Iron is an essential cofactor for many microbial enzymes and, as such, is required for growth of most bacteria (20). This need for iron is, however, complicated by the low solubility and, thus, bioavailability of this element in nature (20, 45). Many bacteria overcome this problem by synthesizing high-affinity iron-chelating molecules termed siderophores that, together with cell surface receptors specific for the iron-siderophore complexes, serve to provide the organism with iron under the most nutritionally dilute conditions (7). Significantly, pathogenic organisms also encounter an iron-limited environment in the host, where siderophore-mediated iron uptake plays an equally important role in growth and, thus, pathogenesis of many disease-causing bacteria (21, 55).

Pseudomonas aeruginosa is an opportunistic human pathogen associated with infections of compromised individuals (15). The organism produces two known siderophores, pyoverdine (11) and pyochelin (10), and can utilize a number of siderophores produced by other microorganisms (9, 38, 52), which likely explains the large number of ferric-siderophore receptors identified in the recently completed genome sequence (66). Production of pyoverdine has been documented in vivo (22), consistent both with the in vivo induction of pyoverdine biosynthetic genes (24) and a demonstrated role for this siderophore in promoting in vivo growth and pathogenesis (41, 45, 57). Pyoverdine is, by far, the superior iron chelator in aqueous medium (62), exhibiting a stability constant of $10^{25}$ at pH 7 (40). A mixed hydroxamate-catecholate siderophore, pyoverdine is characterized by a conserved hydroxquinoline chromophore bound to an amino acid tail of variable length and composition (8). The latter appears to be responsible for the strain specificity of pyoverdine utilization in P. aeruginosa (42). Two gene clusters involved in pyoverdine biosynthesis have been identified, which are responsible for synthesis of the chromophore (pvc) (64, 65) and peptide (pvd) (13, 25, 35, 56, 69) moieties of the siderophore, respectively. The fpvA gene (51) encoding the ferric pyoverdine receptor (50) also maps to the pvd gene cluster (37).

Pyoverdine production occurs in response to iron limitation, and its production decreases with increasing iron availability (39). This iron-regulated expression is mediated by a homologue of the Fur repressor protein shown to regulate iron-siderophore systems in Escherichia coli (4, 54). Fur only indirectly regulates pyoverdine biosynthesis in P. aeruginosa, via the alternate sigma factor PvdS (74), which positively regulates several pyoverdine biosynthetic genes (12, 36, 44) but is itself regulated by Fur (12, 36, 44, 47). More recently, a LysR-type transcriptional activator of the pvc genes, PtxR, has been described in P. aeruginosa (23, 65) and is itself regulated by PvdS (71).

An interesting feature of FpvA is its N-terminal extension that may be unique to certain receptors involved in signal transduction. Both the FecA ferric dicitrate receptor of E. coli (53, 73) and the PupB ferric pseudobactin BN7/8 receptor of Pseudomonas putida WCS358 (33) share this extension and are involved in receptor-dependent expression of the corresponding receptor genes (26, 34). Such receptor-dependent signaling of gene expression involves an atypical two-component regulatory system, including a cytoplasmic membrane-associated component (FecR and PupR) and a cytoplasmic alternate sigma factor of the extracytoplasmic factor family (FecI and PupI) (2, 34, 70). Signaling involves the novel N termini of these receptors (32, 34), which has, in the case of FecA, been shown to physically associate with the cytoplasmic membrane component FecR (14), which itself interacts with FecI (14, 63). Ligand binding to the outer membrane receptor ultimately activates or releases the alternate sigma factors, which then stimulate receptor gene expression (6). In the present study, we assessed the significance of FpvA vis-a-vis expression of genes associated with pyoverdine-mediated iron uptake and noted that pyoverdine production and expression of pyoverdine biosynthetic genes are reduced in fpvA knockouts. As
TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>Wild-type PA01</td>
<td>This study</td>
</tr>
<tr>
<td>K767</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K1660</td>
<td>K767 ΔfpvA</td>
<td></td>
</tr>
<tr>
<td>K1661</td>
<td>K1660 attB::fpvA</td>
<td></td>
</tr>
<tr>
<td>K1662</td>
<td>K1660 attB::fpvA-wt</td>
<td></td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>endA hsdR17 supE44 thi-1 relA gyrA relA Δ(lacZYA-argF)U169 deor [8808lacΔlacz(M15)]</td>
<td>(3)</td>
</tr>
<tr>
<td>DH5α</td>
<td>supE44 rfbD1 spoT1 thi-1 endA hsdR17 pro</td>
<td>(59)</td>
</tr>
<tr>
<td>MM294</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Plasmids**

