2 or paraquat, a 2.3- to 3.7-fold increase in *algT* expression was observed, respectively (Fig. 6). Longer incubation periods (4 h) did not result in further stimulation of *algT* gene expression (data not shown). Furthermore, a basal level of *algT* transcriptional activity (ca. 400 U of GUS/mg of protein) was necessary to see further induction of the *algT* promoter when ROI-generating compounds were added; otherwise both H_2O_2 and paraquat were toxic (data not shown).

When actively growing FF5(palgTA) cells were subjected to a temperature upshift (30 to 50°C), a significant increase in *algT* transcriptional activity was apparent within 15 min and was twofold higher than in control cells (which were not heat shocked) at 60 min (Fig. 7). GUS activity in FF5(pBBR.Gus) remained low, regardless of temperature (Fig. 7).

DISCUSSION

In the present study, the Alg$^-$ mutant FF5.36 contained a Tn*5* insertion in *nadB*, which encodes L-aspartate oxidase, a flavoprotein in the pathway for NAD biosynthesis (12). The *nadB* gene in both *P. syringae* and *P. aeruginosa* (12) is encoded

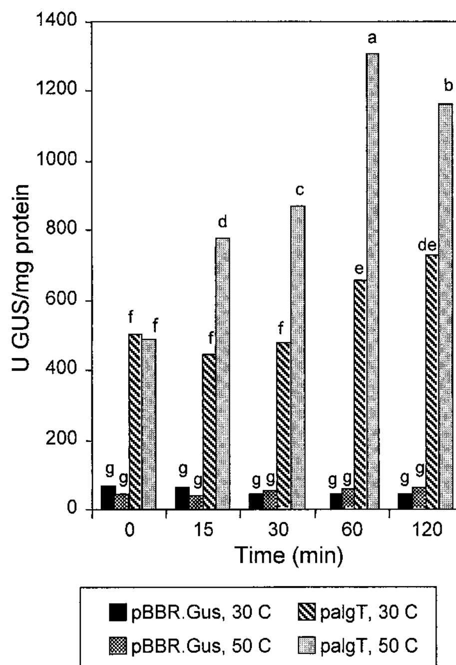

FIG. 7. GUS activity in *P. syringae* pv. syringae FF5 subjected to heat shock (temperature shift from 30 to 50°C). Prior to GUS assays, all strains were grown at 30°C as described in Fig. 5. The bacteria were incubated at 30°C until the concentration had an A_{600} of 0.5 and were then exposed to heat shock by rapid transfer to 50°C. palgTA and pBBR.Gus are described in Fig. 5. Values represent the mean from one experiment containing three replicates, and the experiment was repeated with similar results. Treatments accompanied by the same lowercase letter were not significantly different at a *P* of 0.01 as determined by Duncan's multiple-range test.

upstream of *algT* and divergently transcribed with respect to *algT*. In *P. aeruginosa*, *nadB* was not essential for NAD production, and a Tn*501* insertion in *nadB* did not affect alginate biosynthesis (12). However, in the present study, the *nadB*::Tn*5* insertion in FF5.36 was unstable with respect to alginate production. Although we could not identify additional Tn*5* insertions in FF5.36, it remains possible that additional point mutations may have occurred, leading to a nonmucoid phenotype. Therefore, the *algT* mutant FF5.LK1 was constructed in the present study, and all subsequent experiments were conducted with this mutant.

The *algT* genes in *P. syringae* and *P. aeruginosa* are highly homologous (81% nucleotide identity) and closely related to *rpoE*, which encodes σ^E, an alternate sigma factor involved in high-temperature gene expression in *E. coli* (11, 13, 14). Our results show that the *algT* promoter region in *P. syringae* contained two motifs conserved in promoters transcribed by the RNAP-σ^E complex (11) (Fig. 3). In *P. aeruginosa*, these two promoters were AlgT-dependent and designated P_1 and P_3 (52). The conservation of these promoters in *P. syringae* and *P. aeruginosa* and the complementation of FF5.LK1 with *algT* from both species suggest that the *algT* homologs in these two pseudomonads may be functionally interchangeable.

