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Global control in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco

(secondary metabolites/hydrogen cyanide/2,4-diacetylphloroglucinol/two-component regulatory systems/*uvrC*)

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ABSTRACT **_Pseudomonas fluorescens_ CHA0 colonizes plant roots, produces several secondary metabolites in stationary growth phase, and suppresses a number of plant diseases, including _Thielaviopsis basicola_-induced black root rot of tobacco. We discovered that mutations in a _P. fluorescens_ gene named _gacA_ (for _g_lobal _a_ntibiotic and _c_yanide control) pleiotropically block the production of the secondary metabolites 2,4-diacetylphloroglucinol (Phl), HCN, and pyoluteorin. The _gacA_ mutants of strain CHA0 have a drastically reduced ability to suppress black root rot under gnotobiotic conditions, supporting the previous observations that the antibiotic Phl and HCN individually contribute to the suppression of black root rot. The _gacA_ gene is directly followed by a _uvrC_ gene. Double _gacA–uvrC_ mutations render _P. fluorescens_ sensitive to UV irradiation. The _gacA–uvrC_ cluster is homologous to the _orf-2_ (= _uvrY_)–_uvrC_ operon of _Escherichia coli_. The _gacA_ gene specifies a trans-active 24-kDa protein. Sequence data indicate that the GacA protein is a response regulator in the FixJ/DegU family of two-component regulatory systems. Expression of the _gacA_ gene itself was increased in stationary phase. We propose that GacA, perhaps activated by conditions of restricted growth, functions as a global regulator of secondary metabolism in _P. fluorescens_.**

Soil bacteria of the genus *Pseudomonas* synthesize a wide variety of antibiotic compounds (1–3). There is growing evidence that some of these compounds play an important role in the biological control of plant diseases (2–5). Strains of *Pseudomonas fluorescens* that effectively suppress root diseases caused by soil-borne fungi have been isolated from plant roots in various parts of the world. Most, if not all, of these strains synthesize one or several antibiotic compounds (3). Phenazine-1-carboxylic acid, an antibiotic having activity against phytopathogenic fungi, is produced by the fluorescent *Pseudomonas* strains 2-79 and 30-84 in the rhizosphere of wheat. Phenazine-nonproducing mutants have a significantly reduced ability to suppress take-all, a wheat disease caused by *Gaeumannomyces graminis* var. *tritici* (6–8). 2,4-Diacetylphloroglucinol (Phl) is another antibiotic whose production by *P. fluorescens* can be detected in the rhizosphere of wheat (9). A mutant of *P. fluorescens* strain CHA0 blocked in Phl synthesis lacks part of the suppressive effect on take-all of wheat and on black root rot of tobacco. *In vitro*, the Phl$^-$ mutant shows diminished inhibition of the corresponding fungal pathogens, *G. graminis* var. *tritici* and *Thielaviopsis basicola* (2, 9). HCN (10), oomycin (5, 11), pyoluteorin (12), and pyrrolnitrin (13) are further antibiotic compounds that are produced by rhizosphere isolates of *Pseudomonas* species and that may contribute to the suppression of root diseases.

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Conditions of restricted growth or stationary phase generally favor the production of antibiotics and other secondary metabolites. In *Bacillus* and *Streptomyces* species global control elements involved in antibiotic synthesis have been identified (14–17). In *Pseudomonas* species, by contrast, little is known about the regulatory mechanisms governing antibiotic production. We have therefore decided to characterize a mutant of *P. fluorescens* CHA0 that is pleiotropically defective in the synthesis of three secondary metabolites—Phl, pyoluteorin, and HCN. We have previously shown that Phl as well as cyanide are important for the suppression of black root rot of tobacco by strain CHA0 (9, 10), whereas pyoluteorin probably helps suppress *Pythium ultimum*-induced root disease of cucumber (18). Here we show that the pleiotropic loss of the three secondary metabolites in *P. fluorescens* CHA0 is due to a mutation in a putative activator¶ of a two-component regulatory system. This defect essentially disables *P. fluorescens* to suppress black root rot of tobacco.