| pAK1900          | E. coli-P. aeruginosa shuttle cloning vector; Ap r/Cb r | A. Kropinski, Queen’s University |
| pFVR2            |            |                     |
| pEX18Tc          | pAK1900::fpvA | (51) |
| pJS2             | Broad-host-range gene replacement vector; Tc r | (27) |
| pRKR2013         | pEX18Tc::ΔfpvA | This study |
| pJS4             | Broad-host-range cloning vector; Tc r | (16) |
| pJS5             | pRKR415::fpvA | This study |
| mini-CTX1        | pRKR415::fpvA-wt | This study |
| pJS6             | Mini-CTX1::fpvA | This study |
| pJS8             | Mini-CTX1::fpvA-wt | This study |
| pMP190           | Broad-host-range, low-copy-number lacZ fusion vector; Cm r Sm r | (60) |
| pMP190:pvdD      | pMP190:pvdD-lacZ; Cm r | (57) |

**Materials and Methods**

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. Plasmid pJS4, a pRK415 derivative carrying the fpvA gene in the same orientation as the resident lac promoter of this vector, was constructed by releasing the gene on a ca. 4.7-kb SphI fragment from pFVR2 and, following polishing of the ends with Klenow fragment, cloning it into PstI-restricted and Klenow-treated pRK415. Plasmid pJS5 is a pRK415 derivative carrying a truncated fpvA (dubbed fpvA*) gene that encodes a receptor that retains the signal sequence but lacks 64 of the first 66 amino acids of the mature protein.

Expected, FpvA-mediated pyoverdine production requires the N-terminal extension of the protein.

In the proper orientation, pJS5, expressed a FpvA protein of the expected size, as confirmed by Western immunoblotting, Nucleotide sequencing of fpvA* confirmed its proper construction. The ΔfpvA derivative of PA01 strain K767, dubbed K1660, was constructed with gene replacement vector pEX18Tc, in which sequences upstream and downstream of fpvA were cloned. Sequences upstream of the fpvA coding region were amplified by PCR with primers JS7 (5′-CACCGAACGGCTCCATCTG-3′; anneals ca. 750 bp upstream of fpvA start codon) and JS8 (5′-CGGATCCAGGA CTGAGACCGTG-3′; BamHI site underlined; anneals ca. 20 bp upstream of fpvA start codon). Sequences downstream of fpvA were amplified with primers JS9 (5′-GCGTTCTCGTCTCCGTGCTAC-3′; anneals ca. 35 bp downstream of the fpvA stop codon) and JS10 (5′-GCAACCTCGGATGATG-3′; anneals ca. 750 bp downstream of the fpvA stop codon). Reaction mixture formulations and amplification parameters were as described above. The upstream fragment was then purified with the QIAquick-spin PCR Purification Kit (Qiagen, Inc.) and cloned into pCR-Blunt II-TOPO (Invitrogen Corp., Carlsbad, Calif.) with the Zero Blunt PCR Cloning Kit and a protocol supplied by the manufacturer. Following sequencing of the insert to ensure that no errors had been introduced by PCR, the 700-bp upstream fragment was released from the TOPO vector by digestion with EcoRI and BamHI and cloned into EcoRI-BamHI-restricted pEX18Tc to yield pJS1. The downstream PCR product was similarly purified, cloned into pCR-Blunt II-TOPO, and sequenced. This fragment was then released by digestion with BamHI and PstI and cloned into BamHI-PstI-restricted pJS1 to yield pJS2. ΔfpaC- carrying pJS2 was then introduced into E. coli strain S17-1 and mobilized into wild-type P. aeruginosa PA01 strain K767 via conjugation as previously described (49). Cells of K767 carrying pJS2 in the chromosome were selected on tetracycline and chloramphenicol (25 μg/ml; to counterselect the donor E. coli). Following selection on Luria-Bertani (LB) agar containing 10% (wt/vol) sucrose, sucrose-resistant colonies were screened for the presence of the fpvA deletion by PCR with primers JS7 and JS10. Loss of FpvA in K1660 was ultimately confirmed by Western immunoblotting (see below).

