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A Modified Two-Component Regulatory System Is Involved in Temperature-Dependent Biosynthesis of the *Pseudomonas syringae* Phytotoxin Coronatine

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Biosynthesis of the phytotoxin coronatine (COR) in *Pseudomonas syringae* pv. glycinea PG4180 is regulated by temperature at the transcriptional level. A 3.4-kb DNA fragment from the COR biosynthetic gene cluster restored temperature-regulated phytotoxin production to Tn*5* mutants defective in COR production. Nucleotide sequence analysis of this fragment revealed three genes, *corS*, *corP*, and *corR*, which encode a modified two-component regulatory system consisting of one sensor protein, CorS, and two response regulator proteins, CorP and CorR. Although only one response regulator, CorR, had a DNA-binding domain, the phosphate-receiving domains of both response regulator proteins were highly conserved. Transcriptional fusions of the *corP* and *corR* promoters to a promoterless glucuronidase gene (*uidA*) indicated that these two genes are expressed constitutively at 18 and 28°C. In contrast, a *corS*::*uidA* fusion exhibited the temperature dependence previously observed for COR biosynthetic promoters and exhibited maximal transcriptional activity at 18°C and low activity at 28°C. Furthermore, glucuronidase activity for *corS*::*uidA* was decreased in *corP*, *corR*, and *corS* mutants relative to the levels observed for PG4180(*corS*::*uidA*). This difference was not observed for *corP*::*uidA* and *corR*::*uidA* transcriptional fusions since expression of these fusions remained low and constitutive regardless of the genetic background. The three regulatory genes functioned in a *P. syringae* strain lacking the COR gene cluster to achieve temperature-dependent activation of an introduced COR biosynthetic promoter, indicating that this triad of genes is the primary control for COR biosynthesis and responsible for thermoregulation. Our data suggest that the modified two-component regulatory system described in this study might transduce and amplify a temperature signal which results in transcriptional activation of COR biosynthetic genes.

Coronatine (COR) (Fig. 1) is a non-host-specific, chlorosis-inducing phytotoxin produced by several *Pseudomonas syringae* pathovars and functions as an important virulence factor in diseases incited by these bacteria (37, 38, 64). COR induces alterations of the host plant metabolism in a manner analogous to plant growth hormones (13, 27, 50). Striking structural and functional homologies between COR, methyl jasmonate, and 12-oxo-phytodienoic acid suggest that COR mimics the octadecanoid signalling molecules of higher plants (14, 62). Recently, light microscopy and ultrastructural studies showed that COR induces additional unique changes in host cells not obtained with methyl jasmonate, suggesting that the two molecules are not identical in mode of action (43).

The biosynthesis of COR involves one pathway leading to the polyketide component, coronafacic acid (CFA), and a second pathway which results in a cyclized derivative of isoleucine, coronamic acid (CMA; 2-ethyl-1-aminocyclopropane 1-carboxylic acid). Both CFA and CMA function as intermediates in COR biosynthesis and are coupled (CPL) via amide bond formation presumably as the last step in COR biosynthesis (46).

The genes required for COR biosynthesis in *P. syringae* pv. glycinea PG4180 are encoded within a 32-kb region of a 90-kb plasmid designated p4180A (4, 69). Extensive mutational analysis (4), as well as the cloning, expression, nucleotide sequencing, and transcriptional mapping of several genes required for COR production, has resulted in the development of a functional map of the COR biosynthetic gene cluster (32, 58, 59) (Fig. 2A). These studies have indicated a clustering of the genes for CMA and CFA biosynthesis at opposing ends of the COR biosynthetic gene cluster (Fig. 2A). Transposon insertions in the CMA or CFA biosynthetic regions resulted in mutants with CMA$^-$ CFA$^+$ CPL$^+$ or CMA$^+$ CFA$^-$ CPL$^+$ phenotypes; these could be complemented when exogenous CMA or CFA, respectively, was supplied to the bacterial cultures (4). The two biosynthetic regions are joined by a 4.3-kb region in which mutations result in a CMA$^-$ CFA$^-$ CPL$^+$ phenotype (4), suggesting a possible role for this region in the regulation of COR biosynthesis (Fig. 2A).

COR production in *P. syringae* pv. glycinea PG4180 is temperature dependent, with maximal amounts of COR and CFA produced at 18°C and negligible amounts produced at 30°C (41, 42). Similarly, production of CMA was maximal at 18°C and undetectable at 28°C (58). Transcriptional fusions of a promoterless glucuronidase (GUS) gene (*uidA*) to operons containing structural genes for COR biosynthesis revealed that 18°C was an optimal temperature for transcription of the CFA and CMA biosynthetic clusters (31, 41, 58).

In the present study, we focused on the 4.3-kb DNA region of the COR biosynthetic gene cluster which is required for

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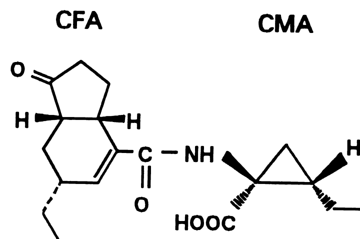

FIG. 1. Structure of the phytotoxin COR showing the polyketide component, CFA, coupled to a cyclized amino acid, CMA, via amide bond formation.

both CFA and CMA biosynthesis. Our aim was to characterize this region and determine whether it performs a regulatory role in COR production. To achieve this, portions of this DNA region were used to complement mutants defective in CFA and CMA biosynthesis. Nucleotide sequence analysis of the region and transcriptional fusions suggest that the region encodes a modified two-component regulatory system which is responsive to temperature.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. *Pseudomonas* strains were routinely cultured on King's medium B (28) or mannitol-glutamate (MG) medium (26) at 24 to 26°C. *Escherichia coli* HB101 and DH5α (51) were used as hosts in cloning experiments and were cultured in Luria-Bertani medium at 37°C. Protein contents of cell lysates were determined with the Bio-Rad (Richmond, Calif.) protein assay kit as recommended by the manufacturer. The following antibiotics were added to media in the indicated concentrations (micrograms per milliliter): tetracycline, 12.5; kanamycin, 12.5; ampicillin, 40; spectinomycin, 25; streptomycin, 25; chloramphenicol, 12.5; and gentamicin, 2.

DNA procedures. Agarose gel electrophoresis, restriction digests, purification of DNA fragments from agarose gels, electroporations, and small-scale plasmid DNA preparations were performed by standard procedures (51). Southern hybridizations were done as described earlier (54). Cloning of *corP* and its upstream promoter region was carried out by PCR, using standard techniques (51) and the following two oligonucleotides: forward primer 5′-GACGGATCCGGG CGTCAAGAAGAACTG-3′ (see reference 58; complement of nucleotides 153 to 170) and reverse primer 5′-ATTGGATCCGCTACCTCCAGATCGAGG-3′ (nucleotides 2495 to 2513 in Fig. 5). Large-scale preparations of plasmid DNA from *E. coli* were isolated by alkaline lysis and purified with Qiagen columns (Qiagen, Chatsworth, Calif.). Plasmid DNA was isolated from *Pseudomonas* strains as described previously (3, 11). Triparental matings using pRK2013 as the mobilizing plasmid were done as described earlier (6), and transconjugants were verified by agarose gel electrophoresis.