In *P. aeruginosa*, the negative regulatory genes *mucA* and *mucB* suppress alginate production, and mutagenesis of these genes results in a mucoid phenotype (19, 36). In the current study, pRTB6.5, which contains *algT*, *mucA*, and *mucB*, did not restore alginate production to the *algT* mutant FF5.LK1. However, palgTA.1, which contains *algT* without extraneous flanking DNA, partially restored alginate production to FF5.LK1. Previous studies have shown that MucA physically binds AlgT (σ^{22}) and functions as an anti-σ factor (53, 61), whereas MucB is presumed to alter the conformation of MucA in such a way that it targets σ^{22} for degradation (39). Therefore, a stoichiometric relationship exists between these three proteins and may explain why alginate production was not fully restored to wild-type levels in FF5.LK1(palgTA.1).

There is growing evidence that the *algT-mucABCD* gene cluster forms a signal transduction system that modulates *algT* activity in response to environmental stress (13, 37, 51, 52, 63). The *algT* gene fusion from *P. syringae* was transcriptionally activated in response to both NaCl and sorbitol (Fig. 5), indicating that osmotic stress is a stimulus for *algT* activation in both *P. syringae* and *P. aeruginosa* (52). Phytopathogenic bacteria are exposed to high osmolarities on the leaf surface (3), and the increased synthesis of alginate is critical to survival during epiphytic colonization (62); therefore, transcriptional activation of *algT* may enhance epiphytic fitness.

The *algT* mutant of *P. syringae* was more sensitive to H_2O_2 and paraquat, and *algT* expression was activated in response to both compounds. Although an *algT* mutant of *P. aeruginosa* showed increased susceptibility to paraquat, no difference in sensitivity to H_2O_2 was detected between the mutant and wild-type strains (37). These results suggest that *P. syringae* and *P. aeruginosa* differ in their response to ROIs. Although aspects of the oxidative burst are similar in animal and plant hosts (32), plant cells produce ROIs (mainly H_2O_2) constitutively throughout the defense response (5), and H_2O_2 has an important role in plant disease resistance (1, 8, 59). In animals, alginate production by *P. aeruginosa* may suppress the oxidative burst in neutrophils and scavenge the ROIs produced by phagocytic cells (22, 55). Therefore, the activation of *algT* by ROIs and the subsequent production of alginate may help *P. syringae* evade the plant defense response.

In previous studies, copper sulfate stimulated *algD* transcriptional activity and alginate production in *P. syringae* (29, 46). However, copper sulfate was not a signal for *algD* gene expression or alginate production in clinical strains of *P. aeruginosa*, possibly because these strains are not repeatedly exposed to toxic levels of copper sulfate (29, 46). In the current study, the *algT* promoter in *P. syringae* pv. syringae FF5 was stimulated by exposure to copper sulfate (Fig. 5C), which is consistent with earlier studies showing *algD* activation in response to copper sulfate (46). In agriculture, bactericidal sprays containing copper sulfate are frequently used for the control of *P. syringae* and other phytopathogenic bacteria, and copper-mediated stress is high. Because copper is known to generate free radicals (58), the increased production of alginate in response to copper sulfate may be caused by oxidative stress. Alternatively, the sequence divergence in the *nadB-algT* intergenic regions of *P. syringae* and *P. aeruginosa* may reflect the unique activation of the *algT* promoter in *P. syringae* by copper sulfate.

The *algT* mutant of *P. syringae* was more sensitive to elevated temperature (Fig. 4), and *algT* expression was activated in response to heat shock (Fig. 7). In contrast to human and animal pathogens, little is known about how phytopathogenic bacteria respond to temperature stress. We recently demonstrated that *P. syringae* responds to heat shock by producing DnaK (28), a molecular chaperone that facilitates the disassembly of proteins that have been damaged by heat stress (34). The present study expands our knowledge of the temperature stress response in *P. syringae* and clearly shows that *algT* increases the heat tolerance of this bacterium. The increased production of alginate in response to elevated temperatures could be advantageous since the alginate capsule could provide some protection from the dehydration and desiccation which develop during heat stress.