Introduction of wild-type or truncated fpvA into the chromosome of strain K1660 was achieved with the mini-CTX1 vector and a protocol developed by Huang and coworkers (26). The wild-type gene was first recovered on a 4.3-kb PstI fragment from plasmid pFVR2 and cloned into PstI-restricted mini-CTX1. The resultant vector, pJS6, was introduced into E. coli S17-1 and mobilized into K1660 via conjugation (49). Following selection on tetracycline and chloramphenicol (25 μg/ml; counterselection), fpvA was recovered in the chromosome (at the 4CTX attB site) in the absence of plasmid backbone sequences with

---

*a* Carries the fpvA genes in the chromosome at the attB site.

*b* 5′ deletion of the fpvA gene, corresponding to 64 of the first 66 amino acids of the mature protein.
RESULTS AND DISCUSSION

Role of FpvA in pyoverdine production. Alignment of FpvA with homologous ferric-siderophore receptors (Fig. 1) demonstrated that the mature FpvA protein, like the ferric pseudobactin receptors of P. putida, possesses an extension at the N terminus of ca. 70 amino acids that is not shared by other ferric-siderophore receptors. In the case of the PupB ferric pseudobactin BN7/BN8 receptor, this extension plays a role in PupB-mediated signal transduction associated with upregulation of pupB gene expression (34). To assess the possible involvement, therefore, of FpvA in receptor-dependent expression of fpvA, the entirety of the fpvA coding region was deleted from the chromosome of wild-type P. aeruginosa PAO1 strain K767. Intriguingly, the resultant strain, K1660, was, unlike its parent, nonfluorescent on iron-deficient minimal succinate plates, suggesting that it was deficient in pyoverdine biosynthesis. Examination of pyoverdine production as a function of growth in an iron-limited medium confirmed the pyoverdine deficiency of the FpvA-deficient K1660 strain (Fig. 2). As expected, then, K1660 also failed to grow in iron-limited minimal medium supplemented with the nonmetabolizable iron chelator EDDHA (Fig. 3), a phenotype typical of P. aeruginosa with defects in pyoverdine production or utilization. Two loci, pvd and pvc, are known to be associated with pyoverdine biosynthesis in P. aeruginosa (65, 69). Using an available pvdD-lacz fusion vector, loss of fpvA in K1660 was correlated with an inconsistent decline in pvd expression of twofold or less (as a measure of expression of genes of the pvd locus, which are expected to be coregulated [12, 36, 44]) (data not shown). Beare and colleagues reported a similar ca. 2.5-fold decline in expression in another pvd gene, pvdF, in an FpvA knockout, also with a lacZ reporter (P. A. Beare et al., Pseudomonas 2001, Brussels, Belgium, abstr. PS146, 2001). With RT-PCR, however, it was clear that the FpvA-deficient strain indeed exhibited reduced pvdD expression during growth under iron limitation relative to its FpvA+ parent (Fig. 4B, c.f. lanes 2 and 4). The FpvA-producing wild-type strain produced surprisingly modest levels of pvdD under iron-limiting conditions (Fig. 4, compare lane 2 with lane 1), likely reflecting the fact that RNA for RT-PCR was harvested from cells early during growth in an iron-limiting medium (at an optical density at 600 nm of 0.7), when they would be only moderately iron limited. The identification of pvdD transcripts in wild-type cells (K767) growing under iron-replete conditions (Fig. 4, lane 1), although perhaps unexpected, was consistent with the earlier demonstration that L-broth-grown (i.e., iron-replete) cells of P. aeruginosa produce some FpvA receptor protein (and other iron-regulated receptors) (46). Thus, P. aeruginosa apparently expresses these iron-regulated genes at low levels under iron-replete conditions and methods such as RT-PCR are sensitive enough to detect this.

