APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2002, p. 4122–4126 Vol. 68, No. 8
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.8.4122–4126.2002

Different Responses of Pyoverdine Genes to Autoinduction in *Pseudomonas aeruginosa* and the Group *Pseudomonas fluorescens-Pseudomonas putida*

Cecilia Ambrosi,[1,2] Livia Leoni,[1] and Paolo Visca[1,2]*

Unità di Microbiologia Molecolare, I.R.C.C.S. "Lazzaro Spallanzani," 00149 Rome,[1] and Dipartimento di Biologia, Università di Roma Tre, 00146 Rome,[2] Italy

Received 21 February 2002/Accepted 8 May 2002

We investigated the regulation of the *psbA* and *pvdA* pyoverdine biosynthesis genes, which encode the L-ornithine N^5-oxygenase homologues in *Pseudomonas* strain B10 and *Pseudomonas aeruginosa* PAO1, respectively. We demonstrate that pyoverdine$_{B10}$, as the end product of its biosynthetic pathway, is a key participant of the control circuit regulating its own production in *Pseudomonas* strain B10. In *P. aeruginosa* PAO1, however, pyoverdine$_{PAO1}$ has no apparent role in the positive regulation of the *pvdA* gene.

The competition for iron is a main determinant for the dynamics of microbial populations in natural ecosystems. Aerobic bacteria living in neutral environments are normally faced with the nutritional iron deficit resulting from the low solubility of iron in its oxidized state (13). Likewise, bacterial parasites, which multiply within higher organisms, must counteract the iron deficiency imposed by the activity of the host's iron binding proteins (15). Iron withholding also affects the interactions between microbial populations in the rhizosphere (14).

Pyoverdines (also referred to as pseudobactins), the fluorescent siderophores produced by the rRNA group I species of genus *Pseudomonas,* constitute a large family of iron chelators that differ in terms of the length and composition of a hydroxamate-containing oligopeptide joined to a structurally conserved dihydroxyquinoline chromophore (1). Due to their high affinity for ferric iron, pyoverdines can suppress the growth of deleterious rhizomicroorganisms, which lack uptake specificities for ferripyoverdine complexes, thereby contributing to the plant defense from parasitic infection (2, 14).

In *Pseudomonas* spp. and many other bacteria, regulation of iron transport is primarily controlled by the Fur repressor protein (22). When the level of intracellular iron is high, transcription of iron-repressible genes is blocked through the binding of Fur-Fe(II) complexes to a highly conserved sequence (the Fur box) located within the promoters of these genes (5). However, Fur is not the only regulator of iron uptake in fluorescent pseudomonads (24). In *Pseudomonas aeruginosa*, the rRNA group I type species, expression of pyoverdine$_{PAO1}$ (*pvd*) genes is controlled by iron through a cascade of negative and positive regulatory proteins (Fig. 1). Briefly, Fur acts as a repressor of the *pvdS* gene, encoding an extra cytoplasmic function (ECF) sigma factor, called PvdS, which is required for transcription initiation at the promoters of *pvd* genes (4, 9). A DNA sequence element, the iron starvation box (ISB), has been identified in PvdS-dependent promoters and has been shown to participate in sequence-specific promoter recognition by PvdS, acting as a −35-like sequence element (27). Several Fur-controlled alternative sigma factors, structurally related to PvdS, have been identified in fluorescent *Pseudomonas* spp. and have been found to be involved in pyoverdine synthesis and/or uptake (7, 20, 23). An additional level of complexity of the siderophore regulatory pathway in these species is inferred by the positive effect of ferric siderophore complexes on the expression of cognate biosynthesis genes. Siderophore-regulated responses have previously been demonstrated for the pyochelin-salicylate operons of *P. aeruginosa* and for the pyoverdine gene systems of *Pseudomonas putida* WCS358 and *Pseudomonas fluorescens* M114 (3, 16, 23, 24).