DNA sequencing and analysis. Nucleotide sequencing reactions were performed by the dideoxynucleotide method (51) with Sequenase 2.0 (U.S. Biochemical, Cleveland, Ohio) and [α-^{35}S]dATP (DuPont-NEN, Boston, Mass.). Automated DNA sequencing was accomplished by using an ABI 373A apparatus and the Prism Ready Reaction Dyedeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, Calif.). Automated sequencing was provided by the Oklahoma State University Recombinant DNA/Protein Resource Facility. A series of subclones (0.5 to 1.0 kb) was generated in pBluescript SK+ (Stratagene, La Jolla, Calif.) and sequenced by using T3 and T7 primers. Sequencing gaps were filled by synthesizing internal primers (approximately 18 bp in length). The precise locations of Tn*5* insertions in CMA$^-$ CFA$^-$ mutants of PG4180 were determined as described previously (58). All oligonucleotide primers used in this study were synthesized by the Oklahoma State University Recombinant DNA/Protein Resource Facility. Sequence data were aligned and processed with MacVector 4.1 (International Biotechnologies Inc., New Haven, Conn.). DNA and protein sequence homology searches of the GenBank, EMBL, PIR, and SWISSPROT databases were performed by using the University of Wisconsin Genetics Computer Group (UWGCG) programs BLASTX, FASTEMBL, and BESTFIT.

Construction of promoter probes and GUS assay. Plasmid pRG960sd, which contains a promoterless GUS gene (*uidA*) downstream of a multiple cloning site (60), was used to identify and characterize promoter sequences upstream of *corR*, *corS*, and *corP*. Subcloned DNA fragments were ligated into pRG960sd, orientations were determined by restriction digests, and constructs were mobilized into *Pseudomonas* strains via triparental matings. Transcriptional activities were initially monitored by spotting bacterial suspensions (A_{590} of 0.05) on MG agar plates containing 20 μg of X-Gluc (5-bromo-4-chloro-3-indolyl glucuronide) per ml followed by incubation at 18 or 28°C for 5 to 7 days. Transcriptional activity was quantified by fluorometric analysis of GUS activity (67) of cells grown for 7 days in 10 ml of HSC broth (described below) at 18 or 28°C. Fluorescence was monitored with a Fluoroskan II version 4.0 microplate reader (ICN Biomedicals, Inc., Costa Mesa, Calif.) in 96-well microtiter plates.

Detection and quantitation of COR biosynthesis. *Pseudomonas* strains were incubated in 10-ml aliquots of Hoitink-Sinden medium optimized for COR production (HSC) (42) at 18 or 28°C as described previously (59). Organic acids were extracted from bacterial supernatants and extracts were analyzed by methods described elsewhere (42).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this study were deposited with GenBank/EMBL under accession numbers U33326 (*corR* and *corS*) and U33327 (*corP*).

RESULTS

Phenotypic characterization of mutants defective in the COR regulatory region. Two Tn*5* mutants with the CMA$^-$ CFA$^-$ CPL$^+$ phenotype, PG4180.F7 (F7) and PG4180.D4 (D4), were used as host strains for pRGMU7, a construct containing a temperature-sensitive CMA biosynthetic promoter fused to a GUS gene (*cmaA*::*uidA* promoter [58]). GUS activities for F7, D4, and PG4180 containing pRGMU7 were compared at 18 and 28°C (Fig. 3A). GUS activity was quite high in PG4180 (pRGMU7) incubated at 18°C and approximately 4.5-fold lower when this construct was incubated at 28°C (Fig. 3A). Interestingly, GUS activity was negligible in F7(pRGMU7) and D4(pRGMU7) at both temperatures (Fig. 3A), suggesting that these mutants are defective in transcriptional activation of the *cmaA*::*uidA* promoter. As controls, we investigated the GUS activity of pRGMU7 in PG4180.C9 (C9; CMA$^-$ CFA$^+$ CPL$^+$) and PG4180.D5 (D5; CMA$^+$ CFA$^-$ CPL$^+$), mutants which are defective in CMA and CFA biosynthesis, respectively (59). Unlike the results obtained for D4 and F7, GUS activities in both C9(pRGMU7) and D5(pRGMU7) were similar to that observed for PG4180(pRGMU7) (data not shown).

Complementation analysis of PG4180 regulatory mutants. Since the Tn*5* insertions in F7 and D4 map within *Sst*I fragment 3 (Fig. 2A), attempts were made to restore COR production to F7 and D4 with pMUS25 and pMUH34, subclones containing *Sst*I fragment 3 and a 3.4-kb *Hin*dIII-*Eco*RI fragment including *Sst*I fragment 3 and some flanking DNA, respectively (Fig. 2B). Although pMUS25 did not restore COR biosynthesis to F7 or D4, both F7(pMUH34) and D4(pMUH34) produced COR at levels approaching that observed for PG4180 at 18°C (Fig. 4B). We also investigated whether pMUH34 was involved in transcriptional activation of the *cmaA*::*uidA* promoter by assaying F7 and D4 transconjugants containing pRGMU7 and pMUH34 for GUS activity at 18 and 28°C (Fig. 3B). The introduction of pMUH34 into F7(pRGMU7) and D4(pRGMU7) resulted in GUS activity comparable to that of PG4180(pRGMU7) at both temperatures (Fig. 3B), indicating that genes encoded by pMUH34 are involved in the transcriptional activation of the *cmaA* promoter.

Nucleotide sequence analysis of the COR regulatory region. The nucleotide sequence of the 3.4-kb *Hin*dIII-*Eco*RI fragment in plasmid pMUH34 and the exact locations of the Tn*5* insertions in F7 and D4 were determined. Figure 5 shows the sequence of the 3.4-kb *Hin*dIII-*Eco*RI fragment beginning from the *Eco*RI site located in *Sst*I fragment 4 (Fig. 2B). Use of an oligonucleotide primer complementary to the IS*50* ends of Tn*5* (58) indicated that the mutations in F7 and D4 were located within *Sst*I fragment 3 at nucleotides 3211 and 1366, respectively (Fig. 2B and 5). Three complete open reading frames (ORFs) were detected in the sequenced region and designated *corR* (582 bp), *corS* (1,305 bp), and *corP* (453 bp). Their authenticity was further indicated by codon preference analysis using the UWGCG program CODONPREFERENCE in its default mode and a *Pseudomonas* codon usage table provided

