Posttranscriptional Regulation of 2,4-Diacetylphloroglucinol Production by *GidA* and *TrmE* in *Pseudomonas fluorescens* 2P24

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Pseudomonas fluorescens 2P24 is a soilborne bacterium that synthesizes and excretes multiple antimicrobial metabolites. The polyketide compound 2,4-diacetylphloroglucinol (2,4-DAPG), synthesized by the *phlACBD* locus, is its major biocontrol determinant. This study investigated two mutants defective in antagonistic activity against *Rhizoctonia solani*. Deletion of the *gidA* (PM701) or *trmE* (PM702) gene from strain 2P24 completely inhibited the production of 2,4-DAPG and its precursors, monoacetylphloroglucinol (MAPG) and phloroglucinol (PG). The transcription of the *phlA* gene was not affected, but the translation of the *phlA* and *phlD* genes was reduced significantly. Two components of the Gac/Rsm pathway, RsmA and RsmE, were found to be regulated by *gidA* and *trmE*, whereas the other components, RsmX, RsmY, and RsmZ, were not. The regulation of 2,4-DAPG production by *gidA* and *trmE*, however, was independent of the Gac/Rsm pathway. Both the *gidA* and *trmE* mutants were unable to produce PG but could convert PG to MAPG and MAPG to 2,4-DAPG. Overexpression of *PhlD* in the *gidA* and *trmE* mutants could restore the production of PG and 2,4-DAPG. Taken together, these findings suggest that *GidA* and *TrmE* are positive regulatory elements that influence the biosynthesis of 2,4-DAPG posttranscriptionally.

Many strains of *Pseudomonas fluorescens* are important plant growth-promoting rhizobacteria (PGPR), enhancing plant growth through various mechanisms (1, 2). Secondary metabolites produced by these bacteria play a key role in the suppression of plant diseases caused by soilborne pathogens (3, 4). One of the most extensively studied metabolites, 2,4-diacetylphloroglucinol (2,4-DAPG), has antifungal, antibacterial, antiviral, antihelminthic, and phytotoxic activities at high concentrations (5, 6, 7, 8). 2,4-DAPG can also act as a signal molecule for plants, inducing systemic resistance, promoting the exudation of amino acids from roots, and enhancing branching of the root system (9, 10, 11).

The biosynthetic locus of 2,4-DAPG has been identified in several *Pseudomonas* strains, including *P. fluorescens* Q2-87, CHA0, F113, Pf-5, and 2P24 (12, 13, 14, 15, 16). The locus contains four genes, *phlA*, *phlC*, *phlB*, and *phlD*, which are transcribed as a single operon (*phlACBD*) (13). The *phlD* gene encodes a type III polyketide synthase, which is responsible for the synthesis of phloroglucinol (PG) from three molecules of malonyl-coenzyme A (CoA) (17, 18, 19). The *phlA*, *phlC*, and *phlB* genes together are required for conversion of PG to monoacetylphloroglucinol (MAPG) and of MAPG to 2,4-DAPG (13, 20). The *phlA* gene encodes a β -ketoacyl-ACP synthase III (FabH). *PhlC* has an acetyl-CoA binding site that contains an active cysteine residue and a glycine-rich C-terminal region, which are typical structural features of condensing enzymes (13). *PhlB* is a small protein and has sequence homology to several nucleic acid-binding proteins in the GenBank database (21). The *phlACBD* operon is flanked on either side by the separately transcribed *phlE* and *phlF* genes (see Fig. S1 in the supplemental material), which code for a putative transmembrane permease and a TetR-like transcriptional repressor, respectively (13, 14, 22). *PhlF* represses *phlA* transcription by binding to the specific binding site *pho*, which is located in the *phlA* promoter region (23). In *P. fluorescens* CHA0, the *phlG* gene, located downstream of the *phlF* gene, encodes a hydrolase that specifically degrades 2,4-DAPG to MAPG (24, 25). The biosynthe-

sis of 2,4-DAPG is highly dependent on bacterial cell density and is controlled by many regulatory elements, including the conserved regulatory system Gac/Rsm. In this regulatory cascade, the GacS/GacA two-component system activates the transcription of one or several small RNAs (sRNAs), including RsmX, RsmY, and RsmZ, which subsequently bind to the translational repressor proteins RsmA and RsmE and dissociate them from the ribosome-binding sites of target genes to relieve translational repression (26, 27). Many other molecules involved in the regulation of 2,4-DAPG biosynthesis have been identified, including the sigma factors RpoS, RpoD, and RpoN (12, 28, 29); the H-NS-related proteins MvaT and MvaV (30); the oxidoreductase DsbA (31); the resistance-nodulation-division efflux pump EmhABC (16), the RNA chaperone Hfq (32); the sigma regulator PsrA (33); and various environmental factors (34, 35).

P. fluorescens 2P24 is a rhizosphere isolate capable of producing the antibiotic 2,4-DAPG. We previously showed that 2,4-DAPG biosynthesis in this strain involved a distinct and complex regulatory network (16, 32, 33). In this study, glucose-inhibited division protein A (*GidA*) and tRNA modification GTPase (*TrmE*) were selected as two novel 2,4-DAPG-positive regulators in strain 2P24,

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as antifungal activity was completely absent in both *gidA* and *trmE* mutants. Previous studies showed that these two proteins form a complex that catalyzes the addition of a carboxymethylaminomethyl (cmnm) group at the 5' position of the wobble uridine (U34) of tRNAs (36, 37, 38). This modification allows base pairing at the wobble position with a purine (A or G) and restricts the misreading of codons that end with a pyrimidine (U or C) (39, 40, 41). The GidA and TrmE proteins may regulate bacterial factors in some strains by posttranscriptionally modifying tRNAs, including tRNA^{Lys}, tRNA^{Glu}, tRNA^{Gln}, tRNA^{Leu}, and tRNA^{Arg} (36, 42). In this study, we present evidence that GidA and TrmE positively control 2,4-DAPG biosynthesis at the posttranscriptional level and that this control is independent of the Gac/Rsm pathway.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *P. fluorescens* strains were grown in Luria-Bertani (LB) (43) or King's B (KB) medium (44) at 28°C. *Escherichia coli* strains were routinely grown at 37°C in LB medium. *Rhizoctonia solani* was cultured in PDA medium (45). Where indicated, the media were supplemented with the antibiotics ampicillin (50 µg/ml), kanamycin (50 µg/ml), gentamicin (10 µg/ml), chloramphenicol (20 µg/ml), and tetracycline 20 (µg/ml) or with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (40 µg/ml).

DNA manipulation and sequence analyses. Standard techniques were used for plasmid and chromosomal DNA preparation, restriction enzyme digestion, agarose gel electrophoresis, purification of DNA fragments, ligation, and other molecular assays (43). Plasmid DNA was introduced into *E. coli* or *P. fluorescens* chemically or by electroporation (43). Nucleotide and deduced amino acid sequences were analyzed with the on-line BLAST search engine in GenBank (<http://www.ncbi.nlm.nih.gov/BLAST>) and DNAMAN software (version 5.2; Lynnon Biosoft).