The pyoverdine$_{B10}$ biosynthesis gene *psbA*, encoding the enzyme L-ornithine (L-Orn) N^5-oxygenase (PsbA) in the plant growth-promoting *Pseudomonas* strain B10, has previously been characterized in our laboratory (2). PsbA expression is regulated at the transcriptional level by a Fur-controlled ECF sigma factor, designated PsbS (8). Herein, we provide evidence that pyoverdine$_{B10}$ itself plays a crucial role in the expression of the *psbA* gene. The comparison of autogenous regulation of pyoverdine biosynthesis in *Pseudomonas* strain B10 and *P. aeruginosa* PAO1 highlighted relevant differences between the two species.

The bacterial strains and plasmids used in this study are listed in Table 1. The media and growth conditions have been described previously (6, 9, 25).

Characterization of the *psbA* promoter in *Pseudomonas* strain B10. To define the structure of the *psbA* promoter (P*psbA*), the initiation sites of the *psbA* transcript were determined by primer extension analysis. The primer PE*psbA*RV (5′-GGGCGATGCAATTGCCGTTGTCAT-3′) was end labeled with [γ-^{32}P]ATP and used for reverse transcription of total RNA from iron-deprived *Pseudomonas* strain B10 cells as described elsewhere (9). The unlabeled primer was used to sequence the DNA region upstream from the *psbA* gene from plasmid pCAΔSh with a T7 sequencing kit (Pharmacia) and [α-^{32}P]dATP. The 5′ ends of the *psbA* mRNA were mapped at four distinct sites (Fig. 2). The most abundant transcripts, T3 and T4, originate at positions −44 and −48 relative to the *psbA*

* Corresponding author. Mailing address: Dipartimento di Biologia, Università di Roma Tre, Viale G. Marconi 446, 00146 Rome, Italy. Phone: 39.6.5517.6347. Fax: 39.6.5517.6321. E-mail: visca@bio.uniroma3.it.

