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Regulation of Alginate Biosynthesis in *Pseudomonas syringae* pv. syringae

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Both *Pseudomonas aeruginosa* and the phytopathogen *P. syringae* produce the exopolysaccharide alginate. However, the environmental signals that trigger alginate gene expression in *P. syringae* are different from those in *P. aeruginosa* with copper being a major signal in *P. syringae*. In *P. aeruginosa*, the alternate sigma factor encoded by *algT* (σ^{22}) and the response regulator AlgR1 are required for transcription of *algD*, a gene which encodes a key enzyme in the alginate biosynthetic pathway. In the present study, we cloned and characterized the gene encoding AlgR1 from *P. syringae*. The deduced amino acid sequence of AlgR1 from *P. syringae* showed 86% identity to its *P. aeruginosa* counterpart. Sequence analysis of the region flanking *algR1* in *P. syringae* revealed the presence of *argH*, *algZ*, and *hemC* in an arrangement virtually identical to that reported in *P. aeruginosa*. An *algR1* mutant, *P. syringae* FF5.32, was defective in alginate production but could be complemented when *algR1* was expressed in *trans*. The *algD* promoter region in *P. syringae* (*PsalgD*) was also characterized and shown to diverge significantly from the *algD* promoter in *P. aeruginosa*. Unlike *P. aeruginosa*, *algR1* was not required for the transcription of *algD* in *P. syringae*, and *PsalgD* lacked the consensus sequence recognized by AlgR1. However, both the *algD* and *algR1* upstream regions in *P. syringae* contained the consensus sequence recognized by σ^{22}, suggesting that *algT* is required for transcription of both genes.

The exopolysaccharide alginate is a copolymer of O-acetylated β-1,4-linked D-mannuronic acid and its C-5 epimer, L-guluronic acid (46). Alginate biosynthesis has been extensively studied in *Pseudomonas aeruginosa*, where it functions as a major virulence factor in strains infecting the lungs of cystic fibrosis patients (45). 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, 46). Structural genes that have been characterized in this region include *algA*, which encodes a bifunctional enzyme which functions as a phosphomannose isomerase and a GDP-mannose pyrophosphorylase (54); *algG*, which encodes a C-5 epimerase (7); *algF*, *algI*, and *algJ*, which are involved in acetylation of the alginate polymer (16, 17, 55); and *algD*, which encodes GDP-mannose dehydrogenase (11). This region also contains *algE* and *algK*, which encode proteins with putative roles in polymer export and synthesis, respectively (1, 9, 22), and *algL*, which encodes alginate lyase (6, 49). Other genes which map within this region include *alg44*, *alg8*, and *algX* (*alg60*) (33, 41, 60); however, the functional role of the proteins encoded by these genes remains unclear. Chitnis and Ohman (8) postulated that the alginate biosynthetic gene cluster in *P. aeruginosa* is organized as an operon with transcription initiating at the *algD* promoter.

A region mapping at 68 min on the *P. aeruginosa* chromosome harbors a gene cluster consisting of *algT* (*algU*), *mucA*, *mucB* (*algN*), *mucC*, and *mucD*. These genes modulate the conversion to constitutive alginate production; at the head of this regulatory hierarchy is *algT* (*algU*). The alternative sigma factor encoded by *algT*, σ^{22}, is required for transcription of *algD*, *algT*, and *algR1* (21, 51). *mucA* is a negative regulator of *algT* transcription and encodes an antisigma factor with affinity for σ^{22} (52, 62). Mutations in *mucA* inactivate the MucA protein and result in the Alg^+ phenotype; however, these mutations are unstable and spontaneous reversion to the Alg^- phenotype often occurs due to suppressor mutations in *algT* (14, 50, 52). The remaining *muc* genes also modulate the expression of *algT* and have been described elsewhere (19, 34, 52, 62).

Other genes controlling the regulation of alginate production include *algR1* (*algR*), *algR2* (*algQ*), *algR3* (*algP*), and *algB* (20, 53). AlgR1 functions as a response regulator member of the two-component signal transduction system and binds to multiple sites upstream of *algC* and *algD* (12, 24, 39, 65). Both the *algD* and *algR1* promoters show a consensus sequence at the −35/10 region which is consistent with recognition by σ^{22}, suggesting that an RNA polymerase-σ^{22} complex binds to both promoters and positively regulates transcription (51).

Like *P. aeruginosa*, phytopathogenic strains of *P. syringae* are normally nonmucoid in vitro. Kidambi et al. (28) previously showed that exposure to copper ions stimulated alginate production in selected strains of *P. syringae*. Furthermore, an indigenous plasmid designated pPSR12 conferred constitutive alginate production to *P. syringae* pv. syringae FF5. 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 remain uncharacterized (28). Mutagenesis of FF5(pPSR12) with Tn*5* resulted in the isolation of alginate-defective (Alg^-) mutants, including FF5.31 and FF5.32 (28). The Tn*5* insertion in FF5.31 was located in *algL*, which encodes alginate lyase. Alginate production in FF5.31 was restored by pSK2, a cosmid clone containing homologues of *algD*, *alg8*, *alg44*, *algG*, *algX*, *algL*, *algF*, and *algA*. The order and arrangement of the alginate structural gene cluster were virtually identical to those