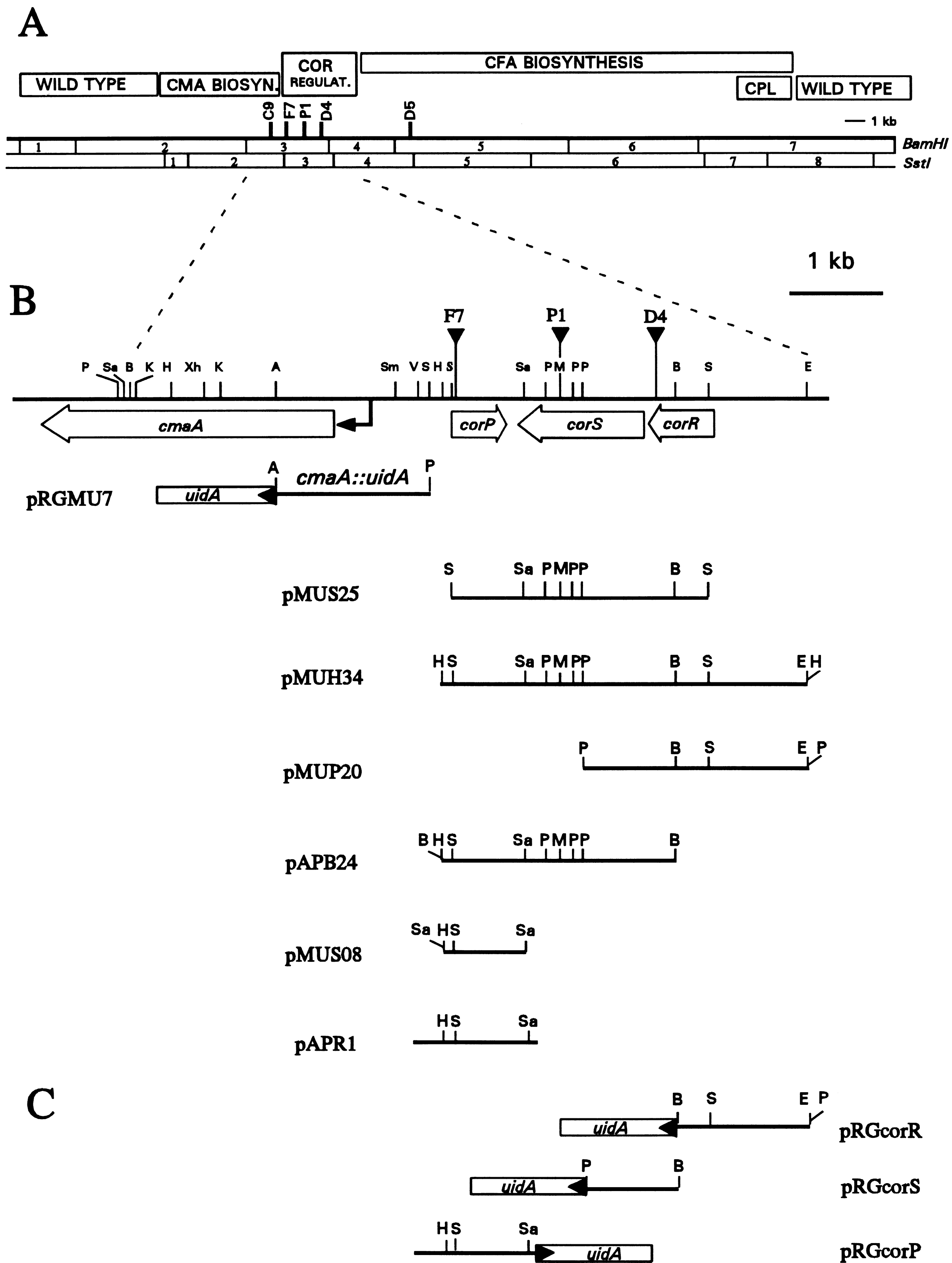

FIG. 2. (A) Partial restriction map of plasmid p4180A from *P. syringae* pv. glycinea PG4180 showing the COR biosynthetic cluster and functional regions. Vertical bars labeled by letter-number combinations indicate the location of Tn*5* and antibiotic cassette insertions. Rectangles above the physical map represent the functional regions of the COR biosynthetic gene cluster. Insertions in the region marked "WILD TYPE" had no effect on COR biosynthesis. (B) Physical map showing a portion of the COR gene cluster (enlargement of area indicated by dashed lines between panels A and B). The locations and orientations of *corP*, *corS*, *corR*, and *cmaA* are indicated. The location of pRGMU7, which contains the *cmaA* promoter fused to the GUS gene (*cmaA*::*uidA*) is shown (open rectangle and adjacent arrow). Horizontal bars below the map show the subclones used for complementation analysis. (C) Locations and orientations of promoter probe constructs used in this study. Restriction enzymes used: A, *Aat*I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; M, *Mcs*I; P, *Pst*I; S, *Sst*I; Sa, *Sal*I; Sm, *Sma*I; V, *Eco*RV; Xh, *Xho*I.

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant characteristics	Reference or source
E. coli		
HB101		51
DH5α		51
P. syringae pv. glycinea		
PG4180	COR$^+$ CMA$^+$ CFA$^+$ CPL$^+$	4, 69
PG4180.D4	COR$^-$ CMA$^-$ CFA$^-$ CPL$^+$ Km^r	4
PG4180.F7	COR$^-$ CMA$^-$ CFA$^-$ CPL$^+$ Km^r	4
PG4180.C9	COR$^-$ CMA$^-$ CFA$^+$ CPL$^+$ Km^r	4
PG4180.D5	COR$^-$ CMA$^+$ CFA$^-$ CPL$^+$ Km^r	59
PG4180.P1	COR$^-$ CMA$^-$ CFA$^-$ CPL$^+$ Gm^r	This study
P. syringae pv. syringae FF5	COR$^-$ CMA$^-$ CFA$^-$ CPL$^-$	54
Plasmids		
p4180A	Contains COR genes	4
pBluescript SK+	Ap^r; ColE1 origin, cloning vehicle	Stratagene
pRG960sd	Sm^r Sp^r; contains promoterless *uidA* with start codon and Shine-Dalgarno sequence	60
pRK2013	Km^r Mob$^+$ Tra$^+$	15
pMUH34	Tc^r; contains a 3.4-kb *Hin*dIII-*Eco*RI insert derived from p4180A in pRK415; required for complementation of COR$^-$ CMA$^-$ CFA$^-$ CPL$^+$ PG4180 mutants	This study
pMUH34R	Tc^r; contains a 3.4-kb blunt-ended *Hin*dIII-*Eco*RI insert derived from p4180A in pRK415; insert has orientation opposite that in pMUH34	This study
pMUS25	Tc^r; contains a 2.5-kb *Sst*I insert derived from p4180A in pRK415	This study
pMUS40	Tc^r; contains a 4.0-kb *Sst*I insert derived from p4180A in pRK415	This study
pMUP20	Tc^r; contains a 2.0-kb *Pst*I insert derived from p4180A in pRK415; contains *corR*	This study
pMUS08	Tc^r; contains a 0.8-kb *Sal*I insert derived from pMUH34 in pRK415	This study
pAPB24	Tc^r; contains a 2.4-kb *Bam*HI insert derived from pMUH34 in pRK415; contains *corS*	This study
pAPR1	Tc^r; contains a 1.1-kb *Bam*HI insert derived from p4180A by PCR cloning in pRK415; contains *corP*	This study
pBSB24	Ap^r; contains a 2.4-kb *Bam*HI insert derived from pAPB24 in pBluescript SK+; contains *corS*	This study
pRGcorS	Sm^r Sp^r; contains a 0.85-kb *Pst*I insert derived from pAPB24 in pRG960sd	This study
pRGcorR	Sm^r Sp^r; contains a 1.1-kb *Bam*HI insert derived from pMUP20 in pRG960sd	This study
pRGcorP	Sm^r Sp^r; contains a 1.1-kb *Bam*HI insert derived from pAPR1 in pRG960sd	This study
pRGMU7	Sm^r Sp^r; contains a blunt-ended 1.5-kb *Pst*I-*Aat*I fragment derived from p4180A and cloned into the *Sma*I site of pRG960sd (*Pst*I-*Aat*I-*uidA*)	58

by Cherry (9). Although *corR* and *corS* shared a common orientation for translation, our analyses indicated that translation of *corP* proceeded in the opposite orientation (Fig. 2B and 5).