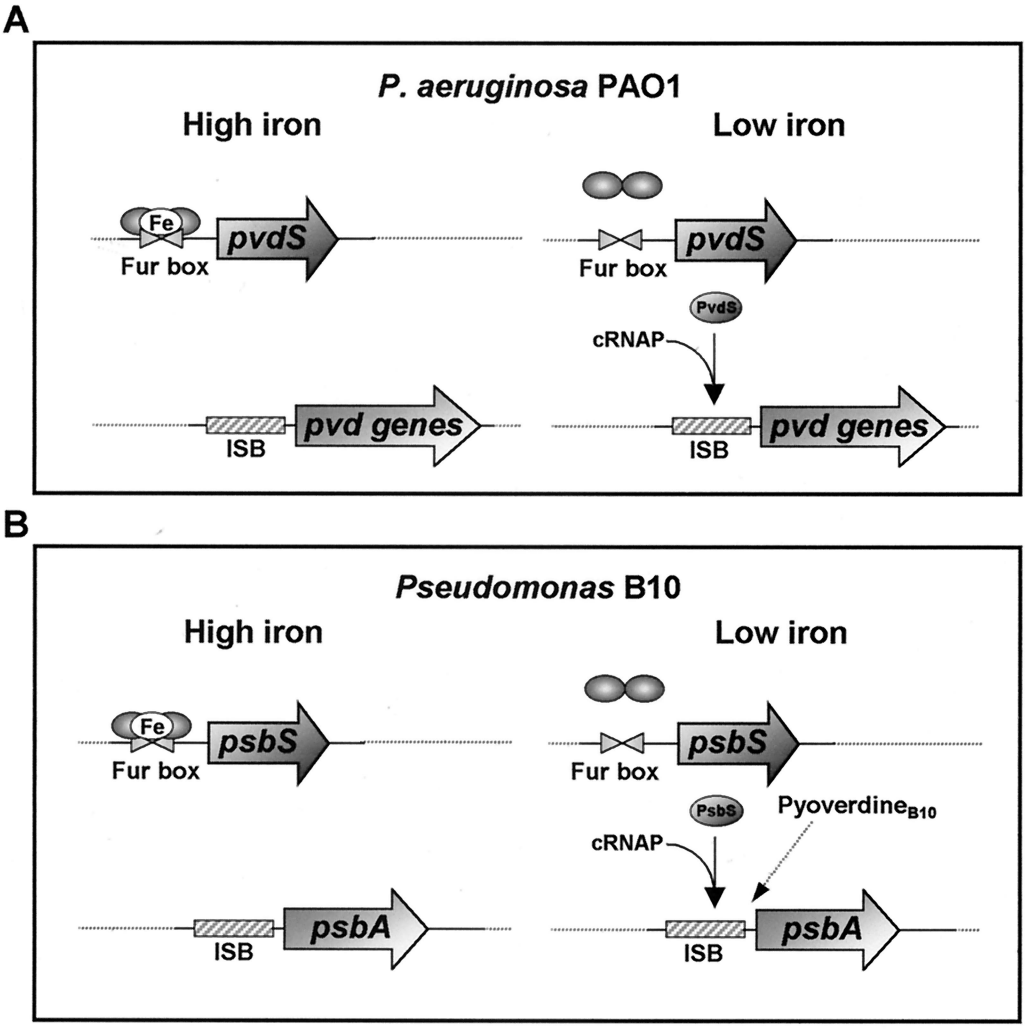

FIG. 1. Regulatory models of pyoverdine genes in *P. aeruginosa* PAO1 and in *Pseudomonas* strain B10. (A) In iron-replete *P. aeruginosa* PAO1 cells, transcription of *pvdS* is prevented by the binding of dimeric Fur-Fe(II) complexes to Fur boxes overlapping the *pvdS* promoter. In the absence of sufficient iron, release of Fur from DNA allows *pvdS* to be transcribed. The ECF sigma factor PvdS mediates recognition of the ISB, thereby conferring specificity to core RNA polymerase (cRNAP) for transcription initiation at the *pvd* promoters. (B) In *Pseudomonas* strain B10, expression of the *psbA* gene is similarly regulated by the Fur-controlled ECF sigma factor PsbS. An additional level of regulation is provided by exogenous pyoverdine$_{B10}$, which exerts a positive induction effect on expression of the *psbA* biosynthetic gene.

start codon, respectively, while two minor transcripts, T1 and T2, originate at positions −87 and −89, respectively. No primer extension product was detected with total RNA from bacteria grown in iron-rich medium (data not shown), consistent with the observation that *psbA* is not transcribed in iron-rich cells (2). The initiation pattern of *psbA* mRNA is consistent with the presence of multiple transcription start sites in the homologous *pvdA* promoter (P*pvdA*) and in *P. aeruginosa* iron-regulated promoters directly or indirectly controlled by the PvdS sigma factor, including *pvdD, regAB*, and *toxA* (9, 17, 22). As shown in Fig. 2, the 158-bp DNA sequence region preceding the *psbA* start codon is 68% identical to the corresponding P*pvdA* region (9). Both sequences contain regulatory motifs shared by ECF-dependent promoters, namely the ISB and the TCCTA element, which is also present in the corresponding region of *algD* and *algU* promoters of *P. aeruginosa* (18). It was also noticed that the *psbA* T2 and T4 transcripts correspond to the *pvdA* T1 and T2 transcripts, respectively. Interestingly, in *Pseudomonas* strain B10, the most abundant *psbA* transcript was T4, while, under the same experimental conditions, the major *pvdA* transcript was T1 (9). The alignment highlights the presence of a 14-nucleotide (nt) deletion and an 18-nt insertion at positions −137 and −49 upstream of the *psbA* start codon, respectively. Due to the presence of the 18-nt insertion in P*psbA*, the T4 start point is located at a longer distance from the ISB compared with the T2 start point of *pvdA* (Fig. 2). This insertion is endowed with remarkable features, consisting of two heptameric direct repeats (TCAGGCC), followed by a third partially conserved repeat (cCAGGCt).

