AN ABSTRACT OF THE DISSERTATION OF

Sierra L. Hartney for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on November 28, 2011.

Title: TonB-dependent Outer-membrane Proteins of *Pseudomonas fluorescens*: Diverse and Redundant Roles in Iron Acquisition.

Abstract approved:

_____

Joyce E. Loper

*Pseudomonas* is a diverse genus of Gram-negative bacteria that includes pathogens of plants, insects, and humans as well as environmental strains with no known pathogenicity. *Pseudomonas fluorescens* itself encompasses a heterologous group of bacteria that are prevalent in soil and on foliar and root surfaces of plants. Some strains of *P. fluorescens* suppress plant diseases and the genomic sequences of many biological control strains are now available. I used a combination of bioinformatic and phylogenetic analyses along with mutagenesis and biological assays to identify and compare the TonB-dependent outer-membrane proteins (TBDPs) of ten plant-associated strains of *P. fluorescens* and related species. TBDPs are common in Gram-negative bacteria, functioning in the uptake of ferric-siderophore complexes and other substrates into the cell. I identified 14 to 45 TBDRs in each strain of *P. fluorescens* or *P. chlororaphis*. Collectively, the ten strains have 317 TBDPs, which were grouped into 84 types based upon sequence similarity and phylogeny. As many as 13 TBDPs are unique to a single strain and some show evidence of horizontal gene transfer. Putative functions in the uptake of diverse groups of microbial siderophores, sulfur-esters, and other substrates were assigned to 28 of these TBDP types based on similarity to characterized orthologs from other *Pseudomonas* species. Redundancy of TBDP function was evident in certain strains of *P. fluorescens*, especially Pf-5, which has three TBDPs for ferrichrome/ferrioxamine uptake, two for ferric-citrate uptake and three for heme uptake.

Five TBDP types are present in all ten strains, and putative functions in heme, ferrichrome, cobalamin, and copper/zinc uptake were assigned to four of the conserved TBDPs.

The fluorescent pseudomonads are characterized by the production of pyoverdine siderophores, which are responsible for the diffusible UV fluorescence of these bacteria. Each of the ten plant-associated strains of *P. fluorescens* or *P. chlororaphis* has three to six TBDPs with putative roles in ferric-pyoverdine uptake (Fpv). To confirm the roles of the six Fpv outer membrane proteins in *P. fluorescens* Pf-5, I introduced deletions into each of the six *fpv* genes in this strain and evaluated the mutants and the parental strain for heterologous pyoverdine uptake. I identified at least one ferric-pyoverdine that was taken up by each of the six Fpv outer-membrane proteins of Pf-5. By comparing the ferric-pyoverdine uptake assay results to a phylogenetic analysis of the Fpv outer-membrane proteins, I observed that phylogenetically-related Fpv outer-membrane proteins take up structurally-related pyoverdines. I then expanded the phylogenetic analysis to include nine other strains within the *P. fluorescens* group, and identified five additional types of Fpv outer-membrane proteins. Using the characterized Fpv outer-membrane proteins of Pf-5 as a reference, pyoverdine substrates were predicted for many of the Fpv outer-membrane proteins in the nine other strains. Redundancy of Fpv function was evident in Pf-5, as some pyoverdines were recognized by more than one Fpv. It is apparent that heterologous pyoverdine recognition is a conserved feature, giving these ten strains flexibility in acquiring iron from the environment.

Overall, the TBDPs of the *P. fluorescens* group are a functionally diverse set of structurally-related proteins present in high numbers in many strains. While putative functions have been assigned to a subset of the proteins, the functions of most TBDPs remain unknown, providing targets for further investigations into nutrient uptake by *P. fluorescens* spp.. The work presented here provides a template for future studies using a combination of bioinformatic, phylogenetic, and molecular genetic approaches to predict and analyze the function of these TBDPs.