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
Escherichia coli		
DH5α		48
Pseudomonas syringae pv. syringae		
FF5	Cu^s; no detectable plasmids, nonmucoid	28
FF5.31	Cu^r Km^r; contains pPSR12, nonmucoid, *algL*::Tn*5*	44
FF5.32	Cu^r Km^r; contains pPSR12, nonmucoid, *algR1*::Tn*5*	28
Plasmids		
pPSR12	Cu^r Sm^r; 200 kb, confers constitutive alginate production to *P. syringae* pv. syringae FF5	28
pSK2	Tc^r; contains alginate biosynthetic cluster from *P. syringae* pv. syringae FF5 in pRK7813	44
pRK2013	Km^r; helper plasmid	15
pRK415	Tc^r; RK2-derived cloning vector	26
pRK7813	Tc^r; cosmid vector	23
pCP13	Tc^r; cosmid vector	10
pBluescript SK(+)	Ap^r; ColE1 origin, cloning vehicle	Stratagene
pRG960sd	Sm^r Sp^r; contains promoterless *uidA* with start codon and Shine-Dalgarno sequence	58
pBBR1MCS	Cm^r; 4.7-kb broad-host-range cloning vector	30
pBBR.Gus	Cm^r; 6.6-kb promoter probe vector containing *uidA* in pBBR1MCS	43
pCR2.1	Ap^r Km^r; 3.9-kb cloning vector	Invitrogen
pADP	Ap^r Km^r; contains *PsalgD* as a 2.7-kb PCR product in pCR2.1	This study
pAPDP	Cm^r; 7.4-kb contains *PsalgD* as a 2.7-kb *Hin*dIII-*Eco*RV fragment in pBBR.Gus	This study
pAPDPΔ15	Cm^r; derivative of pAPDP containing a 1.5-kb deletion from the 5′ end of *PsalgD*	This study
pAPDPΔ23	Cm^r; derivative of pAPDP containing a 2.3-kb deletion from the 5′ end of *PsalgD*	This study
pSK3	Sm^r Sp^r; contains a 1.0-kb fragment from *PsalgD* in pRG960sd in the transcriptionally active orientation	44
pSK4	Sm^r Sp^r; contains a 1.0-kb fragment from *PsalgD* in pRG960sd in the transcriptionally inactive orientation	44
pAP32	Tc^r Km^r; contains Tn*5*-inactivated alginate genes from FF5.32 in pRK7813	This study
pAP32.1	Ap^r Km^r; contains a 5.3-kb *Bam*HI fragment consisting of 2.8 kb from Tn*5* and 2.5 kb of FF5.32 DNA in pBluescript SK(+)	This study
pMF4	Tc^r; cosmid clone from FF5(pPSR12) in pRK7813	This study
pMF6	Tc^r; cosmid clone from FF5(pPSR12) in pRK7813	This study
pMF6.1	Ap^r; contains a 2.7-kb *Eco*RI fragment from pMF6	This study
pMF6.2	Ap^r; contains a 2.0-kb *Pst*I fragment from pMF6	This study
pMF6.21	Tc^r; 2.0-kb *Pst*I fragment from pMF6.2 in pRK415 in the transcriptionally active orientation with respect to *lacZ* and *algR1*	This study
pMF6.22	Tc^r; 2.0-kb *Pst*I fragment from pMF6.2 in pRK415 in the transcriptionally inactive orientation with respect to *lacZ* and *algR1*	This study
pAD1039	Tc^r; contains *algR1* from *P. aeruginosa* in pCP13	V. Kapatral

previously described for *P. aeruginosa*. Complementation analyses, however, indicated that the structural gene clusters in *P. aeruginosa* and *P. syringae* were not functionally interchangeable when expressed from their native promoters (44).

In the present study, the Alg^- mutant FF5.32 was shown to contain a Tn*5* insertion in *algR1*. Unlike *P. aeruginosa*, expression from the *P. syringae algD* promoter (*PsalgD*) did not require a functional copy of *algR1*. Nucleotide sequence analysis indicated that *PsalgD* did not contain recognizable AlgR1 binding sites, which helps explain the differential regulation of alginate gene expression in *P. aeruginosa* and *P. syringae*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Table 1 lists the bacterial strains and plasmids used in the present study. *Pseudomonas* spp. were routinely maintained at 28°C on King's medium B (29), mannitol-glutamate (MG) medium (25), or MG medium supplemented with yeast extract at 0.25 g/liter (MGY); *Escherichia coli* strains were grown on Luria-Bertani (LB) medium (36) at 37°C. Antibiotics were added to the media at the following concentrations: ampicillin, 100 μg/ml; tetracycline, 25 μg/ml; kanamycin, 25 μg/ml; spectinomycin, 25 μg/ml; streptomycin, 25 μg/ml; piperacillin, 250 μg/ml; and chloramphenicol, 25 μg/ml.

Molecular genetic techniques. Plasmid DNA was isolated from *Pseudomonas* spp. by alkali lysis (48). Restriction enzyme digests, agarose gel electrophoresis, Southern transfers, and isolation of DNA fragments from agarose gels were performed by standard methods (48). Genomic DNA was isolated from *P. syringae* by established procedures (56), and a total genomic library of FF5.32 was constructed in pRK7813 as described previously (2). Clones were mobilized into nonmucoid recipient strains by using a triparental mating procedure and the mobilizer plasmid pRK2013 (4).

DNA fragments were isolated from agarose gels by electroelution (48) and labelled with digoxigenin (Genius labelling and detection kit; Boehringer Mannheim, Indianapolis, Ind.) or with [α-^{32}P]dCTP by using the 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 72 h. Each plate was handled separately for quantification of alginate. The cells were washed from each plate and resuspended in 0.9% NaCl. Removal of cellular material from the mucoid growth and estimation of the alginate content and total cellular protein were performed as described previously (35). Alginic acid from seaweed (*Macrocystis pyrifera*; Sigma Chemical Co., St. Louis, Mo.) was used as a standard in these experiments. Mean values of three replicate determinations were expressed as micrograms of alginate per milligram of protein.

Construction of transcriptional fusions. *PsalgD* was initially cloned in pCR2.1 as a 2.7-kb PCR product. Plasmid pSK2 was used as template, and the following oligonucleotides were used as primers: forward primer, 5′ TGGTGCTGGAAA TATCCACACC (located 100 bp downstream of the presumed translational start site of *algD* [P1 in Fig. 1A]); and reverse primer, 5′ AATTCTGCCAGTCCAG CCACTGAC (P2 in Fig. 1A). Following amplification of the 2.7-kb PCR product, ligation in pCR2.1, and transformation into *E. coli* DH5α, plasmid pAPD was recovered. The promoter probe construct, pBBR.Gus, which contains a promoterless glucuronidase gene (*uidA*) downstream of the polylinker in pBBR1MCS (43), was used to more precisely define the promoter region upstream of *algD*. pAPD was digested with *Hin*dIII and *Eco*RV, and the 2.7-kb insert was isolated, end-filled with Klenow, and ligated into pBBR.Gus. Transformants were selected on LB agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and chloramphenicol, and pAPDP was found to contain the *algD*::*uidA* fusion in the transcriptionally active orientation.