In contrast to pvdD, the entirety of the fpvA coding region was deleted from the chromosome of wild-type P. aeruginosa PAO1 strain K767. Intriguingly, the resultant strain, K1660, was, unlike its parent, nonfluorescent on iron-deficient minimal succinate plates, suggesting that it was deficient in pyoverdine biosynthesis. Examination of pyoverdine production as a function of growth in an iron-limited medium confirmed the pyoverdine deficiency of the FpvA-deficient K1660 strain (Fig. 2). As expected, then, K1660 also failed to grow in iron-limited minimal medium supplemented with the nonmetabolizable iron chelator EDDHA (Fig. 3), a phenotype typical of P. aeruginosa with defects in pyoverdine production or utilization. Two loci, pvd and pvc, are known to be associated with pyoverdine biosynthesis in P. aeruginosa (65, 69). Using an available pvdD-lacz fusion vector, loss of fpvA in K1660 was correlated with an inconsistent decline in pvd expression of twofold or less (as a measure of expression of genes of the pvd locus, which are expected to be coregulated [12, 36, 44]) (data not shown). Beare and colleagues reported a similar ca. 2.5-fold decline in expression in another pvd gene, pvdF, in an FpvA knockout, also with a lacZ reporter (P. A. Beare et al., Pseudomonas 2001, Brussels, Belgium, abstr. PS146, 2001). With RT-PCR, however, it was clear that the FpvA-deficient strain indeed exhibited reduced pvdD expression during growth under iron limitation relative to its FpvA+ parent (Fig. 4B, c.f. lanes 2 and 4). The FpvA-producing wild-type strain produced surprisingly modest levels of pvdD under iron-limiting conditions (Fig. 4, compare lane 2 with lane 1), likely reflecting the fact that RNA for RT-PCR was harvested from cells early during growth in an iron-limiting medium (at an optical density at 600 nm of 0.7), when they would be only moderately iron limited. The identification of pvdD transcripts in wild-type cells (K767) growing under iron-replete conditions (Fig. 4, lane 1), although perhaps unexpected, was consistent with the earlier demonstration that L-broth-grown (i.e., iron-replete) cells of P. aeruginosa produce some FpvA receptor protein (and other iron-regulated receptors) (46). Thus, P. aeruginosa apparently expresses these iron-regulated genes at low levels under iron-replete conditions and methods such as RT-PCR are sensitive enough to detect this.