# TonB-dependent Outer-membrane Proteins of *Pseudomonas fluorescens*: Diverse and Redundant Roles in Iron Acquisition

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APPROVED:

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Major Professor, representing Botany and Plant Pathology

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Chair of the Department of Botany and Plant Pathology

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Sierra L. Hartney, Author

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# CONTRIBUTION OF AUTHORS

Dr. Sylvie Mazurier performed crossfeeding assays between the Pf-5 siderophore mutant and 39 of the 61 strains of *Pseudomonas* presented in Chapter 2. Sylvie performed crossfeeding assays between *fpv* mutants of Pf-5 and 19 of the 37 strains of *Pseudomonas* presented in Chapter 3. She also supervised the purification of pyoverdines from selected *Pseudomonas* spp. that were used in Chapter 3.

Dr. Teresa A. Kidarsa made the Δ*pvdI-pchC* mutant of Pf-5 used in crossfeeding assays presented in Chapter 2.

Dr. Maria Carolina Quecine observed the presence of a zone of clearing around colonies of Pf-5 on CAS agar as discussed in Chapter 2.

Neal Wilson did Bayesian analysis to generate the tree of *Pseudomonas* species based on multi-locus sequence analysis presented in Chapter 4.

Neal C. Goebel ran the HPLC analysis on the samples prepared from the Pf-5 siderophore mutants presented in Appendices 5 and 6.

Dr. Philippe Lemanceau advised the work of Sylvie Mazurier and provided encouraging support and critical review of the research presented in Chapters 2 and 3.

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**TonB-dependent Outer-membrane Proteins of *Pseudomonas fluorescens*: Diverse and Redundant Roles in Iron Acquisition**

## Chapter 1: Introduction

*Pseudomonas* species are ubiquitous inhabitants of soil, water, and plant surfaces (2, 51). Members of the genus live in association with roots, seeds, fruit, foliar and floral surfaces. These commensal species can have profound effects on plants by suppressing pests, enhancing access to key nutrients, altering physiological processes, or degrading environmental pollutants. The processes by which commensal microorganisms exert beneficial effects on plant health are poorly understood, and knowledge of these processes is needed to exploit microbe-plant interactions for the benefit of agriculture and the environment.

Plant diseases pose significant constraints to agriculturae and their management requires large inputs of time and money to maintain crop productivity. With the ever increasing costs of existing methods for disease control in agriculture, new options, such as biological control agents, are needed. In addition to the possibility of lowering costs, the use of naturally-occurring beneficial microorganisms could result in more stable and sustainable agricultural practices by reducing the current applications of compounds with detrimental effects on the environment. For example, certain *Pseudomonas* spp. are associated with disease suppressive soils (47), a natural process of biological control manifested in soils where plant disease remains negligible even when a pathogen is present. In addition, certain strains of *Pseudomonas* spp. suppress plant disease when used as microbial inoculants, and two strains of *Pseudomonas fluorescens* are currently used in US agriculture for disease management (17). The primary focus of the research presented in this dissertation is *Pseudomonas fluorescens* strain Pf-5, a model organism widely studied by the biological control research community and distinguished as the first fully sequenced biological control agent for plant disease (43).

*Pseudomonas fluorescens* Pf-5 is a soil bacterium that suppresses a number of soilborne pathogens (30). To suppress soilborne plant diseases, biological control bacteria like Pf-5 must colonize seed and root surfaces. The capacity to acquire iron is one factor contributing to the competitive fitness of bacteria in the rhizosphere (46). Understanding the mechanisms by which iron is taken up into the cell by *P. fluorescens* Pf-5 is of scientific interest, as iron is essential to cellular metabolism. Additionally, the mechanisms employed to enhance environmental fitness by a biological control strain could be applied to human and plant health. Within the genus *Pseudomonas* is the opportunistic human pathogen *Pseudomonas aeruginosa*, which has a severe impact on cystic fibrosis sufferers, and devastating plant pathogens like *Pseudomonas syringae*, which infects a wide range of economically-important crops. These bacteria share commonalities at the genomic level, so information generated through studies of Pf-5 can be extended to other *Pseudomonas* spp.