MATERIALS AND METHODS

Microorganisms and Plasmids. Derivatives of *P. fluorescens* CHA0 (19) isolated in this study are shown in Fig. 1. The sources of *Escherichia coli* strains used for cloning and of *T. basicola* have been given previously (10, 19). Recombinant plasmids (Fig. 1) were constructed in cosmid pVK100 (21) and mobilized with the helper plasmid pME497 (10, 22) from *E. coli* to *P. fluorescens*. Tn*5-259* mutagenesis was carried out with pME12 (22). Expression vectors pMS119EH and pMS119HE were kindly provided by E. Lanka (Max-Planck-Institut für Molekulare Genetik, Berlin).

Growth Conditions and DNA Manipulation. Growth media have been described (10, 19, 22). Standard methods were used for plasmid extraction by alkaline lysis and for cloning (23). Nucleotide sequences were determined for both strands by the chain-termination method with Sequenase Version 2.0 and 7-deaza-dGTP (United States Biochemical) according to the manufacturer's instructions; single-stranded DNAs generated from derivatives of M13mp18 and M13mp19 (24) were purified on Sephaglas (PhagePrep Kit, Pharmacia). Gene replacements in *P. fluorescens* were performed with a ColE1-based, mobilizable suicide vector (details to be given elsewhere) essentially as described (10). The CLUSTAL and FASTSCAN programs (PC-Gene, Release 6.5; IntelliGenetics) were used to align amino acid sequences and to find homologies with proteins in the Swiss-Prot data bank.

Abbreviations: IPTG, isopropyl β-D-thiogalactoside; ORF, open reading frame; Phl, 2,4-diacetylphloroglucinol.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M80913).