Exonuclease III (ExoIII) was used to determine the minimal size of the *PsalgD* promoter. pAPDP was digested with *Cla*I and *Apa*I, which generate ExoIII-sensitive and ExoIII-resistant sites, respectively. Staggered deletions in the

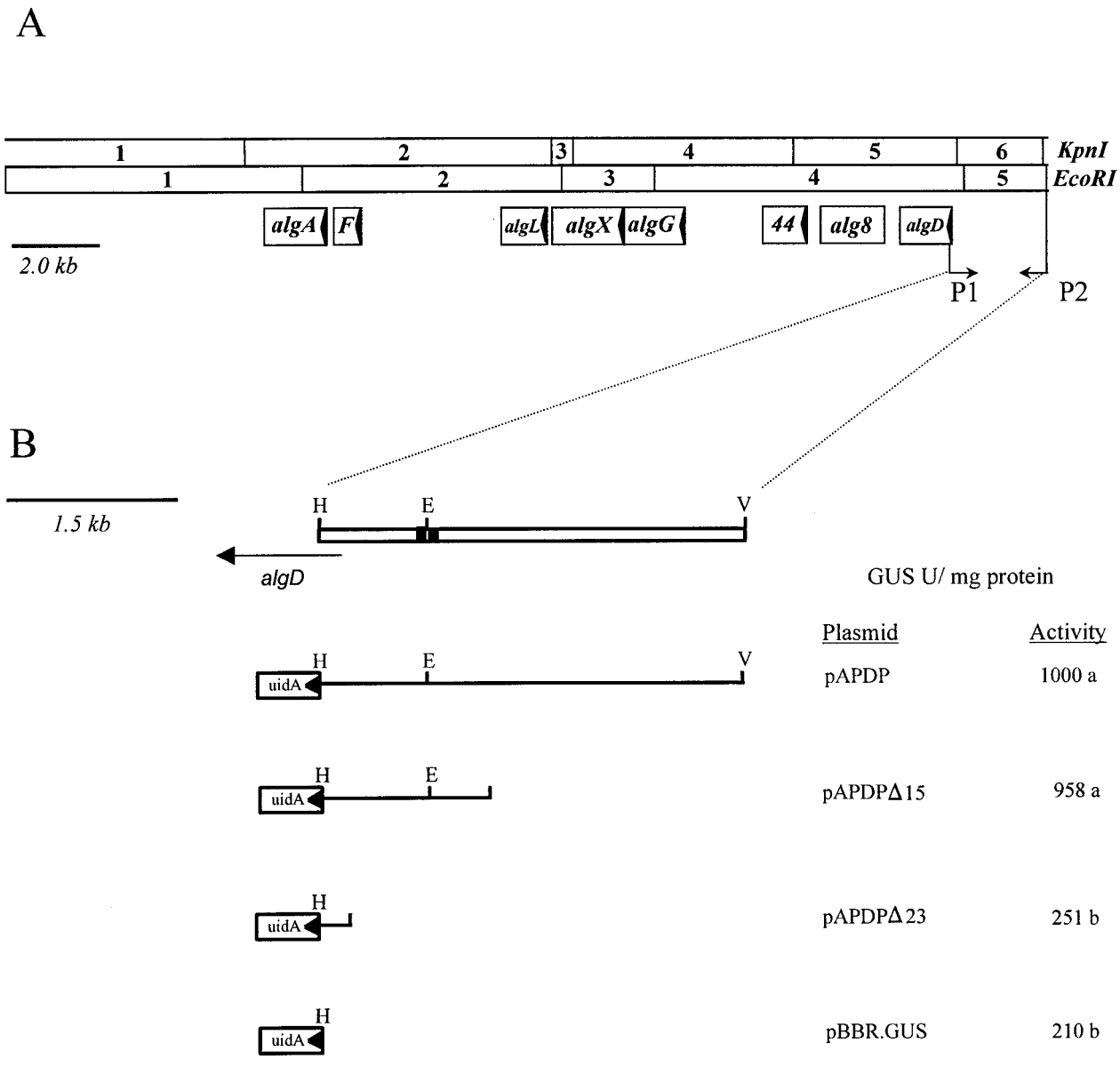

FIG. 1. (A) Physical and functional map of the alginate structural gene cluster in *Pseudomonas syringae* pv. syringae FF5. The arrows within each open reading frame indicate the direction of translation. The locations of the primers (P1 and P2) used to amplify the *algD* promoter region are indicated. Abbreviations: *F*, *algF*; *44*, *alg44*. (B) Expanded view of the region amplified with primers P1 and P2. The location and orientation of the coding region for *algD* are shown (horizontal arrow). The black boxes flanking the *Eco*RI site indicate the consensus sequence recognized by AlgT (σ^{22}). The location and orientation of the *algD*::*uidA* transcriptional fusions are indicated; GUS activity is shown in the column adjacent to each construct. Values followed by the same letter were not significantly different ($P = 0.01$). Abbreviations: E, *Eco*RI; H, *Hin*dIII; V, *Eco*RV.

PsalgD promoter region were generated by following the protocols supplied with the Erase-a-Base kit (Promega, Madison, Wis.). Transcriptional fusions were then mobilized into FF5(pPSR12) and assayed for glucuronidase activity as described below.

GUS assays. Transcriptional activity was initially screened by spotting bacterial suspensions (absorbance at 600 nm of 0.1) on MG agar medium amended with spectinomycin and 20 μg of X-Gluc (5-bromo-4-chloro-3-indolylglucuronide) per ml; the plates were then incubated at 28°C for 24 to 72 h. Glucuronidase (GUS) activity was quantified by fluorometric analysis of cells grown for 18 to 20 h in 3 ml of MG medium. Fluorescence was monitored with a Fluoroscan II version 4.0 microplate reader (ICN Biomedicals, Inc., Costa Mesa, Calif.) in 96-well microtiter plates. GUS activity was expressed in units per milligram of protein, with 1 U being equivalent to 1 nmol of methylumbelliferone formed per min. Values presented for GUS activity represent the average of three replicates per experiment. When significant differences in GUS activity were detected, the experiment was repeated.