In *P. syringae*, *algT* is required for alginate production and increases the survival of the bacterium during environmental stress. Copper and H_2O_2 are toxic compounds that *P. syringae* encounters during colonization of host plant tissues, and these substances may be unique signals for *algT* activation in this bacterium. However, heat shock is a conserved signal for activation of *algT* expression in both *P. aeruginosa* (52) and *P. syringae*. In *P. aeruginosa*, AlgT (σ^{22}) is required for transcription of *algD*, which encodes GDP-mannose dehydrogenase, the first committed step in the alginate biosynthetic pathway (10). In *P. syringae*, the *algD* promoter region contains a putative recognition site for σ^{22} (15), but the requirement of σ^{22} for *algD* transcription has not yet been demonstrated. However, the transcriptional activation of the *algT* and *algD* (46) promoters in response to heat, osmotic stress, and copper sulfate supports the hypothesis that *algT* may control activation of *algD* transcription in *P. syringae*. Studies are currently under way to examine this hypothesis and other possible roles for *algT* in the pathogenicity and fitness of *P. syringae*.

ACKNOWLEDGMENTS

C.B. acknowledges support from the Oklahoma Agricultural Experiment Station and Public Health Service grant AI 43311-01 from the National Institutes of Health.

We thank A. M. Chakrabarty and A. Peñaloza-Vázquez for reviewing this manuscript prior to publication.

REFERENCES

1. **Alvarez, M. E., R. I. Pennel, P. J. Meijer, A. Ishikawa, R. A. Dixon, and C. Lamb.** 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell **92:**773–784.
2. **Barta, T. M., T. G. Kinscherf, and D. K. Willis.** 1992. Regulation of tabtoxin production by the *lemA* gene in *Pseudomonas syringae*. J. Bacteriol. **174:**3021–3029.
3. **Beattie, G. A., and S. E. Lindow.** 1995. The secret life of foliar bacterial pathogens on leaves. Annu. Rev. Phytopathol. **33:**145–172.