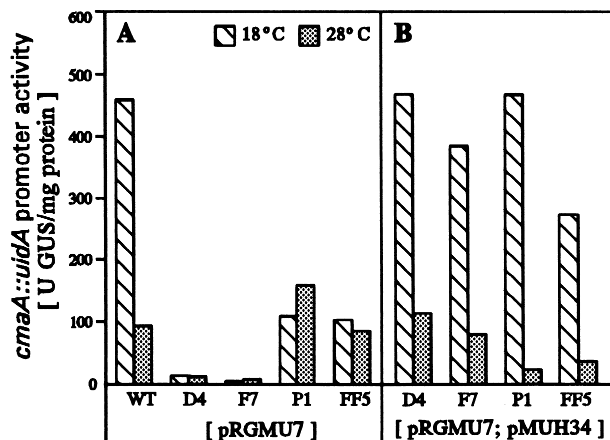

FIG. 3. Effects of temperature and genetic background on *cmaA*::*uidA* promoter activity in *P. syringae*. GUS activities for transconjugants harboring pRGMU7 (A) and pRGMU7 and pMUH34 (B) were determined. WT, wild-type PG4180; D4, F7, and P1, mutants defective in thermoregulation of COR biosynthesis; FF5, *P. syringae* pv. syringae FF5, a strain lacking the COR biosynthetic cluster. Quantities represent the averages of four experiments with two replicates.

Although *corP* and *corR* differed in length, they showed a high degree of nucleotide sequence relatedness (92% identity) in their 5′ regions; upon translation, the relatedness of these two ORFs was striking (Fig. 6). To eliminate the possibility of cloning or sequencing artifacts as a reason for two very similar ORFs, DNA adjacent to pMUH34 was sequenced. The nucleotide sequence of *Sst*I fragment 2 (Fig. 2A) was previously

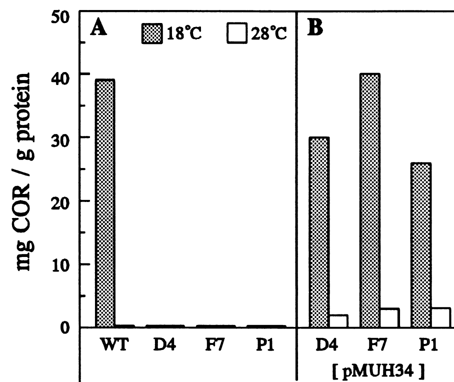

FIG. 4. Temperature effects on COR production by derivatives of *P. syringae* pv. glycinea PG4180. (A) Amount of COR produced by the wild-type PG4180 (WT) and mutants D4, F7, and P1. (B) COR production by D4, F7, and P1 transconjugants containing pMUH34. Quantities represent the averages of four experiments with two replicates.

published (58) and used to derive an oligonucleotide situated within *Sst*I fragment 2 near the right border of this fragment. This oligonucleotide was used to prime a sequencing reaction which extended into *corP*, thus verifying the location of this ORF. Additional oligonucleotide primers were synthesized to confirm the presence of the unique *Bam*HI site in *corR* (Fig. 5, nucleotides 1153 to 1158) and to verify the positions of the *Sst*I sites in *corP* and *corR* (Fig. 5, nucleotides 3253 to 3258 and 818 to 823, respectively).

Sequence homology analyses. Nucleotide sequencing data were compared with GenBank, EMBL, SWISSPROT, and PIR database entries by using the FASTEMBL, BLASTX, and BESTFIT programs. The deduced protein products, CorR (21.5 kDa) and CorP (16.5 kDa), showed significant sequence homologies (25 to 39% identity) to response regulators of two-component regulatory systems, especially members of the RO_{III} group (45), which includes NarL, AlgR1 (AlgR), BvgA, UhpA, DegU, and VsrC (1, 12, 17, 20, 23, 39). Response regulator proteins control the adaptive response in two-component regulatory systems and are characterized by an N-terminal receiver domain which functions as the phosphorylation site and a C-terminal effector domain with a DNA-binding, helix-turn-helix motif (44, 45). Both domains are strongly conserved in CorR; CorP, however, contains the highly conserved receiver domain but lacks a DNA-binding, helix-turn-helix motif. The absence of a DNA-binding motif has been noted for other response regulators such as CheY and Spo0F (33, 68), which were previously assigned to group R (45). The N-terminal receiver domains of CorR and CorP are almost identical when aligned (Fig. 6), suggesting a shared specificity for the same phospho-donor sensor protein(s). Furthermore, the Tn*5* insertions in the COR^- mutants D4 and F7 mapped to *corR* and *corP*, respectively (Fig. 2B), demonstrating the importance of both genes in the regulation of COR production.

The predicted 47.5-kDa protein product of *corS* showed sequence relatedness (24 to 29% identity over the entire sequence) to histidine protein kinases (HPKs) of two-component regulatory systems (45), including VsrB, BvgC, DegS, RcsB, and LemA (1, 19, 21, 39, 56). Because of the strong sequence conservation in the C terminus of HPK sensor proteins, there was an even higher percentage of identity (28 to 33% identity over 285 amino acids) between CorS and other HPKs when the C-terminal regions were aligned. HPKs are histidyl autokinases; they are phosphorylated at the expense of ATP and can transfer the phosphoryl group to an aspartyl residue present in the cognate response regulator (45). Blocks H, G1, F, and G2, which are four of the five conserved motifs present in sensor kinases (45, 55), were detected in CorS (Fig. 5) in the spatial arrangement previously reported for these motifs (45). The sequence of CorS was further subjected to hydropathy index calculations by using PEPPLOT (UWGCG software) and the method of Kyte and Doolittle (29). The N-terminal region of CorS (approximately 160 amino acid residues) contained more hydrophobic zones (data not shown) than the C terminus, indicating a potential membrane location for this portion of the protein.

Construction and characterization of the *corS* insertional mutant, PG4180.P1. To specifically address the potential role of *corS* in regulation, we inserted the gentamicin resistance (Gm^r) gene from pMGm (40) into the *Mcs*I site of *corS* (Fig. 5, nucleotides 2174 to 2179). pBSB24, a clone containing this mutant allele (*corS*::Gm^r), was electroporated into the PG4180 genome; this was followed by screening for Ap^s Gm^r *Pseudomonas* derivatives. Recombination of the Gm^r cassette into *corS* was verified by plasmid isolation and Southern analysis (data not shown). The new mutant, designated PG4180.P1 (P1), did not produce any COR when examined for phytotoxin synthesis at 18 and 28°C (Fig. 4A). However, like F7 and D4, COR production was restored to P1 at 18°C when it contained pMUH34 (Fig. 4B).

To examine whether *corS* was involved in transcriptional activation of the *cmaA*::*uidA* promoter, pRGMU7 was introduced into P1 and P1(pMUH34). GUS activity in P1(pRGMU7) was significantly lower than in the wild-type PG4180(pRGMU7) at 18°C, whereas GUS activities were similar for both transconjugants at 28°C (Fig. 3A). The introduction of pMUH34 into P1(pRGMU7) (Fig. 3B) restored GUS activity to the levels observed for PG4180(pRGMU7) (Fig. 3A), demonstrating that *corS* has a role in activation of the *cmaA* promoter.

Transcriptional organization of the COR regulatory region. To identify the number of transcripts present on pMUH34, three clones containing portions of pMUH34 (pMUP20, pAPB24, and pMUS08; Fig. 2B) were conjugated into D4, F7, and P1, which contain mutations in *corR*, *corP*, and *corS*, respectively. COR production was partially restored to D4 by pMUP20, a construct containing an intact copy of *corR*, upstream DNA, and the N-terminal portion of *corS* (Table 2). P1 was complemented by pAPB24, a clone lacking the N-terminal portion of *corR* but containing an intact copy of *corS* (Table 2). Therefore, although *corR* and *corS* share a common orientation for transcription, these data indicate the possible location of these two genes on separate transcripts.