DNA sequencing and analysis. Nucleotide sequencing reactions were performed by the dideoxynucleotide method with Ampli*Taq* DNA polymerase (Perkin-Elmer, Foster City, Calif.). Automated DNA sequencing was performed with 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 FF5.32 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*. Sequence data were aligned and homology searches were executed by using the University of Wisconsin Genetics Computer Group (UWGCG) sequence analysis package, version 9.0. Sequences associated with σ^{22} and AlgR1 binding were located by using the MOTIFS program included with the UWGCG software.

Nucleotide sequence accession numbers. The nucleotide sequences described in this study were deposited in GenBank under accession no. AF131199 (*fimS-algR1-hemC*) and AF131068 (*PsalgD*).

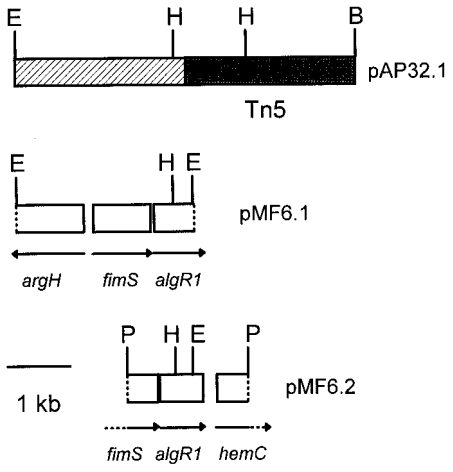

FIG. 2. Constructs used for the cloning and sequencing of *algR1* from *P. syringae* pv. syringae FF5. pAP32.1 is a subclone containing Tn*5* (shaded region) and flanking DNA from *P. syringae* pv. syringae FF5.32 (hatched region). The *Hin*dIII-*Eco*RI fragment in pAP32.1 was used as a probe for *algR1* in the current study. pMF6.1 and pMF6.2 are subclones derived from pMF6, a cosmid which complemented FF5.32 for alginate production. The 2.0-kb *Pst*I fragment in pMF6.2 was sequenced on both strands and shown to contain an intact copy of *algR1*. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I.

RESULTS

Location of Tn*5* insertion in FF5.32. A genomic library of FF5.32 was constructed in pRK7813, and a clone containing the Tn*5* insertion from FF5.32 was recovered and designated pAP32. The internal *Bam*HI site in Tn*5* and 2.5 kb of FF5.32 DNA were cloned from pAP32 into pBluescript SK(+), resulting in a clone named pAP32.1 (Fig. 2). A primer specific for the border region of IS*50* was used to sequence approximately 300 bp of FF5.32 DNA flanking the Tn*5* insertion site. This sequence showed 76% nucleotide identity to *algR1* from *P. aeruginosa*, and the Tn*5* insertion was located at nucleotide 51 of *algR1* from *P. aeruginosa* (12).

Genomic DNA from FF5(pPSR12) and FF5.32 was digested with *Eco*RI and analyzed by Southern blotting with the 2.3-kb *Hin*dIII-*Eco*RI fragment from pAP32.1 as a probe (Fig. 2). The probe hybridized to 2.7- and 8.4-kb *Eco*RI fragments in FF5(pPSR12) and FF5.32, respectively (data not shown). These results indicated that the region associated with *algR1* was located in a 2.7-kb *Eco*RI fragment, and the 2.7-kb fragment was inactivated by Tn*5* (5.7 kb) in FF5.32.

Cloning of *algR1* from *P. syringae*. A genomic library of *P. syringae* pv. syringae FF5(pPSR12) was previously constructed in pRK7813 (44). In the current study, the 2.3-kb *Hin*dIII-*Eco*RI fragment from pAP32.1 (Fig. 2) was used to screen the library for clones containing the complete *algR1* coding region. Seven cosmid clones hybridized with the probe; two clones designated pMF4 and pMF6 were chosen for further study and contained a 2.7-kb *Eco*RI fragment which hybridized with the probe. This fragment was subcloned from pMF6 in pBluescript SK(+), resulting in pMF6.1 (Fig. 2). Sequence information for pMF6.1 was generated with the T7 and T3 primers and indicated that this fragment contained DNA homologous to *argH*, *fimS*, and *algR1*. In previous studies, the *fimS* gene showed relatedness to sensor kinases of two-component systems and mapped immediately upstream of *algR1* in *P. aeruginosa* (61). It is important to note that *fimS*, which was also named *algZ* (63), is distinct from the *algZ* described by Baynham and Wozniak (3). To avoid further confusion in nomenclature, the name "*fimS*" will be used hereafter to describe the sensor kinase which maps adjacent to *algR1*. In *P. syringae*, *argH*, which encodes arginosuccinate lyase, mapped adjacent to *fimS*; in *P. aeruginosa*, *argH* was divergently transcribed with respect to both *fimS* and *algR1* (37, 63). Sequence analysis of pMF6.1 indicated that this arrangement is conserved in *P. syringae* (Fig. 2).

Sequence analysis indicated that pMF6.1 contained 560 bp of *algR1* but lacked approximately 180 bp located at the 3′ end. Southern blot analysis of pMF6 and pMF6.1 suggested that the intact *algR1* was probably contained in a 2.0-kb *Pst*I fragment; this was subcloned in pBluescript SK(+) and designated pMF6.2 (Fig. 2). pMF6.2 was completely sequenced on both strands and shown to contain DNA homologous to the 3′ end of *fimS* (585 bp), an intact copy of *algR1* (747 bp), and the 5′ end of *hemC* (432 bp). In *P. aeruginosa*, *hemC* encodes porphobilinogen deaminase and maps adjacent to *algR1* (40). The *P. syringae* homologues showed a high degree of relatedness to the corresponding *P. aeruginosa* genes; for example, nucleotide identity between *fimS*, *algR1*, and *hemC* in the two species was 88, 84, and 80%, respectively. Furthermore, the *algR1* homologue in *P. syringae* showed extensive relatedness (86 to 88% nucleotide identity) to *algR* from *Azotobacter vinelandii* (42) and to *pprA*, an *algR1* homologue in *P. putida* (59). In *P. aeruginosa*, AlgR1 contains two aspartate residues (D54 and D85) which have been suggested to function as phosphorylation sites (32, 61); both aspartate residues were present in the predicted translation product of *algR1* from *P. syringae*. A consensus sequence for σ^{22} was located 108 bp upstream of the *algR1* translational start site, a location which is also conserved in *P. aeruginosa* (63).