4. **Bender, C. L., S. A. Young, and R. E. Mitchell.** 1991. Conservation of plasmid DNA sequences in coronatine-producing pathovars of *Pseudomonas syringae*. Appl. Environ. Microbiol. **57:**993–999.
5. **Bolwell, G. P., and P. Wojtaszek.** 1997. Mechanisms for the generation of reactive oxygen species in plant defence—a broad perspective. Physiol. Mol. Plant Pathol. **51:**347–366.
6. **Boucher, J. C., J. Martinez-Salazar, M. J. Schurr, M. H. Mudd, H. Yu, and V. Deretic.** 1996. Two distinct loci affecting conversion to mucoidy in *Pseudomonas aeruginosa* in cystic fibrosis encode homologs of the serine protease HtrA. J. Bacteriol. **178:**511–523.
7. **Boucher, J. C., M. J. Schurr, H. Yu, D. W. Rowen, and V. Deretic.** 1997. *Pseudomonas aeruginosa* in cystic fibrosis: role of *mucC* in the regulation of alginate production and stress sensitivity. Microbiology **143:**3473–3480.
8. **Chamnongpol, S., H. Willekens, W. Moeder, C. Langebartels, H. Sandermann, M. Van Montagu, D. Inzé, and W. Van Camp.** 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. Proc. Natl. Acad. Sci. USA **95:**5818–5823.
9. **Chitnis, C. E., and D. E. Ohman.** 1993. Genetic analysis of the alginate biosynthetic gene cluster of *Pseudomonas aeruginosa* shows evidence of an operonic structure. Mol. Microbiol. **8:**583–590.
10. **Deretic, V., J. F. Gill, and A. M. Chakrabarty.** 1987. *Pseudomonas aeruginosa* infection in cystic fibrosis: nucleotide sequence and transcriptional regulation of the *algD* gene. Nucleic Acids Res. **15:**4567–4581.
11. **Deretic, V., M. J. Schurr, J. C. Boucher, and D. W. Martin.** 1994. Conversion of *Pseudomonas aeruginosa* to mucoidy in cystic fibrosis: environmental stress and regulation of bacterial virulence by alternative sigma factors. J. Bacteriol. **176:**2773–2780.
12. **DeVries, C. A., D. J. Hassett, J. L. Flynn, and D. E. Ohman.** 1995. Genetic linkage in *Pseudomonas aeruginosa* of *algT* and *nadB*: mutation in *nadB* does not affect NAD biosynthesis or alginate production. Gene **156:**63–67.
13. **DeVries, C. A., and D. E. Ohman.** 1994. Mucoid-to-nonmucoid conversion in alginate-producing *Pseudomonas aeruginosa* often results from spontaneous mutations in *algT*, encoding a putative alternative sigma factor, and shows evidence of autoregulation. J. Bacteriol. **176:**6677–6687.
14. **Erickson, J. W., and C. A. Gross.** 1989. Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate σ factor involved in high-temperature gene expression. Genes Dev. **3:**1462–1471.
15. **Fakhr, M. K., A. Peñaloza-Vázquez, A. M. Chakrabarty, and C. L. Bender.** 1999. Regulation of alginate biosynthesis in *Pseudomonas syringae* pv. syringae. J. Bacteriol. **181:**3478–3485.
16. **Farr, S., and T. Kogoma.** 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol. Rev. **55:**561–585.
17. **Figurski, D., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided *in trans*. Proc. Natl. Acad. Sci. USA **76:**1648–1652.
18. **Gacesa, P.** 1998. Bacterial alginate biosynthesis—recent progress and future prospects. Microbiology **144:**1133–1143.
19. **Goldberg, J. B., W. L. Gorman, J. L. Flynn, and D. E. Ohman.** 1993. A mutation in *algN* permits *trans* activation of alginate production by *algT* in *Pseudomonas* species. J. Bacteriol. **175:**1303–1308.
20. **Govan, J. R. W., and V. Deretic.** 1996. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Microbiol. Rev. **60:**539–574.
21. **Hershberger, C. D., R. W. Ye, M. R. Parsek, Z.-D. Xie, and A. M. Chakrabarty.** 1995. The *algT* (*algU*) gene of *Pseudomonas aeruginosa*, a key regulator involved in alginate biosynthesis, encodes an alternative σ factor (σ^E). Proc. Natl. Acad. Sci. USA **92:**7941–7945.
22. **Jensen, E. T., A. Kharazmi, K. Lam, J. W. Costerton, and N. Høiby.** 1990. Human polymorphonuclear leucocyte response to *Pseudomonas aeruginosa* grown in biofilms. Infect. Immun. **58:**2382–2385.
23. **Jones, J. D. G., and N. Gutterson.** 1987. An efficient mobilizable cosmid vector, pRK7813, and its use in a rapid method for marker exchange in *Pseudomonas fluorescens* strain HV37a. Gene **61:**299–306.
24. **Kado, C. I., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
25. **Kato, J., and A. M. Chakrabarty.** 1991. Purification of the regulatory protein AlgR1 and its binding in the far upstream region of the *algD* promoter in *P. aeruginosa*. Proc. Natl. Acad. Sci. USA **88:**1760–1764.
26. **Keane, P. J., A. Kerr, and P. B. New.** 1970. Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. Aust. J. Biol. Sci. **23:**585–595.
27. **Keen, N. T., S. Tamaki, D. Kobayashi, and D. Trollinger.** 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene **70:**191–197.
28. **Keith, L. M. W., J. E. Partridge, and C. L. Bender.** 1999. *dnaK* and the heat stress response of *Pseudomonas syringae* pv. *glycinea*. Mol. Plant-Microbe Interact. **12:**563–574.
29. **Kidambi, S. P., G. W. Sundin, D. A. Palmer, A. M. Chakrabarty, and C. L. Bender.** 1995. Copper as a signal for alginate synthesis in *Pseudomonas syringae* pv. syringae. Appl. Environ. Microbiol. **61:**2172–2179.
30. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44:**2172–2179.