Cloning of *gidA* and *trmE* genes. To screen for novel positive regulators of 2,4-DAPG production, *P. fluorescens* 2P24 was subjected to random Tn5 insertional mutagenesis using the pUT-Km plasmid (46). Two out of approximately 3,000 kanamycin-resistant colonies of the 2P24 strain showed decreased levels of antagonistic activity, which is linked to the production of 2,4-DAPG. The genomic DNA fragment that flanks the kanamycin resistance gene in transposon Tn5 was cloned into the pBlue-script II SK(+) (Stratagene) vector at the EcoRI site and sequenced with zhang-O and zhang-I primers (see Table S1 in the supplemental material) localized at the two ends of the Tn5 transposon. The Tn5-flanking sequences of one PM121 mutant were identified as part of a deduced *gidA* gene using BLAST. The upstream and downstream sequences of the entire *gidA* gene were then determined in the ongoing genomic sequence of strain 2P24 (see Fig. S1 in the supplemental material).

Construction of *P. fluorescens* 2P24 mutants and complementation. To construct the pBSKm suicide vector with kanamycin resistance, the gene that encodes ampicillin with the vector pBluescript II SK(+) (Stratagene) was replaced by a gene encoding kanamycin. An in-frame deletion of the *gidA* gene was made using a two-step homologous-recombination strategy. Briefly, an upstream 959-bp fragment and a downstream 836-bp fragment were PCR amplified from the genomic DNA of strain 2P24 using the primer pairs Gid-F1/Gid-R1 and Gid-F2/Gid-R2, respectively. The upstream fragment was digested with Sall plus BamHI and the downstream fragment with BamHI plus EcoRI, and the two fragments were cloned into the suicide vector pBSKm to generate pBSKΔgidA. The same approach was used to create in-frame deletion mutants of the *trmE*, *phlA*, and *phlD* genes (see Table S1 in the supplemental material), yielding the plasmids pBSKΔtrmE, pBSKΔphlA, and pBSKΔphlD, respectively. Allelic exchange using these plasmids and wild-type strain 2P24 resulted in the mutants PM701 (ΔgidA), PM702 (ΔtrmE), PM915 (ΔphlA), and PM920 (ΔphlD), respectively (47). The double mutants PM917 (ΔgidA ΔphlA), PM918 (ΔtrmE ΔphlA), and PM919 (ΔphlD ΔphlA) were generated by

using the plasmids pBSKΔgidA, pBSKΔtrmE and pBSKΔphlD to delete the *gidA*, *trmE*, and *phlD* genes, respectively, from the *phlA* mutant PM915 (ΔphlA). For complementation analysis, the complete *gidA* and *trmE* genes were PCR amplified and inserted into the shuttle vector pBBR1MCS-5 (48), yielding the complementation plasmids pBBR5-gidA and pBBR5-trmE, respectively. The resultant plasmids were introduced into their respective deletion mutants to generate complementation strains. For overexpression analysis, the complete *phlD* gene and the complete *phlD* gene tagged at its 3' end with a sequence encoding the vesicular stomatitis virus glycoprotein (VSV-G) epitope 5'-TATACAGATATTGA AATGAATAGATTAGGAAAA-3' (49) were PCR amplified and inserted into the vector pBBR1MCS-5, yielding the complementation plasmids pBBR5-phlD and pBBR5-phlDV, respectively.

Extraction and detection of 2,4-DAPG, MAPG, and PG. The production of 2,4-DAPG and MAPG by *P. fluorescens* 2P24 and its mutant derivatives was measured after growth for 48 h in KB medium, and the production of PG was measured in strains grown in LB medium supplemented with 2% glucose. To analyze the conversion of PG to MAPG and of MAPG to 2,4-DAPG, synthetic PG or MAPG dissolved in methanol was added to cultures grown for 24 h to yield a final concentration of 10 µg/ml, followed by culture for an additional 24 h. 2,4-DAPG and MAPG production was quantified by analytical high-performance liquid chromatography (HPLC) (50). For HPLC analysis, the sample was dissolved in 0.05 ml methanol and analyzed using a symmetric C₁₈ reverse-phase column (4.6 by 150 mm; Agilent TC-18). Fractions were separated isocratically by elution with 55:45 (vol/vol) acetonitrile-0.1% H₃PO₄ at a flow rate of 1 ml min⁻¹. 2,4-DAPG (1 µg/ml) was added to induce PG production when the growth of strains reached an optical density at 600 nm (OD₆₀₀) of 0.8. After incubation for another 36 h, PG was quantified according to the method of Kidarsa et al. (20).

Fungal-inhibition assay. Fungal-inhibition assays were performed on one-fifth-strength PDA, with *R. solani* as the target pathogen. A 0.6-cm circular plug from a 48-h culture of *R. solani* was placed in the center of the plate. Aliquots (5 µl) of suspensions of washed bacterial cells, prepared from exponential-growth-phase LB cultures and adjusted to an OD₆₀₀ of 0.8, were spotted onto the plates 2.5 cm from the edge of the fungal colony. The plates were incubated for 36 h at 28°C, and the growth situation of *R. solani* was determined.

β-Galactosidase assays. *P. fluorescens* 2P24 and its derivatives were grown in 100-ml Erlenmeyer flasks containing 20 ml LB at 28°C with shaking at 150 rpm. Aliquots were collected after certain time periods, and β-galactosidase activity was measured as described previously (44).

Western blot analysis. To construct a C-terminal VSV-G epitope-PhlA fused gene, a PCR-generated fragment with the VSV-G sequence inserted in frame at the 3' end of the *phlA* gene was cloned into pBSKm. The resulting plasmid, pBSKPhlA-VSV, was used to transform wild-type 2P24 and its *gidA* and *trmE* mutants to generate the PhlA-marked strains PM108, PM109, and PM110, respectively. The same technique was used to construct the PhlD-marked plasmid pBSKPhlD-VSV, the RsmA-marked plasmid pBSKRsmA-VSV, and the RsmE-marked plasmid pBSKRsmE-VSV. Homologous recombination using these plasmids in strain 2P24 and the *gidA* and *trmE* mutants produced strains PM111 to PM120 (Table 1).

Then, strains with VSV-G epitope sequence tags were grown in KB at 28°C with shaking. The bacterial cells were collected by centrifugation at 12,000 rpm, resuspended in 100 µl lysis buffer (100 mM Tris-HCl, pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 200 mM dithiothreitol [DTT]), and incubated at 100°C for 10 min. Proteins were separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were incubated with primary antibodies to VSV-G (1:2,000; Sigma) and 3-phosphoglycerate kinase (PGK) (1:5,000; Invitrogen), followed by incubation with anti-mouse horseradish peroxidase-conjugated secondary antibodies (1:5,000; Sigma). Antibody-tagged protein bands were detected using an ECL Western Blot detection kit (Cowin Biotech, Beijing, China).