Complementation experiments. pMF4 and pMF6, the cosmid clones containing *argH*, *fimS*, *algR1*, and *hemC*, were evaluated for their ability to complement *P. syringae* pv. syringae FF5.32 for alginate production. Transconjugants of FF5.32 containing pMF4 or pMF6 were visibly mucoid and produced significantly more alginate than the mutant FF5.32 did (Table 2). Since Tn*5* frequently causes polar mutations on downstream genes, the 2.0-kb *Pst*I fragment in pMF6.2 was used to investigate whether the Alg$^-$ phenotype in FF5.32 was caused by the mutation in *algR1*. pMF6.2 contains an intact copy of *algR1* with the cognate σ^{22} recognition site and truncated copies of *fimS* and *hemC* (Fig. 2). The 2.0-kb *Pst*I fragment in pMF6.2 was subcloned in pRK415 to form pMF6.21 and pMF6.22, which contain *algR1* in the transcriptionally active and inactive orientations with respect to the *lac* promoter (Table 1). Both pMF6.21 and pMF6.22 restored alginate production to FF5.32 (Table 2), indicating that the Alg$^-$ phenotype of FF5.32 was caused by the Tn*5* insertion in *algR1*. FF5.32 was complemented with both clones irrespective of the orientation of the *lac* promoter and without the addition of isopropyl-β-D-thiogalactopyranoside (IPTG), indicating that a functional promoter for *algR1* was present on the 2.0-kb *Pst*I fragment. To

TABLE 2. Alginate production by derivatives of *P. syringae* pv. syringae FF5

Strain	Alginate production (μg/mg of protein)[a]
FF5(pPSR12)	3,791a
FF5.32	401b
FF5.32(pMF4)	2,635a
FF5.32(pMF6)	2,619a
FF5.32(pMF6.21)	3,450a
FF5.32(pMF6.22)	3,804a

[a] Mean values followed by the same letter are not significantly different at $P = 0.05$ by Duncan's multiple-range test.

TABLE 3. GUS activity for *P. syringae* pv. syringae FF5 and FF5.32 containing various promoter constructs with the *algD* upstream region

Strain[a]	GUS activity (U/mg of protein)[b] in strains containing:		
	pSK3	pSK4	pRG960sd
FF5(pPSR12)	537a	88b	66b
FF5.32	398a	82b	64b

[a] FF5(pPSR12) is the wild type, and FF5.32 is an *algR1* mutant derived from FF5(pPSR12).

[b] Mean values followed by the same letter are not significantly different at $P = 0.05$ by the Student-Newman Keuls test. pSK3 contains the *algD* promoter in the transcriptionally active orientation, pSK4 contains *algD* in the transcriptionally inactive orientation, and pRG960sd is the vector used for construction of pSK3 and pSK4. FF5(pPSR12, pSK3) and FF5.32(pRG960sd) were regarded as positive and negative controls for the GUS assay, respectively.

further confirm that FF5.32 was indeed an *algR1* mutant, we investigated whether this mutant could be complemented by *algR1* from *P. aeruginosa*. Plasmid pAD1039, which contains *algR1* from *P. aeruginosa* (Table 1), complemented FF5.32 and restored alginate production in the mutant to a level equivalent to FF5(pPSR12) (data not shown).

Expression of the *PsalgD* promoter does not require AlgR1. In *P. aeruginosa*, AlgR1 is required for expression of the *algD* promoter (*PalgD*) and has been shown to bind *PalgD* at several conserved sites (24, 39). A portion of *PsalgD* was previously cloned as a 1-kb fragment in the promoter probe vector, pRG960sd, creating pSK3 (*PsalgD*::*uidA*; transcriptionally active orientation) and pSK4 (*uidA*::*PsalgD*; transcriptionally inactive) (44). In the present study, we investigated whether *PsalgD* was transcriptionally active in FF5.32, the *algR1* mutant. GUS activities in FF5(pPSR12) and FF5.32(pSK3) were not significantly different (Table 3), indicating that a functional copy of *algR1* was not required for transcription of *algD* in *P. syringae*.

Analysis of the *PsalgD* promoter. To more fully characterize the minimum sequence necessary for *algD* expression in *P. syringae*, we constructed a series of deletions from the 5′ (*Eco*RV) end of the *PsalgD* promoter (Fig. 1B). A new construct, pAPDP (Fig. 1B), was designed for this purpose since the pBBR.Gus polylinker was more amenable to deletion analysis than was the multicloning site in pRG960sd, the vector used for construction of pSK3. Two deletion derivatives of pAPDP, pAPDPΔ15 and pAPDPΔ23, proved useful for delineating the *algD* promoter region; sequence analysis indicated that these two constructs lacked 1.5 and 2.3 kb of DNA downstream of the *Eco*RV site, respectively. FF5(pPSR12, pAPDPΔ15) (Fig. 1B) retained the full level of GUS activity exhibited by FF5 (pPSR12, pAPDP) (Fig. 1B), suggesting that the 1.5-kb region downstream of the *Eco*RV site was dispensable for promoter activity. However, GUS activity in FF5(pPSR12, pAPDPΔ23) was 3.8-fold lower than in FF5(pPSR12, pAPDPΔ15), demonstrating that deletion of an additional 0.8 kb from the 5′ end of pAPDPΔ15 virtually eliminated *PsalgD* promoter activity (Fig. 1B).

Sequence analysis of the *PsalgD* promoter in pAPDPΔ15 indicated that it contained a putative AlgT (σ^{22}) recognition site 508 bp upstream of the predicted *algD* translational start site (Fig. 3). In this respect, *PsalgD* is similar to the *algD* promoter in *P. aeruginosa* where a long, untranslated leader sequence is located between the *algD* translational start site and the σ^{22} binding region (11, 51). However, *PsalgD* lacked the AlgR1 binding sites, which are located upstream of the *algD* transcriptional start in *P. aeruginosa* (Fig. 3) (24, 38). The absence of these conserved motifs for AlgR1 binding could explain why the *P. syringae algD* promoter does not require a functional copy of *algR1* for transcriptional activity.