In contrast to pvdD, the entirety of the fpvA coding region was deleted from the chromosome of wild-type P. aeruginosa PAO1 strain K767. Intriguingly, the resultant strain, K1660, was, unlike its parent, nonfluorescent on iron-deficient minimal succinate plates, suggesting that it was deficient in pyoverdine biosynthesis. Examination of pyoverdine production as a function of growth in an iron-limited medium confirmed the pyoverdine deficiency of the FpvA-deficient K1660 strain (Fig. 2). As expected, then, K1660 also failed to grow in iron-limited minimal medium supplemented with the nonmetabolizable iron chelator EDDHA (Fig. 3), a phenotype typical of P. aeruginosa with defects in pyoverdine production or utilization. Two loci, pvd and pvc, are known to be associated with pyoverdine biosynthesis in P. aeruginosa (65, 69). Using an available pvdD-lacz fusion vector, loss of fpvA in K1660 was correlated with an inconsistent decline in pvd expression of twofold or less (as a measure of expression of genes of the pvd locus, which are expected to be coregulated [12, 36, 44]) (data not shown). Beare and colleagues reported a similar ca. 2.5-fold decline in expression in another pvd gene, pvdF, in an FpvA knockout, also with a lacZ reporter (P. A. Beare et al., Pseudomonas 2001, Brussels, Belgium, abstr. PS146, 2001). With RT-PCR, however, it was clear that the FpvA-deficient strain indeed exhibited reduced pvdD expression during growth under iron limitation relative to its FpvA+ parent (Fig. 4B, c.f. lanes 2 and 4). The FpvA-producing wild-type strain produced surprisingly modest levels of pvdD under iron-limiting conditions (Fig. 4, compare lane 2 with lane 1), likely reflecting the fact that RNA for RT-PCR was harvested from cells early during growth in an iron-limiting medium (at an optical density at 600 nm of 0.7), when they would be only moderately iron limited. The identification of pvdD transcripts in wild-type cells (K767) growing under iron-replete conditions (Fig. 4, lane 1), although perhaps unexpected, was consistent with the earlier demonstration that L-broth-grown (i.e., iron-replete) cells of P. aeruginosa produce some FpvA receptor protein (and other iron-regulated receptors) (46). Thus, P. aeruginosa apparently expresses these iron-regulated genes at low levels under iron-replete conditions and methods such as RT-PCR are sensitive enough to detect this.
In contrast to that of \textit{pvdD}, expression of the \textit{pvc} genes did not appear to be altered in K1660 relative to its wild-type parent strain under conditions of iron limitation (data not shown), although the very weak expression of \textit{pvcA} detected likely precluded detection of modest changes in \textit{pvc} gene expression. In any case, FpvA control of pyoverdine biosynthesis occurs via the peptide biosynthetic genes at least.

Interestingly, introduction of the cloned wild-type \textit{fpvA} gene (on plasmid pJSS4) into K1660 failed to restore pyoverdine production (Fig. 2) or growth on EDDHA-supplemented (i.e., iron-restricted) minimal medium (Fig. 3) despite the restored production of FpvA in the mutant (Fig. 5, lane 4). Still, introduction of this gene into the chromosome of K1660 (at the \textit{/H9278\ CTX\ attB} site with the mini-\textit{CTX1} vector of Hoang et al. [28], yielding strain K1661), which similarly restored FpvA production (Fig. 5, lane 5), did restore pyoverdine production (Fig. 2) and growth in EDDHA-supplemented medium (Fig. 3). Not unexpectedly, the restored pyoverdine production paralleled an increase in \textit{pvdD} gene expression (Fig. 4B, lane 6). One explanation for the failure of the plasmid-borne \textit{fpvA} gene to complement the pyoverdine and growth deficiencies of K1660 is that the presence of multiple copies of this gene, in particular its upstream promoter region, might have titrated out a positive regulator associated with expression of both \textit{fpvA} and genes of pyoverdine biosynthesis. Still, a previous report showed that plasmid-borne \textit{fpvA} was able to complement the growth defect of an FpvA-deficient mutant (31), although the \textit{fpvA} mutant in this instance was constructed by insertional inactivation and no gene sequences were deleted. It was possible, therefore, that any growth restoration seen resulted from recombination between chromosomal \textit{fpvA} sequences and the plasmid-borne gene. Screening of a \textit{P. aeruginosa} gene library for sequences able to restore pyoverdine biosynthesis and growth (on EDDHA) of pJSS4-harboring K1660, in an attempt to identify such a regulatory gene, have so far failed.