TABLE 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Description ^a	Reference or source
<i>P. fluorescens</i>		
2P24	Wild type; Ap ^r	64
PM121	<i>gidA</i> ::Tn5 in strain 2P24; Ap ^r Km ^r	This study
PM701	<i>gidA</i> gene in-frame deletion in strain 2P24; Ap ^r	This study
PM702	<i>trmE</i> gene in-frame deletion in strain 2P24; Ap ^r	This study
PM703	<i>trmE</i> gene in-frame deletion in mutant PM701; Ap ^r	This study
PM915	<i>phlA</i> gene in-frame deletion in strain 2P24; Ap ^r	This study
PM917	<i>gidA</i> gene in-frame deletion in mutant PM915; Ap ^r	This study
PM918	<i>trmE</i> gene in-frame deletion in mutant PM915; Ap ^r	This study
PM919	<i>phlD</i> gene in-frame deletion in mutant PM915; Ap ^r	This study
PM920	<i>phlD</i> gene in-frame deletion in strain 2P24; Ap ^r	This study
PM108	2P24 with a VSV-G epitope sequence at the C terminus of PhlA; Ap ^r	This study
PM109	PM701 with a VSV-G epitope sequence at the C terminus of PhlA; Ap ^r	This study
PM110	PM702 with a VSV-G epitope sequence at the C terminus of PhlA; Ap ^r	This study
PM111	2P24 with a VSV-G epitope sequence at the C terminus of PhlD; Ap ^r	This study
PM112	PM701 with a VSV-G epitope sequence at the C terminus of PhlD; Ap ^r	This study
PM113	PM702 with a VSV-G epitope sequence at the C terminus of PhlD; Ap ^r	This study
PM114	PM703 with a VSV-G epitope sequence at the C terminus of PhlD; Ap ^r	This study
PM115	2P24 with a VSV-G epitope sequence at the C terminus of RsmA; Ap ^r	This study
PM116	PM701 with a VSV-G epitope sequence at the C terminus of RsmA; Ap ^r	This study
PM117	PM702 with a VSV-G epitope sequence at the C terminus of RsmA; Ap ^r	This study
PM118	2P24 with a VSV-G epitope sequence at the C terminus of RsmE; Ap ^r	This study
PM119	PM701 with a VSV-G epitope sequence at the C terminus of RsmE; Ap ^r	This study
PM120	PM702 with a VSV-G epitope sequence at the C terminus of RsmE; Ap ^r	This study
<i>E. coli</i>		
DH5 α	λ^- ϕ 80 <i>dlacZ</i> Δ M15 Δ (<i>lacZYA-argF</i>)U169 <i>recA1 endA1 hsdR17</i> (r _K ⁻ m _K ⁻) <i>supE44 thi-1 gyrA relA1</i>	65
Fungus		
<i>R. solani</i>	Plant pathogen that causes cotton damping off	Lab stock
Plasmids		
pUTKm	Delivery plasmid for Tn5; R6K replicon; Ap ^r Km ^r	47
pRK600	Helper plasmid for triparental mating; Cm ^r	66
pBluescript II SK(+)	Cloning vector; Ap ^r	Stratagene
pBSKm	Suicide plasmid for <i>Pseudomonas</i> spp., used for homologous recombination; derivative of pBluescript II SK(+); Km ^r	This study
pBSK Δ gidA	pBSKm carrying a deleted <i>gidA</i> gene; Km ^r	This study
pBSK Δ trmE	pBSKm carrying a deleted <i>trmE</i> gene; Km ^r	This study
pBSK Δ phlA	pBSKm carrying a deleted <i>phlA</i> gene; Km ^r	This study
pBSK Δ phlD	pBSKm carrying a deleted <i>phlD</i> gene; Km ^r	This study
pBBR1MCS-5	pBBR1MCS-5 containing pBluescript II KS- <i>lacZ</i> α ; Gm ^r	48
pBBR5- <i>gidA</i>	pBBR1MCS-5 containing <i>gidA</i> gene; Gm ^r	This study
pBBR5- <i>trmE</i>	pBBR1MCS-5 containing <i>trmE</i> gene; Gm ^r	This study
pBBR5- <i>phlD</i>	pBBR1MCS-5 containing <i>phlD</i> gene; Gm ^r	This study
pBBR5- <i>phlDV</i>	pBBR1MCS-5 containing <i>phlD</i> gene with a VSV-G epitope sequence at its C terminus; Gm ^r	This study
pRG970Gm	Cloning vector containing promoterless <i>lacZYA</i> for construction of transcriptional fusion; Gm ^r	32
pRG970Km	Cloning vector containing promoterless <i>lacZYA</i> for construction of transcriptional fusion; Km ^r	67
p970Km- <i>phlAp</i>	pRG970Km containing a <i>phlA-lacZ</i> transcriptional fusion; Km ^r	32
p970Km- <i>rsmXp</i>	pRG970Km containing an <i>rsmX-lacZ</i> transcriptional fusion; Km ^r	33
p970Gm- <i>rsmYp</i>	pRG970Gm containing an <i>rsmY-lacZ</i> transcriptional fusion; Gm ^r	33
p970Km- <i>rsmZp</i>	pRG970Km containing an <i>rsmZ-lacZ</i> transcriptional fusion; Km ^r	33
p970Km- <i>rsmAp</i>	pRG970Km containing an <i>rsmA-lacZ</i> transcriptional fusion; Km ^r	33
p970Gm- <i>rsmEp</i>	pRG970Gm containing an <i>rsmE-lacZ</i> transcriptional fusion; Gm ^r	33
pBSKPhlA-VSV	pBSKm with a VSV-G epitope sequence at the C terminus of PhlA; Km ^r	This study
pBSKPhlD-VSV	pBSKm with a VSV-G epitope sequence at the C terminus of PhlD; Km ^r	This study
pBSKRsmA-VSV	pBSKm with a VSV-G epitope sequence at the C terminus of RsmA; Km ^r	This study
pBSKRsmE-VSV	pBSKm with a VSV-G epitope sequence at the C terminus of RsmE; Km ^r	This study

^a Ap^r, Km^r, Gm^r, and Cm^r indicate resistance to ampicillin, kanamycin, gentamicin, and chloramphenicol, respectively.

Statistical analysis. Data were analyzed and compared with Fisher's least significant difference (LSD) test (a *P* value of <0.001 was considered significant) using SPSS 16.0 software (SPSS Corporation, Chicago, IL, USA).

Nucleotide sequence accession numbers. The sequences of the *gidA* and *trmE* genes from strain 2P24 have been deposited in the GenBank database under accession no. [KC866364](#).

RESULTS

Identification and characterization of *gidA* and *trmE* genes in *P. fluorescens* 2P24. In our previous study, random mutagenesis of *P. fluorescens* 2P24 using the transposon mini-Tn5 was performed to identify the potential regulators of 2,4-DAPG production (16). Similarly, in this study, the mutant PM121 (*gidA*::Tn5), which is defective in antagonistic activity to *R. solani* (Table 1), was selected and further investigated. The mini-Tn5-flanking region in PM121 was cloned, with sequence analysis showing that Tn5 had been inserted into the *gidA* gene (see Fig. S1 in the supplemental material). The deduced amino acid sequence (632 amino acids; 69 kDa) of the *gidA* gene product was highly similar to that of the tRNA uridine 5-carboxymethylaminomethyl modification protein, *GidA*, from *P. fluorescens* Pf0-1 (accession number [YP_351465](#); 97% identity), *Pseudomonas syringae* pv. *syringae* B728a ([YP_238197](#); 93% identity), *Pseudomonas putida* GB-1 ([YP_001671659](#); 91% identity), and *Pseudomonas aeruginosa* PAO1 ([NP_254252](#); 90% identity). Multiple-sequence alignment indicated that *GidA* of *P. fluorescens* 2P24 contains a highly conserved dinucleotide-binding motif at the N terminus (see Fig. S1 in the supplemental material), which is required for tRNA modification (36, 42). Five bases downstream of *gidA*, we identified a deduced *gidB* gene encoding a 16S rRNA methyltransferase and transcribed in the same direction (see Fig. S1 in the supplemental material). In *E. coli*, *GidB* is thought to function as an S-adenosyl-L-methionine (SAM)-dependent methyltransferase in cell division or chromosomal replication (51).