DISCUSSION

The AlgR1 mutant characterized in the present study, FF5.32, was previously shown to be completely defective in alginate synthesis (28), thereby demonstrating that AlgR1 is absolutely required for alginate production in *P. syringae*. However, the role of AlgR1 in *P. syringae* is unclear, since this protein is not required for *algD* expression; it remains possible that AlgR1 is required for transcriptional activation of *algC* in *P. syringae*, which is true in *P. aeruginosa* (65). Alternatively, AlgR1 may function differently in *P. syringae*, perhaps as part of a signal transduction cascade which controls alginate production. A complex regulatory network for alginate synthesis in *P. syringae* seems plausible, since plasmid-encoded regulatory genes are known to mediate the constitutive production of alginate in the *P. syringae* strains which harbor them (28).

The organization of the region flanking AlgR1 is conserved in both *P. aeruginosa* and *P. syringae* (*argH-fimS-algR1-hemC*). In both species, the σ^{22} recognition site preceding *algR1* is located within the 3′ end of *fimS* (63). FimS shows relatedness to the histidine protein kinases which function as environmental sensors, and both AlgR1 and FimS are required for twitching motility in *P. aeruginosa*, a process mediated by type IV pili. Although type IV pili have been identified in *P. syringae* (47), our efforts to demonstrate twitching motility in *P. syringae* pv. syringae FF5 were completely unsuccessful; therefore, the involvement of AlgR1 in twitching motility in *P. syringae* remains unclear. It has also been proposed that FimS may function as the cognate sensor kinase for AlgR1, but the exact role of FimS in alginate production remains unclear (61, 63). Interestingly, phosphorylation of AlgR1 was not required for alginate production in *P. aeruginosa* (32).

Sequence analysis of the *algR1* and *algD* upstream regions in *P. syringae* revealed the presence of σ^{22} recognition sites (Fig. 3). The σ^{22} recognition site identified in the *algR1* upstream region was identical to that identified in *P. aeruginosa*, whereas the σ^{22} recognition sequence in *PsalgD* differed from the corresponding sequence in *P. aeruginosa* by a single nucleotide (51). Although the transcriptional start sites for *algR1* and *algD* were not identified in *P. syringae*, the positions of the σ^{22} recognition sites relative to the translational start site are conserved in both species. The conservation of σ^{22} recognition sequences upstream of *algR1* and *algD* strongly suggests that transcriptional activation of these genes requires a functional copy of *algT*. An *algT* homologue in *P. syringae* has recently been identified, and the role of *algT* in the transcriptional activation of *algD* and *algR1* in *P. syringae* is under investigation (27).

The percent nucleotide identity in the *algD* coding region of *P. syringae* pv. syringae and *P. aeruginosa* ranged from 80 to 90% (Fig. 3 and data not shown); however, upstream of the translational start site, the relatedness between the two species diverged and nucleotide identity decreased to approximately 20% (Fig. 3). This divergence is consistent with the absence of specific sequences in *PsalgD* which are known to be involved in transcriptional activation of *algD* in *P. aeruginosa*. These include the consensus sequences for binding AlgR1 (24), integration host factor (38), and cyclic AMP receptor protein (13). Although some signals for activation of the *algD* promoter are conserved in *P. aeruginosa* and *P. syringae* (5, 31, 44), the *algD* promoter in *P. syringae* is stimulated by exposure to copper