Intriguingly, the only ferric-siderophore receptors to be previously characterized with respect to their involvement in signal transduction are FecA and PupB. Unlike FpvA, however, these receptors transport, and thus respond to, siderophores not produced by the organism itself. Presumably, this provides a way for the cell to sense the presence of these siderophores, upregulating the transport genes only when they are likely to be useful. The need to couple pyoverdine biosynthesis to receptor utilization, presumably of the ferrated siderophore, is less clear. \textit{P. aeruginosa} is capable of producing two siderophores, although only one is likely needed at any given time. Perhaps the coupling of siderophore uptake via \textit{fpvA} to pyoverdine biosynthesis is a way of ensuring that pyoverdine is effective at chelating and, thus, providing iron to the organism in a particular environment before investing energy in production of siderophore-utilizing receptors.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Multiple-sequence alignment of \textit{FpvA} and its homologues. Proteins related to \textit{FpvA} were identified with BLAST (1) (http://www.ncbi.nlm.nih.gov/BLAST/), and the mature sequences (putative signal peptide cleavage sites were identified by the method of Nielsen et al. [46] [http://www.cbs.dtu.dk/services/SignalP/]) were aligned with CLUSTALW (68) (http://www.ebi.ac.uk/clustalw/). Only the N termini are shown. \textit{FptA}, \textit{P. aeruginosa} Fe(III)-pyochelin receptor (accession number P42512); \textit{PbuA}, \textit{P. putida} M114 Fe(III)-pseudobactin M114 receptor (accession number Q08017); \textit{FhuE}, \textit{E. coli} receptor for Fe(III)-coprogen, -ferrioxamine B, and -rhodotorulic acid (accession number P16869); \textit{FauA}, \textit{Bordetella pertussis} Fe(III)-alcaligin receptor (accession number AAD26430); \textit{PupA}, \textit{P. putida} WCS358 Fe(III)-pseudobactin 358 receptor (accession number P25184); \textit{FpvA}, \textit{P. aeruginosa} Fe(III)-pyoverdine receptor (accession number P48632); \textit{PupB}, \textit{P. putida} WCS358 Fe(III)-pseudobactin BN7/BN8 receptor (accession number P38047); PA2466, probable \textit{P. aeruginosa} TonB-dependent receptor (accession number D83337).}
\end{figure}
ing this siderophore. With two endogenous and potentially multiple heterologous siderophores to choose from, this would ensure that the cell invests in pyoverdine only when it is prudent to do so. In the presence, for example, of enterobactin (which is typically a better chelator than pyoverdine [19]) or under conditions in which pyochelin is the better chelator, the resultant failure of pyoverdine to acquire iron would, through its failure to interact productively with FpvA, ensure that energy is not wasted synthesizing a siderophore that is ineffective at acquiring iron. This mirrors what is seen with respect to FpvA expression, inasmuch as strains deficient in pyoverdine production exhibit reduced synthesis of this protein (17). The observation here that the levels of the N-terminally truncated FpvA* protein were noticeably reduced relative to those of wild-type FpvA (Fig. 5, compare lanes 6 and 7 with lanes 4 and 5) is therefore consistent with the reduction in pyoverdine seen in FpvA*-expressing *P. aeruginosa*. That pyoverdine-deficient strain K1660(pJSS4) produced wild-type levels of FpvA (Fig. 5, lane 4) likely reflects the multicopy nature of the plasmid-borne fpvA gene in this instance and promotion of fpvA gene expression.

FIG. 2. Pyoverdine production by *P. aeruginosa* strains K767 (○), K1660 (▲), K1660(pRK415) (□), K1660(pJS55) (■), K1661 (▼), and K1662 (△) as a function of growth in iron-deficient succinate minimal medium. Pyoverdine levels were estimated by measuring the A405 of the cell-free culture supernatant (A) and hydroxamate nitrogen at A520 (B). Values are normalized with respect to culture density, so as to provide a measure of per-cell pyoverdine production. All strains grew equally well in the minimal medium, plateauing at an A600 of ca. 1.4 after 7 h of growth. The results are representative of three independent experiments run on different days. Note that data points for K1662 in panel A are masked by data points of other strains in the lower part of the graph.

FIG. 3. Growth of *P. aeruginosa* strains K767 (○), K1660 (▲), K1660(pRK415) (□), K1660(pJS55) (■), K1661 (▼), and K1662 (△) in EDDHA-supplemented (3 mg/ml) succinate minimal medium. The results are representative of three independent experiments run on different days.

FIG. 4. *rpsL* (A) and *pvdD* (B) expression in *P. aeruginosa* cultured under iron-sufficient (odd-numbered lanes) and iron-deficient (even-numbered lanes) conditions measured by RT-PCR of total RNA (24 ng, top of panel A; 4 ng, bottom of panel A; 4 ng, panel B) isolated from strains K767 (lanes 1 and 2), K1660 (lanes 3 and 4), K1661 (lanes 5 and 6), and K1662 (lanes 7 and 8).
expression from the resident lac promoter of pJSS4 parent plasmid pRK415, both of which would provide some escape from the usual pyoverdine controls.