Activity of *psbA* and *pvdA* promoters in *Pseudomonas* strain B10 and *P. aeruginosa*. The structural similarity between the P*psbA* and P*pvdA*, combined with previous evidence that PsbS and PvdS are functionally interchangeable (8), led us to investigate promoter activity in *Pseudomonas* strain B10 and *P. ae-*

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype and/or relevant characteristics	Source or reference
Strains		
Pseudomonas B10	Prototroph	12
B10CA1	*psbA*::*cat* site-specific insertion mutant	2
P. aeruginosa		
PAO1 (ATCC 15692)	Prototroph	American Type Culture Collection
PALS124	*pvdA*	26
PALS106	*pvdC1*	26
PALS125	*pvdC3*	26
Plasmids		
pMP220	Broad-host-range, low-copy-number promoter-probe vector, IncP replicon, *lacZ* Tc^r Tra^-	21
pMP220::P*pvdA*	*pvdA* promoter directionally cloned in pMP220 (formerly designated pPV51)	9
pMP220::P*psbA*	*psbA* promoter from pMP190::P*psbA* directionally cloned in pMP220	This study
pMP190	Broad-host-range, low-copy-number promoter-probe vector, IncQ replicon, *lacZ* Cm^r Tra^-	21
pMP190::P*psbA*	*psbA* promoter directionally cloned in pMP190	8
pMP190::P*psbS*	470-bp PCR-generated fragment encompassing the *psbS* promoter directionally ligated to the *Kpn*I-*Sal*I sites of pMP190	8
pMP220::P*psbS*	DNA fragment of ca. 530 bp excised from pMP190::P*psbS* by *Hin*dIII digestion and directionally ligated to the *Hin*dIII site of pMP220	This study
pMP220::P*pvdS*	587-bp PCR-generated fragment encompassing the *pvdS* promoter directionally ligated to the *Bgl*II-*Eco*RI sites of pMP220	This study
pUCP18	*Escherichia coli-Pseudomonas* shuttle vector derived from pUC18, pMB1, replicon pRO1600 *lacZ*α *bla* Mob^+ Tra^- Ap^r Cb^r	19
pCAΔSh	pUCP18-derivative containing a 2.7-kb *Pst*I-*Sph*I fragment encompassing the entire *psbA* gene	2

ruginosa PAO1. For this purpose, the β-galactosidase levels expressed by pMP220::P*psbA* and pMP220::P*pvdA* fusions were measured in *P. aeruginosa* PAO1 and *Pseudomonas* strain B10 cells grown under low- and high-iron conditions. Both promoters showed similar activities in iron-poor cultures of *P. aeruginosa* PAO1 (Table 2). Comparable levels of β-galactosidase (ca. 10,000 U) were also expressed by iron-limited cultures of *Pseudomonas* strain B10 carrying the homologous