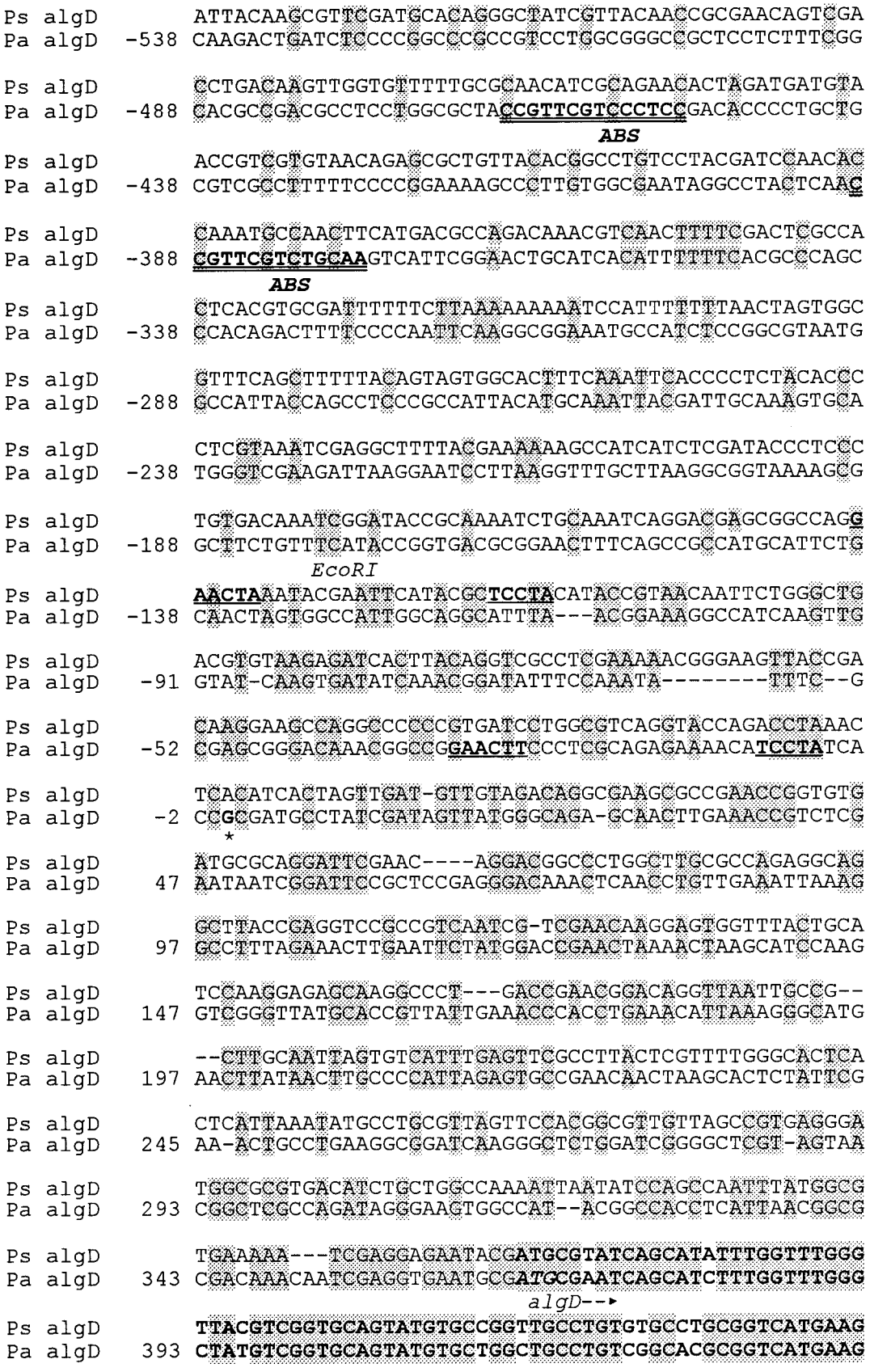

FIG. 3. Alignment of the *algD* promoter sequences from *P. syringae* pv. syringae FF5 (Ps algD) and *P. aeruginosa* (Pa algD). The *P. aeruginosa algD* promoter was previously reported (24, 39); the nucleotides for this sequence are shown on the left, with +1 (asterisk) corresponding to the transcriptional start site. Nucleotides for the *P. syringae* pv. syringae *algD* promoter are shown on the right. The *Eco*RI site in the *P. syringae* sequence corresponds to the left border of *Eco*RI fragment 5 in Fig. 1A. Gaps (−−) were used to maximize the alignment, and identical bases are shaded. The AlgR1 binding sites (ABS) in the *P. aeruginosa algD* promoter are shown in bold and double-underlined. The σ^{22} recognition sequence in both species is indicated in bold and single-underlined. The *algD* translational start site and coding region are shown in bold (*algD*−→).

ions (44) and does not require a functional copy of AlgR1 for transcriptional activation. Recently, Yu et al. (64) provided the first genetic evidence for the role of alginate in the virulence and epiphytic fitness of *P. syringae*. Consequently, the differential regulation of *algD* expression in *P. syringae* and CF isolates of *P. aeruginosa* and the marked divergence in their *algD* promoter regions probably reflect their adaptation to plant and human hosts, respectively.

It remains possible that some unknown regulatory protein binds to *PsalgD* and that this regulator recognizes different signals (such as copper) and activates the *algD* promoter in *P. syringae*. Perhaps this putative DNA binding protein was recruited during the evolutionary divergence of *P. aeruginosa* and *P. syringae* to accommodate a different signal and perhaps another activator. The *algD*::*uidA* transcriptional fusion described in the present investigation could be used to screen for mutants lacking the unknown activator. Such experiments are under way and will probably reveal additional differences in the regulation of alginate biosynthesis in human and phytopathogenic bacteria.

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REFERENCES

1. **Aarons, S. J., I. W. Sutherland, A. M. Chakrabarty, and M. P. Gallagher.** 1997. A novel gene, *algK*, from the alginate biosynthetic cluster of *Pseudomonas aeruginosa*. Microbiology **143:**641–652.
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. **Baynham, P. J., and D. J. Wozniak.** 1996. Identification and characterization of AlgZ, an AlgT-dependent DNA-binding protein required for *Pseudomonas aeruginosa algD* transcription. Mol. Microbiol. **22:**97–108.
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. **Berry, A., J. D. DeVault, and A. M. Chakrabarty.** 1989. High osmolarity is a signal for enhanced *algD* transcription in mucoid and nonmucoid *Pseudomonas aeruginosa* strains. J. Bacteriol. **171:**2312–2317.
6. **Boyd, A., M. Ghosh, T. B. May, D. Shinabarger, R. Keogh, and A. M. Chakrabarty.** 1993. Sequence of the *algL* gene of *Pseudomonas aeruginosa* and purification of its alginate lyase product. Gene **131:**1–8.
7. **Chitnis, C. E., and D. E. Ohman.** 1990. Cloning of *Pseudomonas aeruginosa algG*, which controls alginate structure. J. Bacteriol. **172:**2894–2900.
8. **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.
9. **Chu, L., T. B. May, A. M. Chakrabarty, and T. K. Misra.** 1991. Nucleotide sequence and expression of the *algE* gene involved in alginate biosynthesis by *Pseudomonas aeruginosa*. Gene **107:**1–10.
10. **Darzins, A., and A. M. Chakrabarty.** 1984. Cloning of genes controlling alginate biosynthesis from a mucoid cystic fibrosis isolate of *Pseudomonas aeruginosa*. J. Bacteriol. **159:**9–18.
11. **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.
12. **Deretic, V., R. Kishit, W. M. Konyecsni, A. M. Chakrabarty, and T. K. Misra.** 1989. The *algR* gene, which regulates mucoidy in *Pseudomonas aeruginosa*, belongs to a class of environmentally responsive genes. J. Bacteriol. **171:**1278–1283.
13. **DeVault, J. D., W. Hendrickson, J. Kato, and A. M. Chakrabarty.** 1991. Environmentally regulated *algD* promoter is responsive to the cAMP receptor protein in *Escherichia coli*. Mol. Microbiol. **5:**2503–2509.
14. **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 for autoregulation. J. Bacteriol. **176:**6677–6687.
15. **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.
16. **Franklin, M. J., and D. E. Ohman.** 1993. Identification of *algF* in the alginate biosynthetic gene cluster of *Pseudomonas aeruginosa* which is required for alginate acetylation. J. Bacteriol. **175:**5057–5065.
17. **Franklin, M. J., and D. E. Ohman.** 1996. Identification of *algI* and *algJ* in the *Pseudomonas aeruginosa* alginate biosynthetic gene cluster which are required for alginate O acetylation. J. Bacteriol. **178:**2186–2195.
18. **Gacesa, P.** 1998. Bacterial alginate biosynthesis—recent progress and future prospects. Microbiology **144:**1133–1143.
19. **Goldberg, J. B., W. B. Gorham, 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 sigma factor (σ^E). Proc. Natl. Acad. Sci. USA **92:**7941–7945.
22. **Jain, S., and D. E. Ohman.** 1998. Deletion of *algK* in mucoid *Pseudomonas aeruginosa* blocks alginate polymer formation and results in uronic acid secretion. J. Bacteriol. **180:**634–641.
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. **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.
25. **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.
26. **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.
27. **Keith, L. M. W., and C. L. Bender.** Unpublished results.
28. **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.
29. **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:**301–307.
30. **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.
31. **Leitáo, J. H., A. M. Fialho, and I. Sá-Correia.** 1992. Effects of growth temperature on alginate synthesis and enzymes in *Pseudomonas aeruginosa* variants. J. Gen. Microbiol. **138:**605–610.
32. **Ma, S., U. Selvaraj, D. E. Ohman, R. Quarless, D. J. Hassett, and D. J. Wozniak.** 1998. Phosphorylation-independent activity of the response regulators AlgB and AlgR in promoting alginate biosynthesis in mucoid *Pseudomonas aeruginosa*. J. Bacteriol. **180:**956–968.
33. **Maharaj, R., T. B. May, S.-K. Wang, and A. M. Chakrabarty.** 1993. Sequence of the *alg8* and *alg44* genes involved in the synthesis of alginate by *Pseudomonas aeruginosa*. Gene **136:**267–269.
34. **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.
35. **May, T. B., and A. M. Chakrabarty.** 1994. Isolation and assay of *Pseudomonas aeruginosa* alginate. Methods Enzymol. **235:**295–304.
36. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Mohr, C. D., and V. Deretic.** 1990. Gene-scrambling mutagenesis: generation and analysis of insertional mutations in the alginate regulatory region of *Pseudomonas aeruginosa*. J. Bacteriol. **172:**6252–6260.
38. **Mohr, C. D., and V. Deretic.** 1992. In vitro interactions of the histone-like protein IHF with the *algD* promoter, a critical site for control of mucoidy in *Pseudomonas aeruginosa*. Biochem. Biophys. Res. Commun. **189:**837–844.
39. **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.
40. **Mohr, C. D., S. K. Sonsteby, and V. Deretic.** 1994. The *Pseudomonas aeruginosa* homologs of *hemC* and *hemD* are linked to the gene encoding the regulator of mucoidy AlgR. Mol. Gen. Genet. **242:**177–184.
41. **Monday, S. R., and N. L. Schiller.** 1996. Alginate synthesis in *Pseudomonas aeruginosa*: the role of AlgL (alginate lyase) and AlgX. J. Bacteriol. **178:** 625–632.