31. **Kovach, M. E., R. W. Phillips, P. H. Elzer, R. M. Roop III, and K. M. Peterson.** 1994. pBBR1MCS: a broad-host-range cloning vector. BioTechniques **16:**800–802.
32. **Lamb, C., and R. A. Dixon.** 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:**251–275.
33. **Lukomski, S., R. A. Hull, and S. I. Hull.** 1996. Identification of the O antigen polymerase (*rfc*) gene in *Escherichia coli* O4 by insertional mutagenesis using a nonpolar chloramphenicol resistance cassette. J. Bacteriol. **178:**240–247.
34. **Mager, W. H., and A. J. J. Kruijff.** 1995. Stress-induced transcriptional activation. Microbiol. Rev. **59:**506–531.
35. **Martin, D. W., M. J. Schurr, M. H. Mudd, and V. Deretic.** 1993. Differentiation of *Pseudomonas aeruginosa* into the alginate-producing form: inactivation of *mucB* causes conversion to mucoidy. Mol. Microbiol. **9:**497–506.
36. **Martin, D. W., M. J. Schurr, M. H. Mudd, J. R. Govan, B. W. Holloway, and V. Deretic.** 1993. Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients. Proc. Natl. Acad. Sci. USA **90:**8377–8381.
37. **Martin, D. W., M. J. Schurr, H. Yu, and V. Deretic.** 1994. Analysis of promoters controlled by the putative sigma factor AlgU regulating conversion to mucoidy in *Pseudomonas aeruginosa*: relationship to stress response. J. Bacteriol. **176:**6688–6696.
38. **Martínez-Salazar, J. M., S. Moreno, R. Nájera, J. C. Boucher, G. Espin, G. Soberon-Chávez, and V. Deretic.** 1996. Characterization of the genes coding for the putative sigma factor AlgU and its regulators MucA, MucB, MucC, and MucD in *Azotobacter vinelandii* and evaluation of their roles in alginate biosynthesis. J. Bacteriol. **178:**1800–1808.
39. **Mathee, K., C. J. McPherson, and D. E. Ohman.** 1997. Posttranslational control of the *algT* (*algU*)-encoded σ^{22} for expression of the alginate regulon in *Pseudomonas aeruginosa* and localization of its antagonist proteins MucA and MucB (AlgN). J. Bacteriol. **179:**3711–3720.
40. **May, T. B., and A. M. Chakrabarty.** 1994. Isolation and assay of *Pseudomonas aeruginosa* alginate. Methods Enzymol. **235:**295–304.
41. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Mohr, C. D., J. H. J. Leveau, D. P. Krieg, N. S. Hibler, and V. Deretic.** 1992. AlgR-binding sites within the *algD* promoter make up a set of inverted repeats separated by a large intervening segment of DNA. J. Bacteriol. **174:**6624–6633.
43. **Murillo, J., H. Shen, D. Gerold, A. K. Sharma, D. A. Cooksey, and N. T. Keen.** 1994. Characterization of pPT23B, the plasmid involved in syringolide production by *Pseudomonas syringae* pv. tomato. Plasmid **31:**275–287.
44. **Palmer, D. A., C. L. Bender, and S. B. Sharma.** 1997. Use of Tn*5*-gus*A5* to investigate environmental and nutritional effects on gene expression in the coronatine biosynthetic gene cluster of *Pseudomonas syringae* pv. *glycinea*. Can. J. Microbiol. **43:**517–525.
45. **Peñaloza-Vázquez, A., and C. L. Bender.** 1998. Characterization of CorR, a transcriptional activator which is required for biosynthesis of the phytotoxin coronatine. J. Bacteriol. **180:**6252–6259.
46. **Peñaloza-Vázquez, A., S. P. Kidambi, A. M. Chakrabarty, and C. L. Bender.** 1997. Characterization of the alginate biosynthetic gene cluster in *Pseudomonas syringae* pv. syringae. J. Bacteriol. **179:**4464–4472.
47. **Pier, G. B.** 1998. *Pseudomonas aeruginosa*: a key problem in cystic fibrosis. ASM News **64:**339–347.
48. **Rehm, B. H. A., and S. Valla.** 1997. Bacterial alginates: biosynthesis and applications. Appl. Microbiol. Biotechnol. **48:**281–288.
49. **Rich, J. J., and D. K. Willis.** 1990. A single oligonucleotide can be used to rapidly isolate DNA sequences flanking a transposon Tn*5* insertion by the polymerase chain reaction. Nucleic Acids Res. **18:**6673–6676.
50. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Schurr, M. J., and V. Deretic.** 1997. Microbial pathogenesis in cystic fibrosis: co-ordinate regulation of heat-shock response and conversion to mucoidy in *Pseudomonas aeruginosa*. Mol. Microbiol. **24:**411–420.
52. **Schurr, M. J., H. Yu, J. C. Boucher, N. S. Hibler, and V. Deretic.** 1995. Multiple promoters and induction by heat shock of the gene encoding the alternative sigma factor AlgU (σ^E) which controls mucoidy in cystic fibrosis isolates of *Pseudomonas aeruginosa*. J. Bacteriol. **177:**5670–5679.
53. **Schurr, M. J., H. Yu, J. M. Martinez-Salazar, J. C. Boucher, and V. Deretic.** 1996. Control of AlgU, a member of the σ^E-like family of stress sigma factors, by the negative regulators MucA and MucB and *Pseudomonas aeruginosa* conversion to mucoidy in cystic fibrosis. J. Bacteriol. **178:**4997–5004.
54. **Shankar, S., R. Ye, D. Schlictman, and A. M. Chakrabarty.** 1995. Exopolysaccharide alginate synthesis in *Pseudomonas aeruginosa*: enzymology and regulation of gene expression. Adv. Enzymol. **70:**221–255.
55. **Simpson, J. A., S. E. Smith, and R. T. Dean.** 1989. Scavenging by alginate of free radicals released by macrophages. Free Radical Biol. Med. **6:**347–353.
56. **Staskawicz, B. J., D. Dahlbeck, and N. T. Keen.** 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incom-