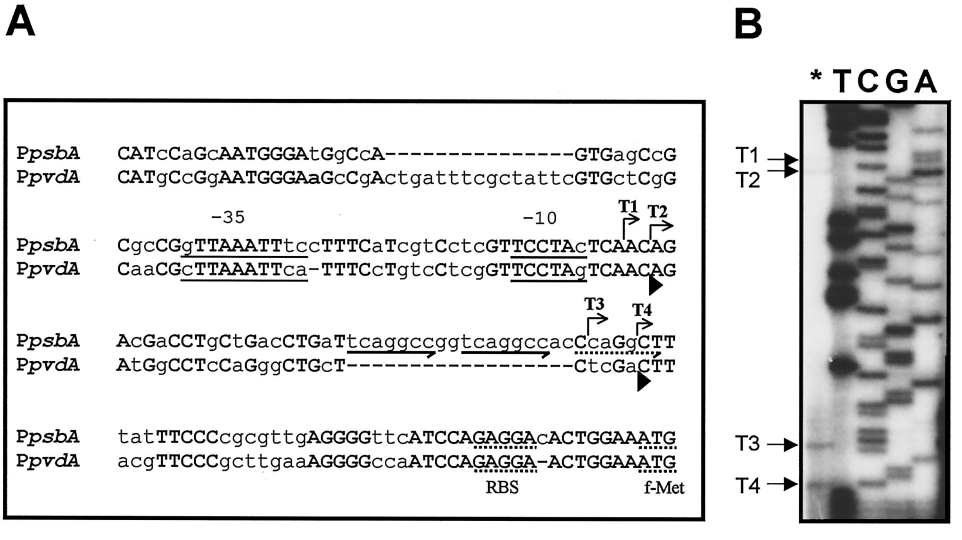

FIG. 2. Structural analysis of the *psbA* promoter. (A) Alignment of the 158-nt region preceding the *Pseudomonas* strain B10 *psbA* start codon with the *P. aeruginosa pvdA* promoter. Identical nucleotides are capitalized and in boldface. The bent arrows indicate the minor (T1 and T2) and major (T3 and T4) transcription start sites of the *psbA* gene and the direction of transcription. The ISB and the TCCTA conserved motifs, which overlap the −35 and −10 regions, respectively, are underlined. The potential ribosome binding sites (RBS) and the transcriptional start codons (f-Met) of *psbA* and *pvdA* are underlined with dots. The locations of the conserved (solid arrows) and partially conserved (dotted arrow) direct repeats are indicated below the *psbA* sequence. Arrowheads below the *pvdA* sequence mark the positions of the 5′ ends of the *pvdA* T1 and T2 transcripts (9). (B) Localization of the transcriptional start sites of *psbA*. The asterisk indicates primer extension analysis of the *psbA* mRNA carried out with the 5′-end-labeled oligonucleotide PE*psbA*RV. Lanes T, C, G, and A represent sequencing ladders of pCAΔSh with the same oligonucleotide. These reactions were run in parallel with primer extension products to determine exactly the 5′ ends of the transcripts. The arrows on the left indicate the origins of *psbA* transcripts T1, T2, T3, and T4.

TABLE 2. Activity of P*psbA*::*lacZ* and P*pvdA*::*lacZ* transcriptional fusions in wild-type *Pseudomonas* strain B10 and *P. aeruginosa* PAO1 and in isogenic pyoverdine-defective mutants

Strain	Plasmid	LacZ activity (Miller units)[a]		
		−Fe(III)	+Fe(III)	−Fe(III) + pyoverdine$_{B10}$
Pseudomonas strain B10	pMP220	38	31	40
	pMP220::P*psbA*	9,828	23	9,614
	pMP220::P*pvdA*	897	29	941
P. aeruginosa PAO1	pMP220	58	50	
	pMP220::P*psbA*	10,015	54	
	pMP220::P*pvdA*	10,317	57	
B10CA1	pMP220	42	35	36
	pMP220::P*psbA*	107	23	6,922
	pMP220::P*pvdA*	48	30	646
PALS124	pMP220	62	55	
	pMP220::P*psbA*	9,624	51	
	pMP220::P*pvdA*	10,186	67	
PALS106	pMP220	50	43	
	pMP220::P*psbA*	8,968	31	
	pMP220::P*pvdA*	9,075	57	
PALS125	pMP220	47	50	
	pMP220::P*psbA*	9,920	39	
	pMP220::P*pvdA*	10,080	35	

[a] Determined in *Pseudomonas* culture lysates grown for 12 h in DCAA [−Fe(III)] and DCAA supplemented with 100 μM $FeCl_3$ [+Fe(III)] (25). LacZ activity is represented by units of β-galactosidase as defined by Miller (11) and correspond to means of four determinations. Standard deviations are <16% of the given value.

[b] Pyoverdine$_{B10}$ was purified from culture supernatants of *Pseudomonas* strain B10 according to a previously described procedure (26) and added at 50 μM.

promoter fusion (pMP220::P*psbA*), while a dramatic (>90%) reduction in reporter gene activity was observed for the heterologous pMP220::P*pvdA* fusion (Table 2). These results suggest that the transcriptional apparatus of *Pseudomonas* strain B10 discriminates between the homologous promoter (P*psbA*) and the heterologous promoter (P*pvdA*), the latter being less efficiently recognized.

Pyoverdine-dependent regulation of *pvdA* and *psbA* promoters. Preliminary reports from independent laboratories have related the down-regulation of pyoverdine promoters in pyoverdine biosynthetic mutants to pyoverdine-mediated induction of cognate siderophore genes (3, 23). On this basis, we compared the pyoverdine-regulated responses in wild-type *Pseudomonas* strain B10 and *P. aeruginosa* PAO1 and in their isogenic pyoverdine-defective derivatives, B10CA1 (*psbA*::*cat*) and PALS124 (*pvdA*) (2, 26). Under low-iron conditions, the activities of both P*psbA*::*lacZ* and P*pvdA*::*lacZ* fusions were strongly reduced in *Pseudomonas* strain B10CA1 compared with those in the parental strain, and this effect was reversed by addition of 50 μM pyoverdine$_{B10}$ to the culture medium (Table 2). In contrast, the activities of both promoter fusions were similar in *P. aeruginosa* PAO1 and in the PALS124 mutant. The pyoverdine-insensitive behavior of the P*pvdA* promoter was confirmed in *P. aeruginosa* mutants blocked at later steps of pyoverdine biogenesis, including PALS106 (*pvdC1*) and PALS125 (*pvdC3*) (Table 2). These results were supported by