42. **Nunez, C. E., S. Moreno, G. Soberon-Chavez, and E. G. Espin.** 1998. GenBank accession no. AF077237.
43. **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.
44. **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.
45. **Pier, G. B.** 1998. *Pseudomonas aeruginosa*: a key problem in cystic fibrosis. ASM News **64:**339–347.
46. **Rehm, B. H. A., and S. Valla.** 1997. Bacterial alginates: biosynthesis and applications. Appl. Microbiol. Biotechnol. **48:**281–288.
47. **Roine, E., D. M. Raineri, M. Romantschuk, M. Wilson, and D. N. Nunn.** 1998. Characterization of type IV pilus genes in *Pseudomonas syringae* pv. *tomato* DC3000. Mol. Plant-Microbe Interact. **11:**1048–1056.
48. **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.
49. **Schiller, N. L., S. R. Monday, C. M. Boyd, N. T. Keen, and D. E. Ohman.** 1993. Characterization of the *Pseudomonas aeruginosa* alginate lyase gene (*algL*): cloning, sequencing, and expression in *Escherichia coli*. J. Bacteriol. **175:**4780–4789.
50. **Schurr, M. J., D. W. Martin, M. H. Mudd, and V. Deretic.** 1994. Gene cluster controlling conversion of alginate-overproducing phenotypes in *Pseudomonas aeruginosa*: functional analysis in a heterologous host and role in the instability of mucoidy. J. Bacteriol. **176:**3375–3382.
51. **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.
52. **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.
53. **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.
54. **Shinabarger, D., A. Berry, T. B. May, R. Rothmel, A. Fialho, and A. M. Chakrabarty.** 1991. Purification and characterization of phosphomannose isomerase-guanosine diphospho-D-mannose pyrophosphorylase. J. Biol. Chem. **266:**2080–2088.
55. **Shinabarger, D., T. B. May, A. Boyd, M. Ghosh, and A. M. Chakrabarty.** 1993. Nucleotide sequence and expression of the *Pseudomonas aeruginosa algF* gene controlling acetylation of alginate. Mol. Microbiol. **9:**1027–1035.
56. **Staskawicz, B. J., D. Dahlbeck, and N. T. Keen.** 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility 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. **Van den Eede, G., R. Deblaere, K. Goethals, M. V. Montagu, and M. Holsters.** 1992. Broad host range and promoter selection vectors for bacteria that interact with plants. Mol. Plant-Microbe Interact. **5:**228–234.
59. **Venturi, V., M. Otten, V. Korse, B. Brouwer, J. Leong, and P. Weisbeek.** 1995. Alginate regulatory and biosynthetic gene homologs in *Pseudomonas putida* WCS358: correlation with the siderophore regulatory gene *pfrA*. Gene **155:**83–88.
60. **Wang, S.-K., I. Sà Correia, A. Darzins, and A. M. Chakrabarty.** 1987. Characterization of the *Pseudomonas aeruginosa* alginate (*alg*) gene region II. J. Gen. Microbiol. **133:**2303–2314.
61. **Whitchurch, C. B., R. A. Alm, and J. S. Mattick.** 1996. The alginate regulator AlgR and an associated sensor FimS are required for twitching motility in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **93:**9839–9843.
62. **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.
63. **Yu, H., M. Mudd, J. C. Boucher, M. J. Schurr, and V. Deretic.** 1997. Identification of the *algZ* gene upstream of the response regulator *algR* and its participation in control of alginate production in *Pseudomonas aeruginosa*. J. Bacteriol. **179:**187–193.
64. **Yu, J., A. Peñaloza-Vázquez, and C. L. Bender.** 1998. Role of the exopolysaccharide alginate in the pathogenicity of *Pseudomonas syringae* pv. *syringae*. Phytopathology **88:**S102.
65. **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.