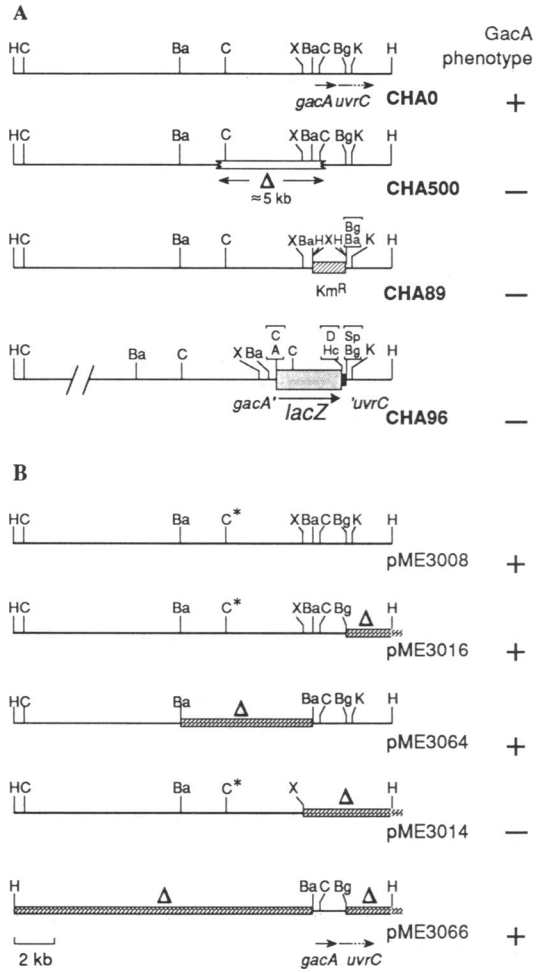

FIG. 1. (*A*) Physical map of the *gacA–uvrC* region in the genome of *P. fluorescens* CHA0 (wild type) and its mutant derivatives CHA500, CHA89, and CHA96 (their isolation is described in the text). The restriction maps were obtained by Southern hybridization experiments (not shown). The precise end points of the spontaneous 5-kilobase (kb) deletion (Δ) in strain CHA500 are uncertain. Km^R, kanamycin resistance cassette from pUC4KIXX (Pharmacia). *'lacZ*, β-galactosidase translational fusion cassette from pNM482 (20), followed by a linker fragment (▮). (*B*) Recombinant plasmids carrying genomic DNA from *P. fluorescens* CHA0 in the vector pVK100 (21). Deletions (Δ) created *in vitro* are indicated by ▨. The GacA phenotype was assessed primarily with a qualitative HCN test (10). Restriction sites are abbreviated as follows: A, *Acc* I; Ba, *Bam*HI; Bg, *Bgl* II; C, *Cla* I; C*, *Cla* I site cleaved in *P. fluorescens* but not in *E. coli*; D, *Dra* I; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn* I; Sp, *Sph* I; X, *Xho* I.

Analysis of Secondary Metabolites and Tryptophan Side Chain Oxidase. HCN, Phl (M_r 210), and pyoluteorin (M_r 268) were determined quantitatively (9, 10). Tryptophan side chain oxidase activity and pyoverdine were assessed qualitatively (19, 25).

Gnotobiotic System. This contained sterilized artificial soil, a tobacco plant, and known numbers of *T. basicola* endoconidia and/or *P. fluorescens* cells (2, 10, 19). Disease symptoms of tobacco were evaluated and analyzed statistically by the procedures described before (9, 10, 19).

RESULTS

Isolation and Characterization of *P. fluorescens* Mutants Pleiotropically Defective in Secondary Metabolism. The wild-type strain CHA0 of *P. fluorescens* was subjected to transposon mutagenesis with pME12, a suicide vector carrying the kanamycin-resistance transposon Tn*5-259* (22). Among 3000 kanamycin-resistant derivatives of strain CHA0, one mutant (CHA500) was found that did not produce HCN, as evidenced by a cyanide indicator paper method (10). Moreover, strain CHA500 lacked Phl and pyoluteorin production, was defective in tryptophan side chain oxidase activity [an enzyme participating in indoleacetate synthesis (25)], and, unexpectedly, was highly sensitive to UV irradiation (Table 1). Production of pyoverdine, the fluorescent siderophore of *P. fluorescens*, was not defective in strain CHA500. According to Southern hybridization, a single Tn*5-259* element was present in the genome of strain CHA500 (data not shown). A genomic library of strain CHA0 established in *E. coli* (10) was mobilized to strain CHA500. Five recombinant cosmids, each containing the same 19-kb genomic *Hin*dIII insert, were found to complement strain CHA500 for cyanide synthesis. One representative cosmid, pME3008, was used to show that in fact all phenotypic defects of the mutant were restored (Table 1). Southern hybridization with pME3008 as the probe revealed that in strain CHA500 the genomic region that corresponded to the complementing 19-kb *Hin*dIII fragment did not contain the Tn*5-259* insertion but had undergone a deletion of about 5 kb (Fig. 1; hybridization data not shown). Thus, it appeared that the deletion rather than the transposon insertion caused the phenotypic defects of strain CHA500. Deletion analysis of pME3008 led to a 1.65-kb *Bam*HI–*Bgl* II fragment in pME3066 (Fig. 1) that complemented strain CHA500 for tryptophan side chain oxidase activity and production of cyanide, Phl, and pyoluteorin but failed to restore wild-type UV resistance (Table 1). The trans-acting function encoded by the 1.65-kb fragment was named *gacA* (for *g*lobal *a*ntibiotic and *c*yanide control). The reason for the inability of the *gacA* fragment to bring back UV resistance will become apparent from the DNA sequence data presented below.

To show that the Tn*5-259* insertion was not relevant to the phenotypes of strain CHA500, we replaced the 1.65-kb $gacA^+$ fragment in the genome of the wild-type CHA0 with a 1.6-kb kanamycin-resistance cassette. The resulting mutant, CHA89 (Fig. 1), was phenotypically indistinguishable from strain CHA500, and the complementation patterns of pME3008 and pME3066 were the same in both mutants (Table 1). Thus, the *gacA* region serves a global regulatory function in *P. fluorescens*. The *hcn* (HCN) and *phl* genes previously mutated and characterized in strain CHA0 (9, 10) are not linked to *gacA*.