Although pMUH34 restored COR production to mutant F7, pMUS08, a clone containing a 0.8-kb *Sal*I fragment from pMUH34 (Fig. 2B), did not complement this mutant (Table 2). When the orientation of *corP* was investigated in these two constructs with respect to the vector promoter, the orientation was *lac* promoter (P_{lac})::*corP* in pMUH34 and *corP*::P_{lac} in pMUS08, indicating that *corP* was probably transcribed from P_{lac} in pMUH34, thus explaining COR production by F7(pMUH34). To further examine this possibility, we constructed pMUH34R, which contains the pMUH34 insert in the opposite orientation relative to P_{lac}. pMUH34R did not complement F7, further verifying that a transcriptional fusion between P_{lac} and *corP* was responsible for COR production in F7(pMUH34). To achieve allelic complementation of F7, a second subclone, designated pAPR1, was constructed by PCR amplification and cloning of a 1,120-bp region encompassing *corP* and 370 nucleotides of upstream DNA (Fig. 2B). Plasmid pAPR1 completely restored COR synthesis to F7 but not to D4 or P1 (Table 2), indicating that the promoter region for *corP* was present in pAPR1.

Temperature response of promoters in the COR regulatory region. The temperature sensitivity of the CMA and CFA biosynthetic promoters justified further studies related to the role of temperature in the transcription of the COR regulatory region. Therefore, selected DNA fragments containing upstream regions of *corR*, *corS*, and *corP* were subcloned in pRG960sd, resulting in pRGcorR, pRGcorS, and pRGcorP

FIG. 5. Nucleotide sequence of a 3.47-kb region required for thermoregulation of COR biosynthesis containing genes *corR*, *corS*, and *corP*, with deduced amino acid sequences indicated below the nucleotide sequence. Nucleotides are numbered on the left; restriction sites and putative Shine-Dalgarno sequences (underlined) are indicated. Inverted triangles above the nucleotide sequence indicate the locations of Tn*5* insertions or antibiotic cassettes. The positions of four conserved motifs (H, G1, F, and G2) common to HPKs (45) are indicated by the shaded amino acids in CorS.

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      EcoRI
   1  GAATTCTCGGCGTAATTAATGGTTATCTGGGCGACCGTGGCTTGTCGTTGCGTCAAGGCACCATCGTCGATGCCACGCTGATTCACGCGCCCAGTTCGAC

 101  CAAGAACAAAGATGGCAATCGCGACCCTGAGATGCATCAAACCAAGAAAGGTAACCAGTACTATTTCGGCGCCAAGGCTCACATCGGCGCCGACTACGAG

 201  TCAGGCCTGGTGCACAGCGTGGTAGTAACAGCGGCCAATGTGGCAGACGTTACGCAGGTTGCCAAACTGCTGCACGGCGAGGAAAACGTAGTCTTGCGCC

 301  GACGCAGGTTACACCGGGGTCGAGAAGCGCGAAGAACATGCTGGGCGCAAGGTCATTTGGCAGATTGCCGCCCGCCGCAGCACTTACAAAAAGCACGGAA

 401  AACGCAGTGTTTTGACCAAGGCGATCCCCACAATCGAGAAGACCAAGGCCCAGGTTCGCGCCAAGGTCGAGCATCCGTTTCGGGTAATCAAACGCCAGTT

 501  TGGCTATGAAAAAGTGCGCTTTCGGGGCTTGCCCAAGAACACCGCGCAGATAGTGACGTTGTTCGCCCTGTCAAACTTGTGGATGGCCCGCCGACATTTG

 601  TTGGCGAGCGCAGGAGAGGGTGCGCGTGTAATGCAGGAAATGGTTACTGCAAAGGGCTTTGCAGCGGCCGAAAGAGCCGAAAAACGAAGAGAATTGAGCA

 701  AATTCCGACATGCATCGTTTTTTGAAAAGTGCTGAGGACTCGGTAGCCGGAAAACATCCGGCTACTTCAGACCTTCCCTAGAAATACTCTTTATATAAGG

                         SstI
 801  TATACGTAGTTCATGCCGAGCTCTTCGATCTTGCTCATCGATGATCACGCCTTGTTCCGTTCCAGCGTGGCCCTGATGTTGGAAATGAGGCTGCCACGGA
      corR ⇒      M  P  S  S  S  I  L  L  I  D  D  H  A  L  F  R  S  S  V  A  L  M  L  E  M  R  L  P  R  T

 901  CGACGGTCAGCGAACGCAGCAGGATCGAGGAGGCGTCTGCCCAGGCGTGCCGGCCCCCCGATCTCATCTTGCTCGATTTGCAAATGAAAGGTACTAACGG
        T  V  S  E  R  S  R  I  E  E  A  S  A  Q  A  C  R  P  P  D  L  I  L  L  D  L  Q  M  K  G  T  N  G

1001  CCTGGAAACGATCGCCTTGCTGCAAGAGCGCTGGTCCTCGGCGAGAGTGGTGATCGTGTCCGCCTTCGACCGGGATCAGGTGTGCGAGGCGATCCAGCGC
       L  E  T  I  A  L  L  Q  E  R  W  S  S  A  R  V  V  I  V  S  A  F  D  R  D  Q  V  C  E  A  I  Q  R
                                                                     BamHI
1101  GGTGCGGTGGAGTTTCACTCTAAGGCAGAATGTCCAGAGCACCTGCTACAGCGGATCCAGGCGCTGCTCTCCGGAGAGCCTCCCGAACCGAGGACTATCG
      G  A  V  E  F  H  S  K  A  E  C  P  E  H  L  L  Q  R  I  Q  A  L  L  S  G  E  P  P  E  P  R  T  I  G

1201  GCGCCACCCCGTTGCCGGTCACTTCACGCCAGCGCCAGGTGCTCGGGTTGCTGTGCCGGGGATTGACGAACAAGGAGATCGTTTTGGAGGTCTGGCGCTC
        A  T  P  L  P  V  T  S  R  Q  R  Q  V  L  G  L  L  C  R  G  L  T  N  K  E  I  V  L  E  V  W  R  S
                                                                                   ▾ D4
1301  GGGGCACACGGTCCGCAGGCATGTCCAGGCGATACTGACTATCCTCCAGACGTCCTGCCGTACGGAGGGTGGTCGATGGCGCGCAGCCCTCTAGGAGTCG
       G  H  T  V  R  R  H  V  Q  A  I  L  T  I  L  Q  T  S  C  R  T  E  G  G  R  W  R  A  A  L  *

1401  GCCTGTGAGTACGACGCTCGCTCATACAAGGAGGTCCTGTGACTCATTCTTACGAACTCACCCAGTCGTTGGTCAGCCCTCGCGCAACTGAGACGGCGAC
                                  corS ⇒    V  T  H  S  Y  E  L  T  Q  S  L  V  S  P  R  A  T  E  T  A  T

1501  TGGGACCTTAACGCCATCCAAACGCAATTGTTGCTTTGGTATGTCGCCGCTCGTGAGTCTTAGGGTGGGCCAAGTCATCTATGCGCGCCAGGTATTGGGA
       G  T  L  T  P  S  K  R  N  C  C  F  G  M  S  P  L  V  S  L  R  V  G  Q  V  I  Y  A  R  Q  V  L  G