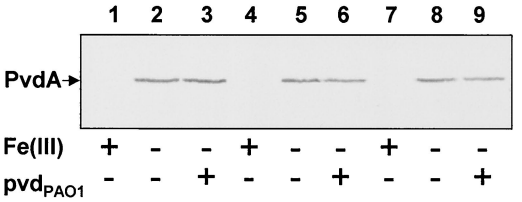

FIG. 3. Western blot analysis of iron-regulated PvdA expression in wild-type and *pvd* mutants of *P. aeruginosa* PAO1. Immunodetection of the 50-kDa PvdA protein in crude lysates (≅20 μg of proteins per lane) of exponentially grown bacteria (A_{600} ≅ 0.5) probed with an anti-PvdA mouse antiserum was performed as previously described (2). Cultures were performed in KB medium supplemented or not with either 100 μM $FeCl_3$ [Fe(III)] or 50 μM pyoverdine$_{PAO1}$ (pvd$_{PAO1}$), as indicated below each lane. Lanes: 1 to 3, *P. aeruginosa* PAO1 (wild type); 4 to 6, PALS106 (*pvdC1*); 7 to 9, PALS125 (*pvdC3*). The position of PvdA is indicated on the left.

Western blot analysis of PvdA expression in the *pvd* mutants (Fig. 3). Under conditions of iron deficiency, comparable levels of PvdA were synthesized by *P. aeruginosa* PAO1, PALS106, and PALS125, irrespective of the presence of exogenous pyoverdine$_{PAO1}$ in the culture medium (Fig. 3). As expected, addition of 100 μM $FeCl_3$ to the iron-poor medium completely repressed P*pvdA*::*lacZ* activity (Table 2) and PvdA expression (Fig. 3). Therefore, it can be deduced that homologous pyoverdine is involved in the activation of the *psbA* promoter in the *Pseudomonas* strain B10 system, while it plays no apparent role in positive regulation of the *pvdA* biosynthesis gene in *P. aeruginosa* PAO1. This effect cannot be ascribed to pyoverdine$_{B10}$-mediated induction of the *psbS* gene, since both *pvdS*::*lacZ* and *psbS*::*lacZ* promoter fusions (as in plasmids pMP220::P*pvdS* and pMP220::P*psbS*) were found to be equally expressed and iron regulated in *P. aeruginosa* PAO1 and *Pseudomonas* strain B10 wild-type strains and in their L-Orn N^5-oxygenase-defective mutants (data not shown).

Conclusions. The data just presented demonstrate that pyoverdine$_{B10}$, as the end product of its biosynthesis pathway, is a key participant in the control circuit regulating its own production in *Pseudomonas* strain B10. In *P. aeruginosa* PAO1, however, extracellular pyoverdine$_{PAO1}$ has no apparent role in the positive regulation of the homologous *pvdA* gene see Fig. 1 for a comparison between the two systems). The lack of *pvdA* response to exogenous pyoverdine is consistent with the observation that *pvd* mutants blocked in late steps of pyoverdine$_{PAO1}$ biogenesis produce wild-type levels of hydroxamate nitrogen in iron-poor medium (26). This implies that PvdA is equally expressed in the pyoverdine$_{PAO1}$-proficient and -deficient backgrounds, and immunoblot analysis of PvdA expression in the wild-type and *pvd* mutants corroborates this conclusion.