***P. fluorescens* CHA0 Has a *gacA–uvrC* Gene Cluster Homologous to the *uvrY–uvrC* Operon of *E. coli*.** The nucleotide sequence of the 1.65-kb *Bam*HI–*Bgl* II fragment of pME3066 was determined. In the proximal part (Fig. 2) an open reading frame (ORF) (*gacA*) was found that was homologous to the *uvrY* gene‖ in the *uvrC* operon of *E. coli* (27, 28). The translation start of *gacA* was tentatively assigned to a TTG codon (Fig. 2), by analogy with the homologous start codon in *uvrY* (28). The deduced amino acid sequence of the GacA protein showed 58% identity with the *uvrY* translation product and 14% additional conservative replacements (Fig. 3). The UvrY protein is a member of the FixJ family of regulators (refs. 29 and 30; see *Discussion*). The (G + C) content of the *gacA* gene was 58%, slightly below the average (G + C) content of 60–62% in the *P. fluorescens*/*Pseudomonas putida* group (32). The *gacA* sequence was directly followed by a second ORF (Fig. 2). The amino acid sequence derived from the first 711 nucleotides of this ORF (data not shown) had 57% identity with the UvrC protein of *E. coli* (28). This similarity and the UV sensitivity of strains CHA500 and

‖We adopt the nomenclature of Kahn and Ditta (26) for this gene; other designations are *orf-2* (27), *24 kD* (28), *uvrC-2* (29), *uvrC-23Kd* (30), and YU23 for the protein (Swiss-Prot data bank).

Table 1. Phenotypic characterization and complementation of *gacA* mutants of *P. fluorescens*

Strain	Phl,* μg/ml	Pyoluteorin,† μg/ml	HCN,‡ μM	TSO§	Survival after UV irradiation¶
CHA0	11.0 ± 1.5	9.0 ± 0.9	161 ± 12	+	8.1×10^{-1}
CHA500	<0.1	<0.1	<5	−	8.1×10^{-6}
CHA89	<0.1	<0.1	<5	−	2.4×10^{-6}
CHA500/pME3008	10.7 ± 2	16.0 ± 0.7	108 ± 8	+	2.2×10^{-1}
CHA500/pME3066	6.8 ± 2.5	12.8 ± 1.2	97 ± 11	+	1.3×10^{-5}
CHA89/pME3008	6.1 ± 2.2	11.9 ± 1.3	115 ± 5	+	3.0×10^{-1}
CHA89/pME3066	14.4 ± 2	14.0 ± 1	88 ± 8	+	2.4×10^{-6}

Data are expressed as mean ± SD of three independent experiments.
*Determined after incubation on malt agar at 18°C for 4 days (9).
†Determined after incubation on King's B agar at 18°C for 4 days (9).
‡Determined after incubation in Castric's medium containing glycine and $FeCl_3$ at 30°C for 40 hr (10).
§Tryptophan side chain oxidase (TSO) was determined qualitatively after incubation on malt agar at 18°C for 4 days by overlaying with tryptophan soft agar (25). +, Black TSO-positive colonies; −, white TSO-negative colonies.
¶Appropriate dilutions were plated on nutrient agar and irradiated with a germicidal lamp at 1 m for 10 sec. Survival is calculated per unirradiated controls.

CHA89 (Table 1) lead us to conclude that the second ORF is a *uvrC* gene. In CHA89, the kanamycin-resistance cartridge extends into the *uvrC* gene. In CHA500, the end points of the 5-kb deletion have not been mapped precisely (Fig. 1). If the right end of the deletion lies in *gacA* and if the promoters for *gacA* and *uvrC* lie upstream of or within *gacA*, then a polar effect may be exerted on *uvrC* expression. Alternatively, the deletion may remove the entire *gacA* gene and the proximal part of *uvrC*. In either case, *uvrABC*-mediated excision repair would be affected, explaining the UV sensitivity of strains CHA89 and CHA500.