1601  CTCGACCTGGACGCTGGCCGAGTGCAGCCAGTGACTCGGGTCCTGATGCTGGCCGGTATCTTGGAAGCAGTCGTCTGGGTAATGCTGTGCGAAATTGCCC
      L  D  L  D  A  G  R  V  Q  P  V  T  R  V  L  M  L  A  G  I  L  E  A  V  V  W  V  M  L  C  E  I  A  L

1701  TCCAACAGGCGTTTCCTACACCCGTCATCGGCTTGCTGTTGGTGCTGTTCGCCACGCTCGGCAATGTCAAGGTGTCGCTGAATTGTGCCGTGCCCAGTCT
        Q  Q  A  F  P  T  P  V  I  G  L  L  L  V  L  F  A  T  L  G  N  V  K  V  S  L  N  C  A  V  P  S  L

1801  TTATCTGGGACGCATCCTGCTGCCGGTGGCACTGATCACTATGTGGTTTCTGCGCGTTGGTGATGACCTGCACAGAATCCTGGGGAGCGCCATGCTGCTT
       Y  L  G  R  I  L  L  P  V  A  L  I  T  M  W  F  L  R  V  G  D  D  L  H  R  I  L  G  S  A  M  L  L

1901  TACGGCTTGGGGCTCTGCCGATTGGCGCACTCGGGTCGGCAGCGCCTAGTGAAGCAATTGAAAAGGCAATTGCTGCTGGATGAGACCACCAAGCGGGCAC
      Y  G  L  G  L  C  R  L  A  H  S  G  R  Q  R  L  V  K  Q  L  K  R  Q  L  L  L  D  E  T  T  K  R  A  R
             PstI
2001  GCCGTGACCTGCAGCAGCTCCGCATCGCCTTGCAACGGGCGCGGCAGTCGAATACGGCGCGCTGTCAGTTGCTGGCTGGAGCTAGCCATGACCTGCACCA
        R  D  L  Q  Q  L  R  I  A  L  Q  R  A  R  Q  S  N  T  A  R  C  Q  L  L  A  G  A  S  H  D  L  H  Q
        PstI                                                                 MscI ▾ P1        (H)
2101  GCCCCTGCAGGCTCAGGGATTTTTTCTGGCAGCCTTGGCCGGCAGTCCGCTCGATTCCCAACAGCGGCAATTGCTGGCCAGGGCCCGAACGGCCGTCAAG
       P  L  Q  A  Q  G  F  F  L  A  A  L  A  G  S  P  L  D  S  Q  Q  R  Q  L  L  A  R  A  R  T  A  V  K
                                                                                             PstI
2201  ACTACTACCGACATGCTAAATACCTTACTGGAACTATCACGCATCGAACTGGGTGCCTTGCAGCCGACATTGCACGTTTGTGCCCTGCAGCCACTGCTGG
      T  T  T  D  M  L  N  T  L  L  E  L  S  R  I  E  L  G  A  L  Q  P  T  L  H  V  C  A  L  Q  P  L  L  D

2301  ATAAACTGGAAATGGAGCTTGCGCCCCTCGCCAACGGCAAGGGCCTCCAGCTACCCGCACGCTGGACACGGAGCTATTGTCTCTATCCGATCCCACCCTT
        K  L  E  M  E  L  A  P  L  A  N  G  K  G  L  Q  L  P  A  R  W  T  R  S  Y  C  L  Y  P  I  P  P  L

2401  GCTGGAGCTTGATACTTGCGAAATCTTTATCGGCAATGCGATCCCGCTCACCCTGGAGGGGCGGCGTCCTGAATCGCTTGCCAGCGGTGCCATGGGCTAC
       L  E  L  D  T  C  E  I  F  I  G  N  A  I  P  L  T  L  E  G  R  R  P  E  S  L  A  S  G  A  M  G  Y

                 SalI
2501  CTCCAGATCGAGGTCGTCGACACCGGCGTCGGTATCGCTCTCCAGCACCAGCAGGAAATCTTTCGCGACTTTCACCAACTCGATCATCCGACCCGGAACA
      L  Q  I  E  V  V  D  T  G  V  G  I  A  L  Q  H  Q  Q  E  I  F  R  D  F  H  Q  L  D  H  P  T  R  N  N
                              (G1)                             (F)

2601  ACCACGAAGGGCTAGGTCTGGGGCTTGCCATCGTTGCTAGGTTGGCTCGCCTATTAGGCCATCCATTGACCTTGGCTTCCCGAGAGGGGCACGGCAGCAC
        H  E  G  L  G  L  G  L  A  I  V  A  R  L  A  R  L  L  G  H  P  L  T  L  A  S  R  E  G  H  G  S  T
                  (G2)
2701  TTTTCGGGTGCATCTTCCCCCTGGTCAGGCCGGTGAGAGCTGATGGTGAGACGGTCATTGTGCCCAATACATCCAAGACCGAGCGAGAGTCTTTGAATGC
       F  R  V  H  L  P  P  G  Q  A  G  E  S  *

2801  CGGTGCCGTTTTAGCGCATTTCAACCAAAGCCTGGCGCGGCGCGGCATCGTAGAGGAGCGACAACGGAGTGGTGATGAGGGGCGCCCGTTGGGAGGAGCC
                  *  R  M  E  V  L  A  Q  R  P  A  A  D  Y  L  L  S  L  P  T  T  I  L  P  A  R  Q  S  S  G

2901  ACCGGAGAGCAGCGCCAAGATCCGCTGTAGCAGGTGCTCTGGACATTCTGTCTTAGAGTGAAACTCCACCGTACCGCGCTGGATCGCCTCGCACACCTGA
        G  S  L  L  A  L  I  R  Q  L  L  H  E  P  C  E  T  K  S  H  F  E  V  T  G  R  Q  I  A  E  C  V  Q

3001  TCCCGGTCGAAGGCGGACACGATCACCACTCTCGCCGAGGACCAGCGCTCTTGCAGCAAGGCGATGCCTTCCAGGCCGTTAGTACCTTTCATTTGCAAAT
       D  R  D  F  A  S  V  I  V  V  R  A  S  S  W  R  E  Q  L  L  A  I  G  E  L  G  N  T  G  K  M  Q  L

3101  CGAGCAAGATGAGATCGGGGGCCGGGCACGCCTGGGCAGACGCCTCCTCGATCCTGCTGCGTTCGCTGACCGTCGTCCGTGGCAGCCTCATTTCCAACAT
      D  L  L  I  L  D  P  A  P  C  A  Q  A  S  A  E  E  I  R  S  R  E  S  V  T  T  R  P  L  R  M  E  L  M
           ▾ F7                                SstI                                                 SmaI
3201  CAGGGCCACGCTGGAACGGAACAAGGCGTGATCATCGATGAGCAAGCTCGAAGAGCTCGGCATGGACTACGTATACCTCCAGTAAAGCGTATCCCCGGGG
        L  A  V  S  S  R  F  L  A  H  D  D  I  L  L  S  S  S  S  P  M      ⇐ corP