In addition to the *gidA* gene, the *trmE* (*mnme*) gene was recently found to be involved in the process of tRNA modification (37, 52, 53). The *trmE* gene of strain 2P24 was located upstream of the *gidA* gene and downstream of the *yidC* gene, which encodes a putative inner membrane protein translocase component (see Fig. S1 in the supplemental material). Sequence analysis showed that the deduced *TrmE* protein (456 amino acids; 49 kDa) exhibited high levels of sequence identity to its orthologs from many other *Pseudomonas* strains (see Table S2 in the supplemental material). In contrast to the *GidA* protein, the *TrmE* protein of *P. fluorescens* 2P24 contains conserved GTP-binding motifs, labeled G-1 to G-4 (see Fig. S1 in the supplemental material), which are important for GTPase activity.

Effects of *GidA* and *TrmE* on 2,4-DAPG production and antimicrobial activity. To confirm the ability of *GidA* and *TrmE* to regulate 2,4-DAPG production, the in-frame deletion mutants PM701 (Δ *gidA*) and PM702 (Δ *trmE*) were constructed in *P. fluorescens* 2P24, and their 2,4-DAPG production was assessed by HPLC. As expected, lack of either the *gidA* or the *trmE* gene significantly decreased the 2,4-DAPG level by about 20-fold compared with wild-type 2P24. The introduction of the complementation plasmid pBBR5-*gidA* into PM701, or of pBBR5-*trmE* into PM702, restored the production of 2,4-DAPG and reached levels that were higher than those of the wild type (Fig. 1A). Furthermore, the production of the precursors of 2,4-DAPG, MAPG and PG, was drastically reduced in PM701 and PM702 compared with the wild-type and complementation strains (Fig. 1B and C). Al-

FIG 1 Effects of *gidA* and *trmE* mutations on 2,4-DAPG, MAPG, and PG production and on antifungal activity toward *R. solani*. (A and B) HPLC analysis of 2,4-DAPG (A) and MAPG (B) production by strain 2P24 and its derivatives in KB medium. (C) PG production of strains in LB medium supplemented with 2% glucose using a colorimetric method (17). Each value is the average of three independent cultures grown for 2 days, with the error bars denoting standard deviations (SD) and the asterisks indicating significantly different results (*P* < 0.001). (D) Measurement of antifungal activity in one-fifth-strength PDA medium, with *R. solani* as the target pathogen.

though strain 2P24 displayed strong antifungal activity against *R. solani* in a dual-culture test, this activity was completely absent in PM701 and PM702 (Fig. 1D). These results indicate that *GidA* and *TrmE* act as positive regulatory elements in the synthesis of 2,4-DAPG, MAPG, and PG.

Regulation of 2,4-DAPG biosynthesis genes by *GidA* and *TrmE* at the posttranscriptional level. In the 2,4-DAPG biosyn-

FIG 2 Regulation of antibiotic-biosynthetic genes *phlA* and *phlD* by GidA and TrmE. (A) The transcriptional levels of the *phlA* gene in *P. fluorescens* 2P24 and its variants were measured using the β -galactosidase assay. Growth is indicated by the dashed lines. (B) Levels of PhlA-VSV and PhlD-VSV proteins were evaluated by Western blotting using an anti-VSV antibody. An antibody directed against 3-phosphoglycerate kinase (α -PGK) was used as a loading control. All experiments were performed in triplicate, with the means $\pm$ SD shown.

thesis pathway, PhlD is responsible for the production of PG, and PhlACB is required for the acetylation of PG to MAPG and of MAPG to 2,4-DAPG (13, 17, 19, 20, 21). To determine the mechanism of action by which GidA and TrmE regulate the expression of 2,4-DAPG biosynthesis genes, the expression of a construct of *lacZ* fused to the *phlA* gene was tested in cultures grown in LB medium. There were no evident differences in the levels of *phlA* gene transcription between the 2P24 strains and the two mutants (Fig. 2A), indicating that GidA and TrmE did not regulate the transcription of the *phlA* gene.

We also assayed the expression of the PhlA-VSV and PhlD-VSV proteins in the PM108 (2P24-*phlA*VSV), PM109 (Δ *gidA*-*phlA*VSV), PM110 (Δ *trmE*-*phlA*VSV), PM111 (2P24-*phlD*VSV), PM112 (Δ *gidA*-*phlD*VSV), and PM113 (Δ *trmE*-*phlD*VSV) strains by Western blotting using an anti-VSV antibody. We found that the expression levels of the PhlA-VSV and PhlD-VSV proteins were obviously lower in PM109, PM110, PM112, and PM113 than in PM108 and PM111 throughout cell growth (Fig. 2B). These findings confirmed that GidA and TrmE modulated the expression of the 2,4-DAPG biosynthesis genes *phlA* and *phlD* at the posttranscriptional level.

The effects of GidA and TrmE on 2,4-DAPG biosynthesis are independent of the Gac/Rsm pathway. The Gac/Rsm pathway in *P. fluorescens* is a conserved signal transduction pathway that tightly controls 2,4-DAPG expression at the posttranscriptional level (26, 27). The system contains two paralogous RNA-binding proteins, RsmA and RsmE, and three sequestering sRNAs, RsmX, RsmY, and RsmZ, all of which are present in the draft genome sequence of *P. fluorescens* 2P24 (33). To determine whether GidA and TrmE affect the Gac/Rsm pathway, the transcription of GacA-dependent sRNA genes and that of their binding proteins were assayed in the wild-type strain and the PM701 (Δ *gidA*) and PM702 (Δ *trmE*) mutants. There were no significant differences between the mutants and the wild type in the levels of *rsmX*, *rsmY*, and *rsmZ* mRNAs (Fig. 3A to C). In contrast, the levels of *rsmA-lacZ* and *rsmE-lacZ* transcripts were significantly lower in the two mutants than in the wild type (Fig. 3D and E). Western blotting results were consistent with those of transcriptional expression analysis, further confirming that GidA and TrmE positively control the expression of the *rsmA* and *rsmE* genes (Fig. 3F). Although RsmA and RsmE in *P. fluorescens* 2P24 have been identified as

translational repressors of 2,4-DAPG production (33), the reduced expression of RsmA and RsmE in the mutants did not increase 2,4-DAPG production (Fig. 1A), suggesting that GidA and TrmE posttranscriptionally affect 2,4-DAPG biosynthesis independently of the Gac/Rsm pathway.

The activity of PhlD is defective in PM701 (Δ *gidA*) and PM702 (Δ *trmE*) mutants. To understand the function of GidA and TrmE in the 2,4-DAPG-biosynthetic pathway, we first evaluated the activities of PhlACB in wild-type 2P24 and its mutants by chemical complementation. Neither 2,4-DAPG nor MAPG was detectable in the *phlA* mutant PM915 (Δ *phlA*), regardless of whether their respective substrates, MAPG and PG, were present. However, addition of their precursors significantly enhanced the production of 2,4-DAPG and MAPG in the PM701 and PM702 mutants (Fig. 4A and B), suggesting that the remaining PhlACB could convert PG to MAPG and MAPG to 2,4-DAPG. To avoid the conversion of PG to MAPG and 2,4-DAPG, PhlD activity was assayed in the *phlA* mutant PM915 (Δ *phlA*), and the synthetic 2,4-DAPG (1 μ g/ml) was added to stimulate *phl* autoinduction. A large amount of PG accumulated in the PM915 mutant, whereas no PG could be detected in the double mutants PM917 (Δ *gidA* Δ *phlA*) and PM918 (Δ *trmE* Δ *phlA*) (Fig. 4C). Since PhlD is responsible for PG production in *P. fluorescens* (17), as shown by the failure of the *phlD* mutant PM920 (Δ *phlD*) to produce PG (Fig. 4C), it is likely that PhlD function was absent from the PM701 and PM702 mutants. Taken together, these findings suggest that the activity of PhlD, rather than PhlACB, is defective in the PM701 and PM702 mutants.