Despite the differences in individual activity between P*psbA* and P*pvdA*, which are likely to reflect their structural diversity, both promoters were found to respond positively to homologous pyoverdine in the *Pseudomonas* strain B10 system, but not in *P. aeruginosa*. The mechanism by which pyoverdine$_{B10}$ increases *psbA* expression does not involve up-regulation of *psbS* transcription, implying that sensing of pyoverdine$_{B10}$ could result in posttranscriptional activation of the PsbS sigma factor. Siderophore-dependent induction has been elucidated in the

pupIR-pupB system of *P. putida* WCS358. The Fur-controlled *pupIR* operon encodes a surface-signaling system. PupI is an ECF sigma factor required for the expression of *pupB*, encoding the receptor for heterologous pyoverdine$_{BN8}$. PupR up-regulates *pupB* expression through activation of PupI in the presence of ferripyoverdine$_{BN8}$, but it prevents the PupI-dependent transcription of *pupB* in its absence (7). Sequencing of the complete genomes of *P. fluorescens* and *P. putida* (available at http://www.jgi.doe.gov/and http://www.ncbi.nlm.nih.gov/, respectively) disclosed the presence in these species of multiple genes encoding putative PupR-like proteins. Whether a similar regulatory device also exists in *Pseudomonas* strain B10, it would account for modulation of PsbS activity through signaling of ferripyoverdine$_{B10}$ binding to its outer membrane receptor (10). This would ensure up-regulation of *psbA* expression under conditions in which pyoverdine$_{B10}$ is effective in delivering iron to the cell: namely, when ferripyoverdine$_{B10}$ is engaged with its receptor.

This investigation was supported by grants from the Department of Biology, University of Roma Tre, and from the Italian Ministry of Health (targeted projects 1999 and 2000) to P.V.