3301  CGCTTTTGGTCAAAAAAGGACGATGGGCGCTCGCGCGGTACGCCTTTAAAGTCGGTTCGTGGTCCAGCGTGGGCCATCTCCCTGAGCGAGCAGATGGCTTT

          HindIII                                    SstI
3401  CGGCGTGAAGCTTACCGATGTGCCGGGGAACCGGCTCCCTTATTGACGACGAGGCATGTAAACCATGAGCTC
```

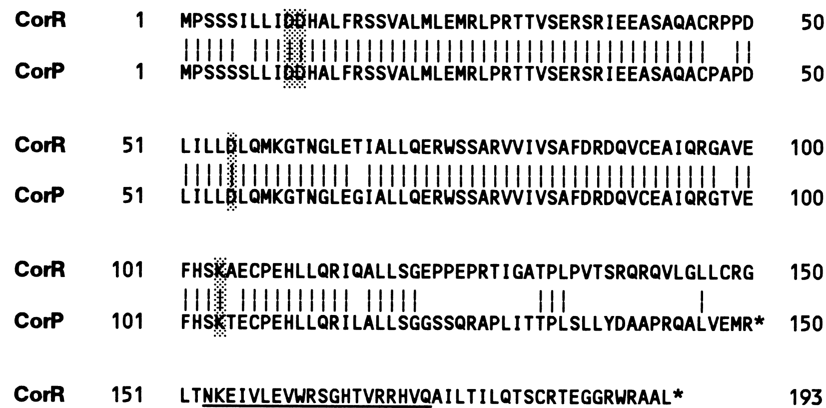

FIG. 6. Sequence alignment of CorR and CorP. Identical amino acid residues are indicated by vertical bars. Shaded amino acids indicate the locations of aspartyl and lysyl residues which are required for phosphorylation of response regulators (45). The putative DNA-binding, helix-turn-helix motif of CorR is underlined.

(Fig. 2C). GUS activities for PG4180 containing these constructs are shown in Table 3. GUS activities for *corP*::*uidA* and *corR*::*uidA* were low in PG4180 and not significantly different at 18 and 28°C, suggesting constitutive expression from these two promoters. However, GUS activity for *corS*::*uidA* was strongly dependent on temperature in PG4180 and showed the high level of expression at 18°C previously observed for pRGMU7, the construct containing the temperature-sensitive *cmaA* promoter (Table 3).

Previous experiments established the involvement of both *corP* and *corR* in the temperature-sensitive activation of the *cmaA* promoter (Fig. 3). This finding raised the interesting possibility that the temperature responsiveness of the *corS* promoter also requires functional copies of *corP* and *corR*. When pRGcorS was introduced into F7 and D4, GUS activity from the *corS*::*uidA* transcriptional fusion was dramatically decreased at 18°C relative to the activity observed for PG4180(pRGcorS) (Table 3), indicating that the temperature-induced transcriptional activation of *corS* required functional copies of both *corP* and *corR*. Furthermore, GUS activity in PG4180(pRGcorS) at 18°C was sixfold higher than that observed for P1(pRGcorS) (Table 3), indicating that a functional CorS is also required for full activation of the *corS* promoter at 18°C. These data indicate that CorS has a positive role in the transcriptional activation of its own gene and also requires functional copies of *corP* and *corR*. These results contrast with those obtained for the *corP* and *corR* promoter regions. When pRGcorP and pRGcorR were introduced into the wild-type PG4180 and three regulatory mutants, transcriptional activity remained low and constitutive regardless of the temperature and genetic background (Table 3).

Heterologous expression of the COR regulatory system. Numerous two-component regulatory systems undergo communication with other regulatory networks to modulate gene expression (8, 20, 53, 61). To investigate the autonomy of *corP*, *corR*, and *corS* within this context, we used *P. syringae* pv. syringae FF5 (54), a COR$^-$ strain lacking all genes required for COR biosynthesis. When the *cmaA*::*uidA* transcriptional fusion, pRGMU7, was introduced into FF5, GUS activity at 18 and 28°C was equivalent to the uninduced level expressed in PG4180(pRGMU7) at 28°C (Fig. 3A). However, when pMUH34 was introduced into FF5(pRGMU7), transcriptional activity of the *cmaA*::*uidA* fusion was approximately fivefold higher at 18°C than at 28°C (Fig. 3B). These results indicate that the regulatory genes encoded by pMUH34 can function in *trans* to achieve the temperature-dependent regulation of the *cmaA* promoter when removed from their native background.

TABLE 2. Restoration of COR production to regulatory mutants of *P. syringae* pv. glycinea PG4180 by genetic complementation

Construct[a]	Amount of COR produced (mg/g of protein)[b]					
	D4 (*corR*::Tn*5*)		F7 (*corP*::Tn*5*)		P1 (*corS*::Gm^r)	
	18°C	28°C	18°C	28°C	18°C	28°C
pRK415	ND[c]	ND	ND	ND	ND	ND
pMUH34	30.9	3.1	41.2	4.5	26.9	4.2
pMUP20 (*corR*)	12.6	ND	ND	ND	ND	ND
pAPB24 (*corS*)	ND	ND	ND	ND	21.7	1.5
pMUS08	ND	ND	ND	ND	ND	ND
pAPR1 (*corP*)	ND	ND	32.1	1.9	ND	ND

[a] All constructs were introduced into the mutants by triparental matings (6) and selection for Tc^r pRK415 was the vector utilized for construction of all recombinant plasmids used in this experiment.

[b] Determined by organic acid extraction and by high-pressure liquid chromatography fractionation as previously described (42). Quantities represent mean amounts recovered from four replicates.

[c] ND, not detected.

TABLE 3. GUS activity in response to temperature

Transconjugant[a]		Mean GUS activity[b] (U/mg of protein)	
Strain	Plasmid	18°C	28°C
PG4180	pRG960sd	1.3	0.9
	pRGcorP	16.9	24.8
	pRGcorR	26.7	35.2
	pRGcorS	395.2	27.1
	pRGMU7	402.1	112.6
D4	pRGcorP	12.9	13.7
	pRGcorR	20.1	52.4
	pRGcorS	6.5	59.0
F7	pRGcorP	16.2	22.3
	pRGcorR	19.7	21.5
	pRGcorS	10.2	11.4
P1	pRGcorP	10.9	23.1
	pRGcorR	21.9	36.4
	pRGcorS	65.8	95.2

[a] Locations and extents of cloned regions are indicated in Fig. 2C and Table 1. D4, F7, and P1 contain mutations in *corR*, *corP*, and *corS*, respectively.

[b] Average of four experiments with two replicates per temperature. One unit equals 1 pmol of methylumbelliferone formed per min.

Furthermore, these data indicate that the *cor* regulatory genes function autonomously when separated from the rest of the COR gene cluster.

DISCUSSION

In this study, we report the existence of three regulatory genes encoded by the COR gene cluster, *corS*, *corP*, and *corR*. The predicted protein products of these genes show interesting sequence homologies to sensor and response regulator proteins in two-component regulatory systems. We propose that this triad of genes constitutes a modified two-component regulatory system consisting of a single sensor (CorS) and two cognate response regulators, CorP and CorR. Several genetic observations support the hypothesis that these three genes function to regulate COR biosynthesis in *P. syringae* pv. glycinea. First, mutations in these genes abolish production of CFA and CMA, the two known intermediates in the COR pathway. Furthermore, all three genes were required for expression of the CMA biosynthetic cluster in *P. syringae* pv. glycinea 18a/90, a strain lacking the COR biosynthetic cluster (59). In the current study, mutations in *corP*, *corR*, or *corS* completely abolished the temperature-dependent activation of the *cmaA* biosynthetic promoter. Results of another study indicate that these three genes are required for the temperature-dependent transcriptional activation of the CFA biosynthetic gene cluster (31). Finally, the three regulatory genes functioned in a different genetic background (*P. syringae* pv. syringae FF5) to achieve temperature-dependent activation of the *cmaA* biosynthetic promoter. Thus, *corP*, *corR*, and *corS* exhibit a high degree of autonomy, suggesting that this triad is the primary control for COR biosynthesis. Furthermore, extensive mutagenesis failed to identify any additional genes required for COR production other than those located within the plasmid-encoded COR biosynthetic gene cluster (5).