The *gacA* gene fused in the correct orientation to the inducible *tac* promoter specified a 24-kDa protein in *E. coli* maxicells after induction with isopropyl β-D-galactoside (IPTG) (Fig. 4). The *uvrY* product has the same molecular weight (28). We constructed a translational *gacA′–′lacZ* fusion at the *Cla* I site in *gacA* (Figs. 1 and 2). The fusion protein had β-galactosidase activity in *E. coli*, confirming *gacA* expression in this host. The *gacA′–′lacZ* fusion was introduced, by gene replacement, into the genome of strain CHA0. The mutant thus created, CHA96 (Fig. 1), was defective for the *gacA* and *uvrC* genes, but the sequences

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           10        20        30        40        50
 1    GGATCCCGATCCAGAGCGGCGGGCTGAGTCAGACCGAGGAAAAGGTCCTG
      BamHI
 51   GATTGCTATCGGGCGCTGTTCAAGGAGGATCAGGATGCTATCTGCCGCCT
101   GACCAGTACCCTGGCCGAGCTTTCGGCAAGCCAGGGCGAGAGGCGCAAGG
151   CCCAGGTACGAGAATGAGGGGCGCTGGCCTCTTTCGATGCGCACGGGGGT
201   AGGACCGTTATTGCGCCCGGATGTTTTTTATATAGCGCTTGTGATTAGGT
251   TTGTACGCCGCTTTTTTGCTAAGGTGTCGGGCAACCTGTAAAGACCATAT
                        → gacA
        SD              MetIleArgValLeuValValAspAspHisAspLeu
301   CGCGAGGTGTCTGCTTGATAAGGGTGCTAGTAGTCGATGACCATGATCTC
      NruI
      ValArgThrGlyIleThrArgMetLeuAlaAspIleAspGlyLeuGlnVa
351   GTTCGTACGGGCATTACACGAATGTTGGCTGACATCGATGGCCTGCAAGT
                                         ClaI
      lValGlyGlnAlaGluSerGlyGluGluSerLeuLeuLysAlaArgGluL
401   AGTCGGGCAGGCGGAGTCAGGGGAAGAATCCCTGCTCAAGGCGCGGGAGC
      euLysProTyrValValLeuMetAspValLysMetProGlyIleGlyGly
451   TGAAGCCCTATGTGGTCCTGATGGACGTCAAGATGCCAGGCATCGGCGGC
      LeuGluAlaThrArgLysLeuLeuArgSerHisProAspIleLysValVa
501   CTCGAAGCCACGCGCAAACTGCTGCGCAGTCATCCGGACATCAAGGTCGT
      lAlaValThrValCysGluGluAspProPheProThrArgLeuLeuGlnA
551   GGCGGTCACCGTCTGCGAGGAAGATCCCTTTCCTACCCGCTTGCTGCAAG
         BstEII
      laGlyAlaAlaGlyTyrLeuThrLysGlyAlaGlyLeuAsnGluMetVal
601   CGGGTGCGGCGGGCTATCTGACCAAGGGCGCCGGTCTGAACGAAATGGTC
      GlnAlaIleArgLeuValPheAlaGlyGlnArgTyrIleSerProGlnIl
651   CAGGCCATCCGCCTGGTATTTGCCGGCCAGCGCTATATCAGCCCGCAGAT
      eAlaGlnGlnLeuValPheLysSerPheGlnProSerSerAspSerProP
701   CGCTCAGCAATTGGTGTTCAAGTCATTCCAGCCTTCCAGTGATTCACCGT
        EspI
      heAspAlaLeuSerGluArgGluIleGlnIleAlaLeuMetIleValGly
751   TCGACGCCCTGTCGGAACGGGAGATCCAGATTGCGCTGATGATTGTCGGC
      CysGlnLysValGlnIleIleSerAspLysLeuCysLeuSerProLysTh
801   TGCCAGAAGGTGCAGATCATCTCCGACAAGCTGTGCCTGTCGCCGAAAAC
      rValAsnThrTyrArgTyrArgIlePheGluLysLeuSerIleSerSerA
851   CGTGAATACCTACCGCTATCGCATTTTCGAGAAGCTCTCGATCAGCAGCG
      snThrTyrArgTyrArgIlePheGluLysLeuSerIleSerSerAlaSer
901   ATGTCGAGTTGACGCTATTGGCGGTTCGTCACGGCATGGTTGATGCCAGC
             HincII
      LeuStopMetThrGluGlnPheAspProSerAlaPheLeuSerThrCys..
951   CTCTGAAATGACCGAACAGTTTGATCCCAGTGCCTTTCTTTCTACCTGCAG
             └→uvrC                                 PstI
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FIG. 2. Nucleotide sequence of the *gacA* gene and deduced amino acid sequence of the GacA protein of *P. fluorescens*. The 1.0-kb *Bam*HI–*Pst* I fragment shown represents the 5′ region of the 1.65-kb *Bam*HI–*Bgl* II genomic insert in pME3066 (Fig. 1*B*). SD, putative ribosome binding site.