Overexpression of PhlD restores the wild-type phenotypes in PM701 (Δ *gidA*) and PM702 (Δ *trmE*) mutants. To further explore the mechanism underlying the reduced production of 2,4-DAPG, MAPG, and PG in the PM701 and PM702 mutants, the plasmid pBBR1MCS-5, carrying *phlD* (pBBR5-*phlD*), was introduced into strain 2P24 and its variants. Overexpression of PhlD in the PM701, PM702, and PM703 (Δ *gidA* Δ *trmE*) double mutants restored the production of PG, MAPG, and 2,4-DAPG to the level observed in the wild-type strain containing pBBR5-*phlD* (Fig. 5A to C). However, the amounts of PhlD produced by the three mutants carrying pBBR5-*phlD* were smaller than that of the wild type carrying pBBR5-*phlD* (Fig. 5D), further indicating that the *gidA* and *trmE* genes affect the translation efficiency of the PhlD protein.

FIG 3 Regulation of the small noncoding RNA genes *rsmX*, *rsmY*, and *rsmZ* and their cognate regulator genes, *rsmA* and *rsmE*, by GidA and TrmE. (A to E) Transcriptional expression profiles of *rsmX* (A), *rsmY* (B), *rsmZ* (C), *rsmA* (D), and *rsmE* (E) were measured in *P. fluorescens* 2P24 and its *gidA* and *trmE* mutants. (F) Measurement of RsmA-VSV and RsmE-VSV proteins by Western blotting using an anti-VSV antibody. An anti-3-phosphoglycerate kinase α (α -PGK) antibody was used as a loading control. All experiments were performed in triplicate. The means $\pm$ SD are shown, and the asterisks indicate significantly different results ($P < 0.001$). Growth is indicated by the dashed lines.

DISCUSSION

This study provides genetic evidence demonstrating that, in the biocontrol agent *P. fluorescens* 2P24, the GidA and TrmE proteins regulate 2,4-DAPG biosynthesis at a posttranscriptional level, independently of the Gac/Rsm signal transduction pathway. Mutations in *gidA* have been found to cause morphological changes in some bacteria, including *E. coli* and *Salmonella enterica* serovar Typhimurium (54). These changes are likely due to a defect in chromosome segregation and cell division in the presence of glucose. We found that PM701 (Δ gidA) mutant cells were filamentous, whereas wild-type 2P24 had a normal rod shape in LB me-

dium supplemented with 0.2% glucose (see Fig. S2 in the supplemental material). Despite this difference, however, the growth of the *gidA* mutant was not markedly deficient. GidA has also been shown to regulate the expression of virulence genes in a wide range of bacteria, including *Aeromonas hydrophila*, *Myxococcus xanthus*, *P. syringae*, *S. enterica*, *Shigella flexneri*, and *Streptococcus pyogenes* (42, 55, 56, 57, 58). In *P. aeruginosa*, GidA selectively regulates the expression of the *rhl*-dependent quorum-sensing gene by modulating RhlR posttranscriptionally (59). In addition, TrmE has been reported to be involved in bacterial adaptation to cold temperatures (60), resistance to acidic pH

FIG 4 Effects of GidA and TrmE on PhlA and PhlD functions. (A) Production of 2,4-DAPG in the absence and presence of 10 $\mu\text{g/ml}$ MAPG. (B) Production of MAPG in the absence and presence of 10 $\mu\text{g/ml}$ PG. (C) Production of PG was determined in strain 2P24 and its derivatives. 2,4-DAPG was added to a final concentration of 1 $\mu\text{g/ml}$. All experiments were repeated three times. The means $\pm$ SD are shown, and the asterisks indicate significantly different results ($P < 0.001$).

(61), and oxidation of certain heterocyclic substrates, such as thiophene and furan (62). We found that, in addition to affecting the production of 2,4-DAPG, MAPG, and PG, mutations in the *gidA* and *trmE* genes affected many other phenotypic traits in *P. fluorescens* 2P24, including quorum-sensing gene expression, fluorescent-pigment production, and biofilm formation (data not shown). These findings indicated that GidA and TrmE are likely global regulators in a great variety of bacteria.

The *phlACBD* gene cluster has been shown to be essential for 2,4-DAPG biosynthesis, with PhlD catalyzing the iterative condensation of three malonyl-CoA units to form 3,5-diketoheptane-

FIG 5 Overexpression of PhlD restores the wild-type phenotypes in *gidA*, *trmE*, and their double mutants. (A to C) 2,4-DAPG (A), MAPG (B), and PG (C) production was detected in *P. fluorescens* 2P24 and its derivatives containing the plasmid pBBR1MCS-5 or pBBR5-phlD. All experiments were repeated three times. The means $\pm$ SD are shown, and the asterisks indicate significantly different results ($P < 0.001$). (D) Western blot analysis of the amount of PhlD-VSV present in the indicated strains using an anti-VSV antibody. The numbers 1 to 8 represent strains PM111(pBBR1MCS-5), PM111(pBBR5-phlDV), PM112(pBBR1MCS-5), PM112(pBBR5-phlDV), PM113(pBBR1MCS-5), PM113(pBBR5-phlDV), PM114(pBBR1MCS-5), and PM114(pBBR5-phlDV), respectively. The blots were incubated with anti-3-phosphoglycerate kinase α ($\alpha\text{-PGK}$) antibody as a loading control.

dioate (17). This polyketide product is then cyclized to form PG (19) and subsequently acetylated to MAPG and 2,4-DAPG by PhlACB (13, 19). Downregulation of the synthesis of PG, MAPG, and 2,4-DAPG as a result of *gidA* and *trmE* mutations may be associated with insufficient production levels of proteins encoded by the *phl* locus. This was supported by the results, which showed that the production of PhlA-VSV and PhlD-VSV proteins was

lower in the PM109 (Δ *gidA-phlAVSV*), PM110 (Δ *trmE-phlAVSV*), PM112 (Δ *gidA-phlDVS*), and PM113 (Δ *trmE-phlDVS*) mutants than in PM108 (2P24-*phlAVSV*) and PM111 (2P24-*phlDVS*). However, 2,4-DAPG and MAPG could be detected in the mutants after their substrates were added, suggesting that the residual PhlA remained functional. The severely impaired production of PG in the PM701 and PM702 mutants may have been due to the absence of PG substrates or defective PhlD biosynthesis. Overexpression of PhlD in these mutants resulted in the recovery of PG, MAPG, and 2,4-DAPG production, suggesting that the impaired 2,4-DAPG production in the PM701 and PM702 mutants was likely due to the presence of a defective PhlD. In addition, the expression of PhlD at 36 h was always lower than that recorded at 24 h, regardless of whether the *gidA* and *trmE* genes were mutated. This phenomenon could be explained by the fact that the production of the PhlD protein might be inhibited when the yield of PG reaches a threshold level, or the instability of PhlD (17) might make PhlD sensitive to an unknown substance when the secondary metabolite is largely synthesized by 36 h. However, the exact reason remains to be further investigated.