Although many explanations exist for temperature-regulated COR biosynthesis, one possibility is that inducing temperatures (i.e., 18°C) may promote autophosphorylation of CorS. This would result in phosphorylated CorR and CorP, which could function in concert or sequentially to activate transcription of *corS*. Multiple copies of CorS could then amplify the temperature signal by accelerating phosphorylation of the response regulators which subsequently activate transcription of the biosynthetic genes. At elevated temperatures, CorS would not be activated (or phosphorylated CorS may be unstable), signal amplification would not occur, and transcription of the COR biosynthetic cluster would decrease to basal levels. This system resembles the standard two-component paradigm with recognition of the stimulus and phosphorylation-dephosphorylation activities. The transcriptional activation of *corS* by CorR and CorP would represent a method of signal amplification which functions to provide more copies of the sensor protein.

The two-component regulatory system reported in this study is modified from the norm because it includes a second essential response regulator, CorP, which lacks an obvious DNA-binding domain. At least three hypothetical schemes can be proposed to describe how such a modified signalling system might operate (Fig. 7). One possible function of CorP would be to modulate the phosphorylation and dephosphorylation of CorS (Fig. 7A). For example, there are several HPKs (e.g., VirA and ArcB) which contain integrated receiver domains with the conserved aspartate residue but lack the DNA-binding domain (24, 65). Parkinson and Kofoid (45) have suggested that these integrated transmitter-receiver modules function to augment stimulus sensitivity, perhaps by acting as low-threshold switches or signal amplifiers. CorP might function in a similar manner to intensify signal transduction and amplification. Another potential role for CorP would be to participate in a phosphorelay cascade (8, 45) and function by transferring the aspartyl phosphate from CorS to CorR (Fig. 7B). Phosphorelays are thought to integrate multiple signals that regulate transcription of a single gene or gene cluster and are involved in the onset of sporulation in *Bacillus subtilis* (8). A third scenario could involve heterodimer formation between CorP and CorR prior to transcriptional activation (Fig. 7C). Transcriptional regulators that function as homodimers include NtrC and DtxR (48, 52) and the LysR-like proteins CatR and AmpR (7, 47). Heterodimer formation has been suggested to occur between HrpR and HrpS, two response regulators required for transcriptional activation of *hrpL* in *P. syringae* pv. syringae (66). A CorP-CorR heterodimer could conceivably mediate transcriptional activation, although only CorR contains a helix-turn-helix motif for DNA binding. Although all three schemes are hypothetical and additional possibilities exist, purified CorP, CorR, and CorS are needed to begin testing the validity of the different models. For example, once each protein is obtained in purified form, phosphorylation assays will help clarify the interactions between CorP, CorR, and CorS. Gel shift assays and DNA footprinting experiments will

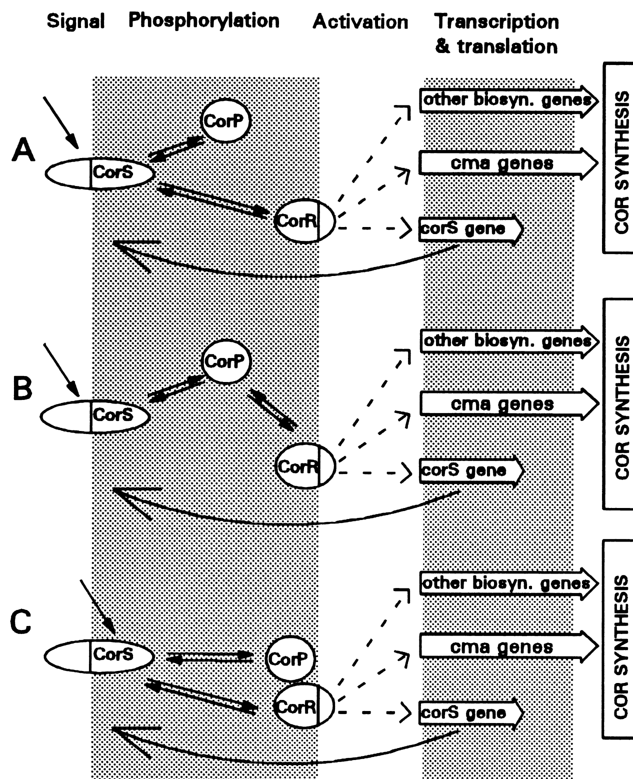

FIG. 7. Three proposed mechanisms (A, B, and C) for thermoregulation of COR biosynthesis via the modified two-component regulatory system described in this report. The three proteins involved in thermoregulation (CorS, CorP, and CorR) are indicated by open circles and ovals. Transcriptional units subjected to thermoregulation are indicated by the open rectangular arrows. Small black arrows indicate possible protein-protein interactions which could result in additional copies of CorS. Possible functions of CorP, a response regulator lacking a DNA-binding motif, include modulating the phosphorylation and dephosphorylation of CorS (A), providing a relay system for directing the phosphorylation signal from CorS to CorR (B), and interacting directly with CorR by forming a heterodimer (C). Ultimately, the result is transcriptional activation (dashed arrows) of the thermally regulated transcripts.

be conducted to determine whether purified CorR binds to DNA upstream of the thermoregulated transcripts.

Very few examples of thermoregulated gene expression in plant-associated bacteria are documented. Transcription of genes encoding pectinases, cellulases, and proteases in *Erwinia carotovora* and *E. chrysanthemi* was enhanced at reduced temperatures, although the mechanism(s) underlying the response to temperature remains obscure (10, 22, 30). In *Agrobacterium tumefaciens*, elevated temperatures were shown to decrease autophosphorylation of VirA activity and subsequent phosphorylation of VirG, thereby resulting in decreased tumor formation (25). The phytotoxin phaseolotoxin, like COR, is produced at optimal levels by *P. syringae* pv. phaseolicola at 18°C, while synthesis at 30°C is undetectable (16, 36). Recently, Rowley et al. (49) reported that thermoregulation of phaseolotoxin synthesis was due to a repressor-like protein(s). The concentration of the putative repressor was higher when cells were incubated at 28°C, a temperature nonconducive to phaseolotoxin production, than that observed at 18°C. Unlike the results reported for phaseolotoxin, our data indicate that COR biosynthesis is controlled by activation at 18°C rather than repression at 28°C.

The role of temperature in the expression of virulence genes in human and animal pathogens is well established (34, 63). Transcription of these genes is activated when microbes enter their warm-blooded hosts, and the mechanisms that bacteria use to sense the increased temperature have been studied intensively (18, 34, 35, 57). Phytopathogens like *P. syringae* are also subjected to temperature fluctuations in nature, and some temperatures are more conducive to disease development than others. In several diseases caused by COR-producing pathovars of *P. syringae*, symptoms are most severe during periods of cool, wet weather (2). Since the chlorosis and stunting caused by COR production in these bacteria could be triggered by temperature, experiments to examine this hypothesis and the mechanisms involved are in progress.

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