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FixJ   MTDYTVHIVDDEEPVRKSLAFMLT-MNGFAVKMH-QSAEAFLAF
GacA   MIR--VLVVDDHDLVRTGITRMLADIDGLQVVGQAESGEESLLK
UvrY   MIN--VLLVDDHELVRAGIRRILEDIKGIKVVGEASCGEDAVKW

FixJ   APDVRNGVLVTDLRMPDMSGVELLRNLGDLKINIPSIVITGHGD
GacA   ARELKPYVVLMDVKMPGIGGLEATRKLLRSHPDIKVVAVTVCEE
UvrY   CRTNAVDVVLMDMSMPGIGGLEATRKIARSTADVKIIMLTVHTE

FixJ   VPMAVEAMKAGAVDFIEKPFEDTVIIEAIER--ASEHLVAAEAD
GacA   DPFPTRLLQAGAAGYLTKGAGLNEMVQAIRLVFAGQRYISPQIA
UvrY   NPLPAKVMQAGAAGYLSKGAAPQEVVSAIRSVYSGQRYIASDIA

                                        helix....
FixJ   VD------DANDIRARLQTLSERERQVLSAVVAGLPNKSIAYDL
GacA   QQLVFKSFQPS-SDSPFDALSEREIQIALMIVGCQKVQIISDKL
UvrY   QQMALSQIEPEKTESPFASLSERELQIMLMITKGQKVNEISEQL

       ...helix..
FixJ   DISPRTVEVHRANVMAKMKAKSLPHLVRMAL------AGGFGPS
GacA   CLSPKTVNTYRYRIFEKLSISSDVELTLLAVRHGMVDA----SL
UvrY   NLSPKTVNSYRYRMFSKLNIHGDVELTHLAIRHGLCNAETLSSQ
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FIG. 3. Alignment of the amino acid sequences of GacA (*P. fluorescens*), UvrY (*E. coli*), and FixJ (*Rhizobium meliloti*). Additional members of the FixJ family of response regulators can be found in refs. 26 and 31. A putative helix–turn–helix motif has been located on FixJ (26). Residues in the N-terminal domain that are strictly conserved among response regulators (29) are indicated by ▼; residues in the C-terminal region that are conserved in the FixJ family (26) are denoted by ●. :, Identical residues; ·, conservative replacements.