REFERENCES

1. **Abdallah, M. A.** 1991. Pyoverdins and pseudobactins, p.139–153. *In* G. Winkelman (ed.), CRC handbook of microbial iron chelates. CRC Press, Inc., Boca Raton, Fla.
2. **Ambrosi, C., L. Leoni, L. Putignani, N. Orsi, and P. Visca.** 2000. Pseudobactin biogenesis in the plant growth-promoting rhizobacterium *Pseudomonas* strain B10: identification and functional analysis of the L-ornithine N^5-oxygenase (*psbA*) gene. J. Bacteriol. **182:**6233–6238.
3. **Callanan, M., R. Sexton, D. N. Dowling, and F. O'Gara.** 1996. Regulation of the iron uptake genes in *Pseudomonas fluorescens* M114 by pseudobactin M114: the *pbrA* sigma factor does not mediate the siderophore regulatory response. FEMS Microbiol. Lett. **144:**61–66.
4. **Cunliffe, H. E., T. R. Merriman, and I. L. Lamont.** 1995. Cloning and characterization of *pvdS*, a gene required for pyoverdine synthesis in *Pseudomonas aeruginosa.* PvdS is probably an alternative sigma factor. J. Bacteriol. **177:**2744–2750.
5. **Escolar, L., J. Pérez-Martìn, and V. de Lorenzo.** 1999. Opening the iron box: transcriptional metalloregulation by the Fur protein. J. Bacteriol. **181:**6223–6229.
6. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanine and fluorescin. J. Lab. Clin. Med. **44:**301–307.
7. **Koster, M., W. van Klompenburg, W. Bitter, J. Leong, and P. Weisbeek.** 1994. Role of the outer membrane ferric siderophore receptor PupB in signal transduction across the bacterial cell envelope. EMBO J. **13:**2805–2813.
8. **Leoni, L., C. Ambrosi, A. Petrucca, and P. Visca.** 2002. Transcriptional regulation of pseudobactin synthesis in the plant growth-promoting *Pseudomonas* B10. FEMS Microbiol. Lett. **208:**219–225.
9. **Leoni, L., A. Ciervo, N. Orsi, and P. Visca.** 1996. Iron-regulated transcription of the *pvdA* gene in *Pseudomonas aeruginosa*: effect of Fur and PvdS on promoter activity. J. Bacteriol. **178:**2299–2313.
10. **Magazin, M. D., J. C. Moores, and J. Leong.** 1986. Cloning of the gene coding for the outer membrane receptor protein for ferric pseudobactin, a siderophore from a plant growth-promoting *Pseudomonas* strain. J. Biol. Chem. **261:**795–799.
11. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 252–255. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Moores, J. C., M. Magazin, G. S. Ditta, and J. Leong.** 1984. Cloning of genes involved in the biosynthesis of pseudobactin, a high-affinity iron transport agent of a plant growth-promoting *Pseudomonas* strain. J. Bacteriol. **157:**53–58.
13. **Neilands, J. B., K. Konopka, B. Schwyn, M. Coy, R. T. Francis, B. H. Paw, and A. Bagg.** 1987. Comparative biochemistry of microbial iron assimilation, p. 3–33. *In* G. Winkelmann, D. van der Helm, and J. B. Neilands (ed.), Iron transport in microbes, plants and animals. VCH Verlagsgesellschaft mbH, Weinheim, Germany.
14. **O'Sullivan, D. J., and F. O'Gara.** 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. **56:** 662–676.
15. **Ratledge, C., and L. G. Dover.** 2000. Iron metabolism in pathogenic bacteria. Annu. Rev. Microbiol. **54:**881–941.
16. **Reimmann, C., L. Serino, M. Beyeler, and D. Haas.** 1998. Dihydroaeruginoic acid synthetase and pyochelin synthetase, products of the *pchEF* genes, are induced by extracellular pyochelin in *Pseudomonas aeruginosa*. Microbiology **144:**3135–3148.
17. **Rombel, I. T., B. J. McMorran, and I. L. Lamont.** 1994. Identification of a DNA sequence motif required for expression of iron-regulated genes in pseudomonads. Mol. Gen. Genet. **246:**519–528.
18. **Schurr, M. J., H. Yu, M. Martinez-Salazar, N. S. Hibler, and V. Deretic.** 1995. Biochemical characterization and posttranslational modification of AlgU, a regulator of stress response in *Pseudomonas aeruginosa.* Biochem. Biophys. Res. Commun. **216:**874–880.
19. **Schweizer, H. P.** 1991. *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19. Gene **97:**109–112.
20. **Sexton, R., P. R. Gill, Jr., M. J. Callanan, D. J. O'Sullivan, D. N. Dowling, and F. O'Gara.** 1995. Iron-responsive gene expression in *Pseudomonas fluorescens* M114: cloning and characterization of a transcription-activating factor, PbrA. Mol. Microbiol. **15:**297–306.
21. **Spaink, H. P., R. J. H. Okker, C. A. Wijffelman, E. Pees, and B. J. J. Lugtenberg.** 1987. Promoters in the nodulation region of the *Rhizobium leguminosarum* Sym plasmid pRL1J1. Plant Mol. Biol. **9:**27–39.
22. **Vasil, M. L., and U. A. Ochsner.** 1999. The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. Mol. Microbiol. **34:**399–413.
23. **Venturi, V., C. Ottewanger, M. Bracke, and P. J. Weisbeek.** 1995. Iron regulation of siderophore biosynthesis and transport in *Pseudomonas putida* WCS358: involvement of a transcriptional activator and of the Fur protein. Mol. Microbiol. **15:**1081–1093.
24. **Venturi, V., P. Weisbeek, and M. Koster.** 1995. Gene regulation of siderophore mediated iron acquisition in *Pseudomonas*: not only the Fur repressor. Mol. Microbiol. **17:**603–610.
25. **Visca, P., G. Colotti, L. Serino, D. Verzili, N. Orsi, and E. Chiancone.** 1992. Metal regulation of siderophore synthesis in *Pseudomonas aeruginosa* and functional effects of siderophore-metal complexes. Appl. Environ. Microbiol. **58:**2886–2893.
26. **Visca, P., L. Serino, and N. Orsi.** 1992. Isolation and characterization of *Pseudomonas aeruginosa* mutants blocked in the synthesis of pyoverdin. J. Bacteriol. **174:**5727–5731.
27. **Wilson, M. J., B. J. McMorran, and I. L. Lamont.** 2001. Analysis of promoters recognized by PvdS, an extracytoplasmic-function sigma factor protein from *Pseudomonas aeruginosa*. J. Bacteriol. **183:**2151–2155.