The *gidA* and *trmE* genes are conserved in both prokaryotes and eukaryotes (42). Their encoded products, the GidA and TrmE proteins, respectively, could regulate cell motility and growth in some strains by posttranscriptionally modifying tRNAs (36, 42). Although we compared the codon usage of PhlA and PhlD, we observed no significant differences in codon types and numbers (data not shown). These findings cannot exclude the possibility, however, that amino acid residues that depend on GidA and TrmE for their synthesis play different roles in the functions of the PhlA and PhlD proteins. Although mutations of the *gidA* and *trmE* genes in strain 2P24 obviously decreased PhlD expression without abolishing it, the residual PhlD was insufficient for the production of PG. Introduction of a plasmid overexpressing the *phlD* gene into these mutants also showed the effects of GidA and TrmE on PhlD. Although these bacteria produced PG, the level of PhlD expression was lower than in the wild type. As GidA and TrmE modify tRNAs, thus contributing to the fidelity of mRNA translation, the amount of correctly translated PhlD in mutant strains that contain the plasmid-borne *phlD* gene, but not the chromosome-borne gene, was sufficient to perform its biochemical function. To further measure the differences in PhlD expression between pBBR5-*phlD*-containing mutants and the wild type, the purified PhlD proteins from these strains were subjected to electrospray ionization-mass spectrometry (ESI-MS) and two-dimensional electrophoresis. However, there were no significant differences in the molecular masses and isoelectric points (pI) of these PhlD proteins (data not shown). Further research is required to evaluate the effects of GidA/TrmE on important amino acids in the catalytic center of PhlD.

The Gac/Rsm signal transduction pathway in *Pseudomonas* has been found to regulate the production of many secondary metabolites and exoenzymes at the posttranscriptional level (27, 63). In *P. syringae* B728a, GidA showed some phenotypic overlap with the GacS/GacA two-component system, but they appear to be separate regulons, although SalA, a downstream regulator within the *gac* regulon, is a member of both (61). Similarly, we found that the regulation of 2,4-DAPG by GidA and TrmE was independent of the Gac/Rsm signal transduction pathway, although the expression of the regulatory proteins RsmA and RsmE was markedly affected by GidA and TrmE mutations. Therefore, the roles of

RsmA/E in the GidA/TrmE regulatory system and the exact relationship between GidA/TrmE and the Gac/Rsm pathway remain to be determined.

In conclusion, the results of this study demonstrated that GidA and TrmE are potential regulators of 2,4-DAPG production in *P. fluorescens* 2P24 by the posttranscriptional regulation of genes involved in the antibiotic biosynthesis pathway but independently of the Gac/Rsm signal transduction pathway. Further research is needed to determine the precise molecular functions of GidA and TrmE relative to their target proteins in *P. fluorescens* 2P24.

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REFERENCES

- Couillerot O, Prigent-Combaret C, Caballero-Mellado J, Moënne-Loccoz Y. 2009. *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Lett. Appl. Microbiol.* 48:505–512. <http://dx.doi.org/10.1111/j.1472-765X.2009.02566.x>.
- Haas D, Défago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* 3:307–319. <http://dx.doi.org/10.1038/nrmicro1129>.
- Dwivedi D, Johri BN. 2003. Antifungals from fluorescent pseudomonads: biosynthesis and regulation. *Curr. Sci.* 85:1693–1703. <http://www.iisc.ernet.in/~currsci/dec252003/1693.pdf>.
- Haas D, Keel C. 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* 41:117–153. <http://dx.doi.org/10.1146/annurev.phyto.41.052002.095656>.
- Reddy TK, Khudiakov I, Borovkov PAV. 1968. *Pseudomonas fluorescens* strain 26-o—producer of phytotoxic substances. *Mikrobiologiya* 38:909–913.
- Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner P, Haas D, Défago G. 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Mol. Plant Microbe Interact.* 5:4–13. <http://dx.doi.org/10.1094/MPMI-5-004>.
- Cronin D, Moënne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O’Gara F. 1997. Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Appl. Environ. Microbiol.* 63:1357–1361.
- Maurhofer M, Keel C, Schnider U, Voisard C, Haas D, Defago G. 1992. Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHA0 on its disease suppressive capacity. *Phytopathology* 82:190–195. <http://dx.doi.org/10.1094/Phyto-82-190>.
- Phillips DA, Fox TC, King MD, Bhuvaneswari TV, Teuber LR. 2004. Microbial products trigger amino acid exudation from plant roots. *Plant Physiol.* 136:2887–2894. <http://dx.doi.org/10.1104/pp.104.044222>.
- Brazelton JN, Pfeufer EE, Sweat TA, Gardener BBM, Coenen C. 2008. 2,4-Diacetylphloroglucinol alters plant root development. *Mol. Plant Microbe Interact.* 21:1349–1358. <http://dx.doi.org/10.1094/MPMI-21-10-1349>.
- Bakker PAHM, Pieterse CMJ, Van Loon LC. 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* 97:239–243. <http://dx.doi.org/10.1094/PHYTO-97-2-0239>.
- Schnider-Keel U, Seematter A, Maurhofer M, Blumer C, Duffy B, Gigot-Bonnefoy C, Reimann C, Notz R, Defago G, Haas D. 2000. Autoinduction of 2,4-diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *J. Bacteriol.* 182:1215–1225. <http://dx.doi.org/10.1128/JB.182.5.1215-1225.2000>.
- Bangera MG, Thomashow LS. 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *J. Bacteriol.* 181:3155–3163.
- Delany I, Sheehan MM, Fenton A, Bardin S, Aarons S, O’Gara F. 2000.