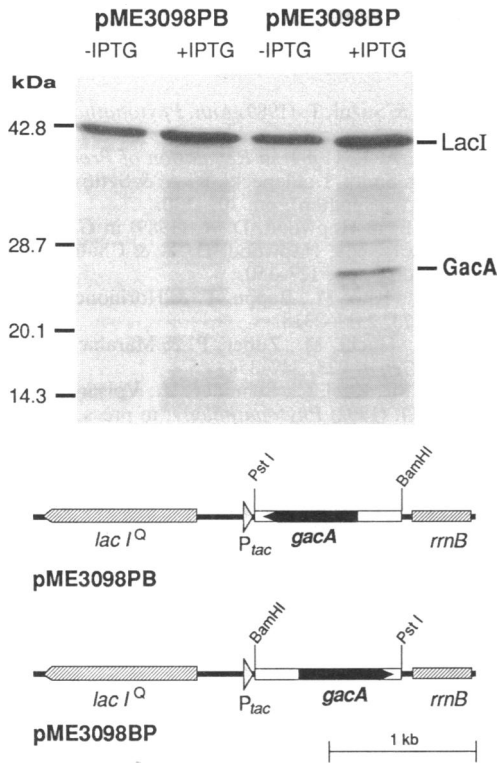

FIG. 4. Expression of the GacA protein in *E. coli* maxicells. The *Bam*HI–*Pst* I fragment carrying the *gacA* gene of *P. fluorescens* (Fig. 2) was inserted into pMS119HE, giving pME3098PB, or into pMS119EH, giving pME3098BP. *E. coli* CSR603 (33) was transformed with the *gacA* plasmids and subjected to maxicell treatment, with or without prior IPTG induction (33, 34). *lacI*^{Q}, Lactose repressor gene; P_{tac}, *tac* promoter; *rrnB*, transcription terminator.

upstream of the *gacA* translation start were the same as in the wild type. Strain CHA96 was phenotypically similar to the other *gacA* mutants CHA89 and CHA500. Thus, a mutation in *gacA* suffices to produce the complex antibiotic-negative phenotype. Strain CHA96 formed "fish-eye" colonies (35) on agar containing 5-bromo-4-chloro-3-indolyl β-D-galactoside (data not shown). In liquid succinate medium β-galactosidase activity of strain CHA96 increased 2-fold from exponential to stationary phase (data not shown). This suggests enhanced expression of GacA during stationary growth phase.

Effective Suppression of Black Root Rot of Tobacco by *P. fluorescens* CHA0 Requires *gacA* Function. The wild-type CHA0 is known to suppress the disease symptoms caused by *T. basicola* on tobacco under gnotobiotic conditions (9, 10, 19). In contrast, the *gacA* mutants CHA500 and CHA89 lacked most of the suppressive ability, as judged by an increase in disease severity (i.e., root surface infected) and a decrease of plant weight (root and total weight) (Table 2). The residual suppressive effect of the *gacA* mutants is due to unknown factors and statistically not significant (Table 2). Plasmid pME3066 largely restored plant protection (Table 2). Instability of pME3066 presumably precluded complete restoration of disease suppression: about 50% of the bacterial cells had lost the plasmid after 4 weeks of incubation in the gnotobiotic system. It appears unlikely that the UV sensitivity of the pME3066 transconjugants should influence the suppressive ability of *P. fluorescens* in soil. In the absence of the pathogen, the bacteria had no significant effect on the growth of tobacco (Table 2). Tobacco roots infected with *T. basicola* were colonized by *gacA*$^+$ bacteria at mean densities of 3×10^8 to 5×10^8 cells per g of root. The mean densities of *gacA*$^-$ strains were not significantly different (about 9×10^8 cells per g of root), indicating that reduced disease suppression by *gacA* mutants is not related to altered root colonization proficiency.

Table 2. Suppression of black root rot of tobacco by *P. fluorescens* in a gnotobiotic system

Microorganisms*	Fresh weight per plant†		Root surface infected,‡ %
	Root, mg	Total, mg	
None	361 a	839 a	—
CHA0	440 a	871 a	—
CHA500	480 a	963 a	—
CHA89	365 a	805 a	—
CHA500/pME3066	453 a	923 a	—
CHA89/pME3066	349 a	736 a	—
Tb	68 c	219 c	76 b
Tb + CHA0	315 a	659 a	31 a
Tb + CHA500	125 c	327 c	65 b
Tb + CHA89	150 c	379 c	65 b
Tb + CHA500/pME3066	281 abc	608 ab	45 a
Tb + CHA89/pME3066	238 b	557 b	34 a

*Ten million colony-forming units of *P. fluorescens* (designated by CHA strain numbers) and/or 5×10^3 endoconidia of *T. basicola* (abbreviated Tb) were added to artificial soil prior to planting a 1-week-old tobacco plant (9, 19).