patibility on *Glycine max* (L.) Merr. Proc. Natl. Acad. Sci. USA **81:**6024–6028.
57. **Sundin, G. W., and C. L. Bender.** 1993. Ecological and genetic analysis of copper and streptomycin resistance in *Pseudomonas syringae* pv. syringae. Appl. Environ. Microbiol. **59:**1018–1024.
58. **Thurman, R. B., and C. P. Gerba.** 1989. The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. Crit. Rev. Environ. Control **18:**295–315.
59. **Van Camp, W., M. Van Montagu, and D. Inzé.** 1998. H_2O_2 and NO: redox signals in disease resistance. Trends Plant Sci. **3:**330–334.
60. **Wozniak, D. J., and D. E. Ohman.** 1994. Transcriptional analysis of the *Pseudomonas aeruginosa* genes *algR*, *algB*, and *algD* reveals a hierarchy of alginate gene expression which is modulated by *algT*. J. Bacteriol. **176:**6007–6014.
61. **Xie, Z., C. D. Hershberger, S. Shankar, R. W. Ye, and A. M. Chakrabarty.** 1996. Sigma factor-anti-sigma factor interaction in alginate synthesis: inhibition of AlgT by MucA. J. Bacteriol. **178:**4990–4996.
62. **Yu, J., A. Peñaloza-Vázquez, A. M. Chakrabarty, and C. L. Bender.** 1999. Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of *Pseudomonas syringae* pv. syringae. Mol. Microbiol. **33:**712–720.
63. **Yu, H., M. J. Schurr, and V. Deretic.** 1995. Functional equivalence of *Escherichia coli* σ^E and *Pseudomonas aeruginosa* AlgU: *E. coli rpoE* restores mucoidy and reduces sensitivity to reactive oxygen intermediates in *algU* mutants of *P. aeruginosa*. J. Bacteriol. **177:**3259–3268.
64. **Zielinski, N. A., R. Maharaj, S. Roychoudhury, C. E. Danganan, W. Hendrickson, and A. M. Chakrabarty.** 1992. Alginate synthesis in *Pseudomonas aeruginosa*: environmental regulation of the *algC* promoter. J. Bacteriol. **174:**7680–7688.