Involvement of the N terminus in FpvA-mediated pyoverdine production. The involvement of FecA and PupB in signal transduction is intimately tied to the unique N-terminal extensions of these receptors (32, 34). To assess, then, the importance of the N terminus of FpvA vis-à-vis the receptor’s involvement in pyoverdine biosynthesis, a gene encoding a truncated version of FpvA lacking 64 of the first 66 amino acids (V3 to Q66) of the mature protein (dubbed FpvA*) was constructed. Like wild-type FpvA-encoding pJSS4, FpvA*-encoding pJSS5 did not promote enhanced pyoverdine production by K1660 (Fig. 2) or growth of K1660 in the presence of EDDHA (Fig. 3), although the truncated protein was expressed (Fig. 5, lane 6). Unlike K1661, however, where introduction of the wild-type fpvA gene at the ΔCTX attB site of K1660 complemented the growth and pyoverdine production defects, the fpvA* gene of K1662 did not promote enhanced pyoverdine production (Fig. 2) or growth in the presence of EDDHA (Fig. 3). As expected, FpvA* was also unable to restore wild-type pvD expression levels in K1662 (Fig. 4B, compare lane 8 with lanes 2 and 6). Nonetheless, the truncated protein was produced in K1662 (Fig. 5, lane 7) and this strain did demonstrate pyoverdine-enhanced growth in EDDHA-supplemented medium, something also seen in wild-type PAO1 strain K767 but not in FpvA-deficient strain K1660 (Fig. 6). Growth of P. aeruginosa in the presence of EDDHA is typically dependent upon the presence of a functional ferric-pyoverdine uptake system and is thus a good measure of pyoverdine-mediated iron acquisition. Thus, FpvA* is presumably competent for transport but defective with regard to its role in pyoverdine biosynthesis. An N-terminally truncated FecA receptor was similarly competent for ferric dicitrate transport but unable to induce fecA gene expression in this case (32). It is likely, therefore, that FpvA, specifically its N terminus, plays a role in a signal transduction process leading to activation of the pvD genes of pyoverdine biosynthesis. PvdS is known to activate pvD gene expression (12), suggesting that this extracytoplasmic sigma factor mediates the influence of FpvA on pyoverdine biosynthesis. Interestingly, homologues of fecIR/pupIR have been identified in the pvD locus (PA2387 and PA2388), as revealed by the recently completed P. aeruginosa genome sequence (66). Deletion of these genes does not, however, impact pyoverdine production by P. aeruginosa (A. Redly, unpublished data).

While FpvA appears to be unique among ferric-siderophore receptors in terms of its role in regulating siderophore biosynthesis, PupA, the receptor for the endogenous pseudobactin of P. putida WCS358, also possesses an N-terminal extension (Fig. 1) and is thus anticipated to play some role in the regulation of gene expression. While this remains to be elucidated, the results of a previous study demonstrating pseudobactin 358-dependent expression of a pseudobactin 358 biosynthetic gene was suggestive of receptor (i.e., PupA) involvement, since utilization of the siderophore was probably needed for this upregulation (72). The involvement, if any, of PupA in pseudobactin 358 synthesis was not, however, examined in this study. Still, an earlier study revealed no difference in siderophore production by PupA-deficient mutants compared to the wild type (3), suggesting that pseudobactin biosynthesis in P. putida WCS358, unlike pyoverdine biosynthesis in P. aeruginosa, has no receptor involvement.

ACKNOWLEDGMENTS

This work was supported by an operating grant from the Canadian Institutes of Health Research (formerly the Medical Research Council of Canada) to K.P. K.P. is a Canadian Cystic Fibrosis Foundation (CCFF) Scholar.

REFERENCES

Pseudomonas aeruginosa outer membrane protein, the Ton system and the electrochemical potential of the cytoplasmic membrane. Mol. Microbiol. 23:333–344.

Downloaded by guest on August 29, 2017 from http://jb.asm.org/