†Plants were weighed after 3 weeks of incubation in the gnotobiotic system. The numbers are mean values obtained in three independent experiments with 10 replicates per experiment and one plant per replicate. Different letters indicate values that are statistically different ($P = 0.05$) according to Student's *t* test.

‡Assessed as described (19). Letters indicate values that are statistically different ($P = 0.05$) in the *t* test after arc sine transformation.

DISCUSSION

We have identified a gene, *gacA*, for regulation of secondary metabolism in *P. fluorescens*. The production of at least three secondary metabolites, HCN, Phl, and pyoluteorin, depended on *gacA* function (Table 1). *In vitro*, these three metabolites are synthesized at the end of exponential growth or during the stationary phase (9, 10, 36). Tryptophan side chain oxidase, which is inducible by stationary phase conditions (25), also required *gacA* for expression (Table 1). These observations point to a global regulatory role of *gacA* during restricted growth and/or limited nutrient supply. The *gacA* gene itself appears to be expressed preferentially under such conditions.

Homology between *gacA* of *P. fluorescens* and *uvrY* of *E. coli* is apparent from sequence similarities (Fig. 3) and from the analogous position of these genes upstream of *uvrC*. The function of *uvrY* in *E. coli* is unknown. A *uvrY* mutant has no obvious phenotype and, compared to an isogenic *uvrY*$^+$ strain, shows no significant difference in survival after UV irradiation of cells grown in minimal medium (28). Extensive (24 hr) starvation of *E. coli* for amino acids, glucose, or phosphate induces *uvrABC*-dependent excision repair (37). It would be interesting to see whether *uvrY* participates in starvation control of *uvrC*.

In prokaryotes, transduction of environmental signals is often mediated by pairs of proteins, a sensor/kinase, and a response regulator. According to the general model of such two-component systems, the sensor, when triggered by the signal, phosphorylates the regulator in the N-terminal domain. This activates the regulator, whose C-terminal part interacts with target gene promoters (26, 29, 30). A sequence alignment (Fig. 3) puts GacA and its homolog UvrY into the FixJ family of response regulators. Other members of this family are, for instance, LasR of *Pseudomonas aeruginosa* (31) and DegU of *Bacillus subtilis* (38), which control the

expression of extracellular proteases and other cellular functions during late growth stages in these bacteria. GacA shares conserved residues with FixJ (22% identity) and DegU (27% identity) but is clearly different from these regulators and from the more distantly related LasR protein. Spo0A, a global regulator of late growth functions including antibiotic synthesis and sporulation in *B. subtilis* (14, 17), also belongs to the response regulator group of two-component systems. However, Spo0A differs in its C-terminal domain from the FixJ family (29). Sensor/kinase components for FixJ, DegU, and Spo0A have been identified (29, 39); those for GacA, UvrY, and LasR remain to be found. *P. fluorescens* and *E. coli* may not be the only bacteria having a *gacA* (*uvrY*) gene. We have preliminary evidence from hybridization experiments that a *gacA*-like sequence also occurs in *P. aeruginosa* PAO, a producer of several secondary metabolites (1).

The *gacA* mutants of *P. fluorescens* had a strongly reduced ability to suppress black root rot of tobacco (Table 2) and to protect cucumbers from *Pythium*, whereas root diseases of wheat could still be suppressed by *gacA* mutants (unpublished results). We conclude that extracellular metabolites (especially antibiotics), which are produced under *gacA* control, are important for the suppression of some root diseases, such as black root rot of tobacco.

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