- Regulation of production of the antifungal metabolite 2,4-diacetylphloroglucinol in *Pseudomonas fluorescens* F113: genetic analysis of *phlF* as a transcriptional repressor. *Microbiology* 146:537–546. <http://mic.sgmjournals.org/content/146/2/537.short>.
15. Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GSA, Mavrodi DV, DeBoy RT, Seshadri R, Ren Q, Madupu R, Dodson RJ, Durkin AS, Brinkac LM, Daugherty SC, Sullivan SA, Rosovitz MJ, Gwinn ML, Zhou L, Schneider DJ, Cartinhour SW, Nelson WC, Weidman J, Watkins K, Tran K, Khouri H, Pierson EA, Pierson LS, III, Thomashow LS, Loper JE. 2005. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat. Biotechnol.* 23:873–878. <http://dx.doi.org/10.1038/nbt1110>.
 16. Tian T, Wu XG, Duan HM, Zhang LQ. 2010. The resistance-nodulation-division efflux pump EmhABC influences the production of 2,4-diacetylphloroglucinol in *Pseudomonas fluorescens* 2P24. *Microbiology* 156:39–48. <http://dx.doi.org/10.1099/mic.0.031161-0>.
 17. Zha W, Rubin-Pitel SB, Zhao H. 2006. Characterization of the substrate specificity of PhlD, a type III polyketide synthase from *Pseudomonas fluorescens*. *J. Biol. Chem.* 281:32036–32047. <http://dx.doi.org/10.1074/jbc.M606500200>.
 18. Ramette A, Moënne-Loccoz Y, Défago G. 2001. Polymorphism of the polyketide synthase gene *phlD* in biocontrol fluorescent pseudomonads producing 2,4-diacetylphloroglucinol and comparison of PhlD with plant polyketide synthases. *Mol. Plant Microbe Interact.* 14:639–652. <http://dx.doi.org/10.1094/MPMI.2001.14.5.639>.
 19. Achkar J, Xian M, Zhao H, Frost JW. 2005. Biosynthesis of phloroglucinol. *J. Am. Chem. Soc.* 127:5332–5333. <http://dx.doi.org/10.1021/ja042340g>.
 20. Kidarsa TA, Goebel NC, Zabriskie TM, Loper JE. 2011. Phloroglucinol mediates cross-talk between the pyoluteorin and 2,4-diacetylphloroglucinol biosynthetic pathways in *Pseudomonas fluorescens* Pf-5. *Mol. Microbiol.* 81:395–414. <http://dx.doi.org/10.1111/j.1365-2958.2011.07697.x>.
 21. Yang F, Cao Y. 2012. Biosynthesis of phloroglucinol compounds in microorganisms—review. *Appl. Microbiol. Biotechnol.* 93:487–495. <http://dx.doi.org/10.1007/s00253-011-3712-6>.
 22. Abbas A, McGuire JE, Crowley D, Baysse C, Dow M, O’Gara F. 2004. The putative permease PhlE of *Pseudomonas fluorescens* F113 has a role in 2,4-diacetylphloroglucinol resistance and in general stress tolerance. *Microbiology* 150:2443–2450. <http://dx.doi.org/10.1099/mic.0.27033-0>.
 23. Abbas A, Morrissey JP, Marquez PC, Sheehan MM, Delany IR, O’Gara F. 2002. Characterization of interactions between the transcriptional repressor PhlF and its binding site at the *phlA* promoter in *Pseudomonas fluorescens* F113. *J. Bacteriol.* 184:3008–3016. <http://dx.doi.org/10.1128/JB.184.11.3008-3016.2002>.
 24. He YX, Huang L, Xue Y, Fei X, Teng YB, Rubin-Pitel SB, Zhao H, Zhou CZ. 2010. Crystal structure and computational analyses provide insights into the catalytic mechanism of 2,4-diacetylphloroglucinol hydrolase PhlG from *Pseudomonas fluorescens*. *J. Biol. Chem.* 285:4603–4611. <http://dx.doi.org/10.1074/jbc.M109.044180>.
 25. Bottiglieri M, Keel C. 2006. Characterization of PhlG, a hydrolase that specifically degrades the antifungal compound 2,4-diacetylphloroglucinol in the biocontrol agent *Pseudomonas fluorescens* CHA0. *Appl. Environ. Microbiol.* 72:418–427. <http://dx.doi.org/10.1128/AEM.72.1.418-427.2006>.
 26. Sonleitner E, Haas D. 2011. Small RNAs as regulators of primary and secondary metabolism in *Pseudomonas* species. *Appl. Microbiol. Biotechnol.* 91:63–79. <http://dx.doi.org/10.1007/s00253-011-3332-1>.
 27. Reimmann C, Valverde C, Kay E, Haas D. 2005. Posttranscriptional repression of GacS/GacA-controlled genes by the RNA-binding protein RsmE acting together with RsmA in the biocontrol strain *Pseudomonas fluorescens* CHA0. *J. Bacteriol.* 187:276–285. <http://dx.doi.org/10.1128/JB.187.1.276-285.2005>.
 28. Péchy-Tarr M, Bottiglieri M, Mathys S, Lejbolle KB, Schnider-Keel U, Maurhofer M, Keel C. 2005. RpoN (σ^{54}) controls production of antifungal compounds and biocontrol activity in *Pseudomonas fluorescens* CHA0. *Mol. Plant Microbe Interact.* 18:260–272. <http://dx.doi.org/10.1094/MPMI.18-0260>.
 29. Heeb S, Valverde C, Gigot Bonnefoy C, Haas D. 2005. Role of the stress sigma factor RpoS in GacA/RsmA-controlled secondary metabolism and resistance to oxidative stress in *Pseudomonas fluorescens* CHA0. *FEMS Microbiol. Lett.* 243:251–258. <http://dx.doi.org/10.1016/j.femsle.2004.12.008>.
 30. Baehler E, de Werra P, Wick LY, Péchy-Tarr M, Mathys S, Maurhofer M, Keel C. 2006. Two novel MvaT-like global regulators control exoproduct formation and biocontrol activity in root-associated *Pseudomonas fluorescens* CHA0. *Mol. Plant Microbe Interact.* 19:313–329. <http://dx.doi.org/10.1094/MPMI-19-0313>.
 31. Mavrodi OV, Mavrodi DV, Park AA, Weller DM, Thomashow LS. 2006. The role of *dsbA* in colonization of the wheat rhizosphere by *Pseudomonas fluorescens* Q8r1-96. *Microbiology* 152:863–872. <http://dx.doi.org/10.1099/mic.0.28545-0>.
 32. Wu XG, Duan HM, Tian T, Yao N, Zhou HY, Zhang LQ. 2010. Effect of the *hfq* gene on 2,4-diacetylphloroglucinol production and the PcoI/PcoR quorum-sensing system in *Pseudomonas fluorescens* 2P24. *FEMS Microbiol. Lett.* 309:16–24. <http://dx.doi.org/10.1111/j.1574-6968.2010.02009.x>.
 33. Wu X, Liu J, Zhang W, Zhang L. 2012. Multiple-level regulation of 2,4-diacetylphloroglucinol production by the sigma regulator PsaA in *Pseudomonas fluorescens* 2P24. *PLoS One* 7:e50149. <http://dx.doi.org/10.1371/journal.pone.0050149>.
 34. Duffy BK, Défago G. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.* 65:2429–2438.
 35. Notz R, Maurhofer M, Schnider-Keel U, Duffy B, Haas D, Défago G. 2001. Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology* 91:873–881. <http://dx.doi.org/10.1094/PHYTO.2001.91.9.873>.
 36. Yim L, Moukadiri I, Björk GR, Armengod ME. 2006. Further insights into the tRNA modification process controlled by proteins MnmE and GidA of *Escherichia coli*. *Nucleic Acids Res.* 34:5892–5905. <http://dx.doi.org/10.1093/nar/gkl752>.
 37. Yim L, Martínez-Vicente M, Villarroja M, Aguado C, Knecht E, Armengod M. 2003. The GTPase activity and C-terminal cysteine of the *Escherichia coli* MnmE protein are essential for its tRNA modifying function. *J. Biol. Chem.* 278:28378–28387. <http://dx.doi.org/10.1074/jbc.M301381200>.
 38. Moukadiri I, Prado S, Píera J, Velázquez-Campoy A, Björk GR, Armengod ME. 2009. Evolutionarily conserved proteins MnmE and GidA catalyze the formation of two methyluridine derivatives at tRNA wobble positions. *Nucleic Acids Res.* 37:7177–7193. <http://dx.doi.org/10.1093/nar/gkp762>.
 39. Shi R, Villarroja M, Ruiz-Partida R, Li Y, Proteau A, Prado S, Moukadiri I, Benítez-Páez A, Lomas R, Wagner J, Matte A, Velázquez-Campoy A, Armengod ME, Cygler M. 2009. Structure-function analysis of *Escherichia coli* MnmG (GidA), a highly conserved tRNA-modifying enzyme. *J. Bacteriol.* 191:7614–7619. <http://dx.doi.org/10.1128/JB.00650-09>.
 40. Meyer S, Scrima A, Versées W, Wittinghofer A. 2008. Crystal structures of the conserved tRNA-modifying enzyme GidA: implications for its interaction with MnmE and substrate. *J. Mol. Biol.* 380:532–547. <http://dx.doi.org/10.1016/j.jmb.2008.04.072>.
 41. Meyer S, Wittinghofer A, Versées W. 2009. G-domain dimerization orchestrates the tRNA wobble modification reaction in the MnmE/GidA complex. *J. Mol. Biol.* 392:910–922. <http://dx.doi.org/10.1016/j.jmb.2009.07.004>.
 42. White DJ, Merod R, Thomasson B, Hartzell PL. 2001. GidA is an FAD-binding protein involved in development of *Myxococcus xanthus*. *Mol. Microbiol.* 42:503–517. <http://dx.doi.org/10.1046/j.1365-2958.2001.02659.x>.
 43. Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
 44. King EO, Ward MK, Raney DE. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301–307.
 45. Sinclair JB, Dhingra OD. 1995. Basic plant pathology methods, 2nd ed. CRC Press, Boca Raton, FL.
 46. Herrero M, de Lorenzo V, Timmis KN. 1990. Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. *J. Bacteriol.* 172:6557–6567.
 47. Mei GY, Yan XX, Turak A, Luo ZQ, Zhang LQ. 2010. AidH, an alpha/beta-hydrolase fold family member from an *Ochrobactrum* sp. strain, is a novel N-acylhomoserine lactonase. *Appl. Environ. Microbiol.* 76:4933–4942. <http://dx.doi.org/10.1128/AEM.00477-10>.
 48. Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, II, Peterson KM. 1995. Four new derivatives of the broad-host-range cloning

- vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 166:175–176. [http://dx.doi.org/10.1016/0378-1119\(95\)00584-1](http://dx.doi.org/10.1016/0378-1119(95)00584-1).
49. Goodman AL, Merighi M, Hyodo M, Ventre I, Filloux A, Lory S. 2009. Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. *Genes Dev.* 23:249–259. <http://dx.doi.org/10.1101/gad.1739009>.
 50. Ahmadzadeh M, Sharifi Tehrani A. 2009. Evaluation of fluorescent pseudomonads for plant growth promotion, antifungal activity against *Rhizoctonia solani* on common bean, and biocontrol potential. *Biol. Control* 48:101–107. <http://dx.doi.org/10.1016/j.biocontrol.2008.10.012>.
 51. Romanowski MJ, Bonanno JB, Burley SK. 2002. Crystal structure of the *Escherichia coli* glucose-inhibited division protein B (GidB) reveals a methyltransferase fold. *Proteins* 47:563–567. <http://dx.doi.org/10.1002/prot.10121>.
 52. Scrima A, Vetter IR, Armengod ME, Wittinghofer A. 2005. The structure of the TrmE GTP-binding protein and its implications for tRNA modification. *EMBO J.* 24:23–33. <http://dx.doi.org/10.1038/sj.emboj.7600507>.
 53. Cabedo H, Macián F, Villarroja M, Escudero JC, Martínez-Vicente M, Knecht E, Armengod ME. 1999. The *Escherichia coli* *trmE* (*mmE*) gene, involved in tRNA modification, codes for an evolutionarily conserved GTPase with unusual biochemical properties. *EMBO J.* 18:7063–7076. <http://dx.doi.org/10.1093/emboj/18.24.7063>.
 54. von Meyenburg K, Jørgensen BB, Nielsen J, Hansen FG. 1982. Promoters of the *atp* operon coding for the membrane-bound ATP synthase of *Escherichia coli* mapped by Tn10 insertion mutations. *Mol. Gen. Genet.* 188:240–248. <http://dx.doi.org/10.1007/BF00332682>.
 55. Sha J, Kozlova EV, Fadl AA, Olano JP, Houston CW, Peterson JW, Chopra AK. 2004. Molecular characterization of a glucose-inhibited division gene, *gidA*, that regulates cytotoxic enterotoxin of *Aeromonas hydrophila*. *Infect. Immun.* 72:1084–1095. <http://dx.doi.org/10.1128/IAI.72.2.1084-1095.2004>.
 56. Kinscherf TG, Willis DK. 2002. Global regulation by *gidA* in *Pseudomonas syringae*. *J. Bacteriol.* 184:2281–2286. <http://dx.doi.org/10.1128/JB.184.8.2281-2286.2002>.
 57. Cho KH, Caparon MG. 2008. tRNA modification by GidA/MnmE is necessary for *Streptococcus pyogenes* virulence: a new strategy to make live attenuated strains. *Infect. Immun.* 76:3176–3186. <http://dx.doi.org/10.1128/IAI.01721-07>.
 58. Shippy DC, Eakley NM, Bochsler PN, Chopra AK, Fadl AA. 2011. Biological and virulence characteristics of *Salmonella enterica* serovar Typhimurium following deletion of glucose-inhibited division (*gidA*) gene. *Microb. Pathog.* 50:303–313. <http://dx.doi.org/10.1016/j.micpath.2011.02.004>.
 59. Gupta R, Gobble TR, Schuster M. 2009. GidA posttranscriptionally regulates *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 191:5785–5792. <http://dx.doi.org/10.1128/JB.00335-09>.
 60. Singh AK, Pindi PK, Dube S, Sundareswaran VR, Shivaji S. 2009. Importance of *trmE* for growth of the psychrophile *Pseudomonas syringae* at low temperatures. *Appl. Environ. Microbiol.* 75:4419–4426. <http://dx.doi.org/10.1128/AEM.01523-08>.
 61. Gong S, Ma Z, Foster JW. 2004. The Era-like GTPase TrmE conditionally activates *gadE* and glutamate-dependent acid resistance in *Escherichia coli*. *Mol. Microbiol.* 54:948–961. <http://dx.doi.org/10.1111/j.1365-2958.2004.04312.x>.
 62. Alam KY, Clark DP. 1991. Molecular cloning and sequence of the *thdF* gene, which is involved in thiophene and furan oxidation by *Escherichia coli*. *J. Bacteriol.* 173:6018–6024.
 63. Humair B, Wackwitz B, Haas D. 2010. GacA-controlled activation of promoters for small RNA genes in *Pseudomonas fluorescens*. *Appl. Environ. Microbiol.* 76:1497–1506. <http://dx.doi.org/10.1128/AEM.02014-09>.
 64. Wei HL, Zhang LQ. 2006. Quorum-sensing system influences root colonization and biological control ability in *Pseudomonas fluorescens* 2P24. *Antonie Van Leeuwenhoek* 89:267–280. <http://dx.doi.org/10.1007/s10482-005-9028-8>.
 65. Hanahan D. 1983. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166:557–580. [http://dx.doi.org/10.1016/S0022-2836\(83\)80284-8](http://dx.doi.org/10.1016/S0022-2836(83)80284-8).
 66. Finan TM, Kunkel B, De Vos GF, Signer ER. 1986. Second symbiotic megaplasmid in *Rhizobium meliloti* carrying exopolysaccharide and thiamine synthesis genes. *J. Bacteriol.* 167:66–72.
 67. Yan Q, Gao W, Wu XG, Zhang LQ. 2009. Regulation of the PcoI/PcoR quorum-sensing system in *Pseudomonas fluorescens* 2P24 by the PhoP/PhoQ two-component system. *Microbiology* 155:124–133. <http://dx.doi.org/10.1099/mic.0.020750-0>.