**Siderophores**

Gram-negative bacteria such as fluorescent *Pseudomonas* spp. are subject to iron limitation in aerobic environments due to the lack of readily-available $Fe^{2+}$ ionsthe form of iron needed for bacterial growth. To overcome the obstacle of limited iron, the fluorescent pseudomonads produce and utilize siderophores. Siderophores are iron-chelating compounds released into the environment to bind $Fe^{3+}$, and taken up into the bacterial cell through TonB-dependent outer-membrane proteins (TBDPs). Inside the cell, the iron can be removed from the siderophore by redox reduction of $Fe^{3+}$ to $Fe^{2+}$, which releases the iron from the siderophore due to reduced affinity for ferrous iron (22). Siderophores, Greek meaning iron carriers, are low molecular weight molecules produced by many organisms, such as plants, bacteria and fungi. Currently, 500 different siderophores are known, with 270 of them structurally characterized (22). Primarily, there are three structural types of siderophores based on the type of iron-binding ligand they contain: catecholates, hydroxamates, and carboxylates (22). In most cases, biosynthesis is by nonribosomal peptide synthetases, which use a modular domain system

to link amino acids together with post-translational modifications by associated proteins to create active siderophores (1). Examples of siderophores made by other biosynthetic mechanisms are aerobactin, which is produced using a different family of synthetases (1), and thioquinolobactin. Thioquinolobactin is the product of two separated metabolic pathways involving tryptophan catabolism and the production of thiocarboxylate (33).

**TonB-dependent outer-membrane proteins**

Characteristics of TonB-dependent outer-membrane proteins (TBDP) include their location on the outer membrane (OM) and a molecular weight of 70-90 kDa. A 22-stranded β-barrel forms the pore through the outer membrane with an internal ~160 amino acid residue plug domain blocking the pore. The plug and β-barrel contribute to substrate binding. The residues binding the substrate vary among TBDPs having different substrate specificities (16, 23, 45). Extracellular loops of the β-barrel close over the channel when the substrate is bound, holding the substrate in place (45). Transport of substrates, siderophores and other bound molecules across the outer-membrane requires energy (16). TBDPs interact with the TonB protein complex to receive the needed energy, which it does through a motif called the TonB-box. The TonB-box is a heptapeptide located near the N-terminus of TBDPs (5, 38, 45). The proteins TonB, ExbB, and ExbD form an energy-transducing complex that harnesses proton motive force to provide energy to move substrates through the TonB-dependent outer-membrane protein (23, 27, 38). The accessory proteins ExbB and ExbD are anchored in the cytoplasmic membrane (CM) where they couple the proton gradient across the CM (16, 45). The domains of TonB are an N-terminal transmembrane domain acting as an anchor, a proline rich region, and residue 160 in the C-terminal region, which is important for interaction with the TBDP (49).

TonB-dependent transducers (TBDTs) are a subclass of TBDPs with an N-terminal extension that interacts with an anti-sigma factor, which then releases a cognate extra-cytoplasmic function (ECF) sigma factor to initiate a signaling cascade (45).

TBDTs are the first proteins of a signaling pathway initiated by substrate binding and ending with expression of substrate-specific transport genes. This signaling pathway was elucidated for *Escherichia coli* FecA, which binds ferric citrate (5) (Fig. 1.1). The binding of TonB to a TBDT is dependent on substrate or siderophore binding at the surface (16) and leads to ligand-dependent transcriptional regulation of an import operon, containing genes for the transport proteins used to move the substrate across the CM. The ~79 residue signaling domain of the TBDT at the N-terminus interacts with the C-terminus of the anti-sigma factor (14, 16). Anti-sigma factors have a leucine rich motif of repeating heptapeptides (residues 247-268) flanked by three leucines and a valine: residues 1-79 of the anti-sigma factor interact with residues 101-317 of the TBDT (5). The anti-sigma factor spans the CM and its N terminus contacts a specific ECF sigma factor in the cytoplasm (5, 23). Upon release from its anti-sigma factor, the ECF sigma factor promotes RNA polymerase binding to the promoter of the import operon (16) (Fig. 1).

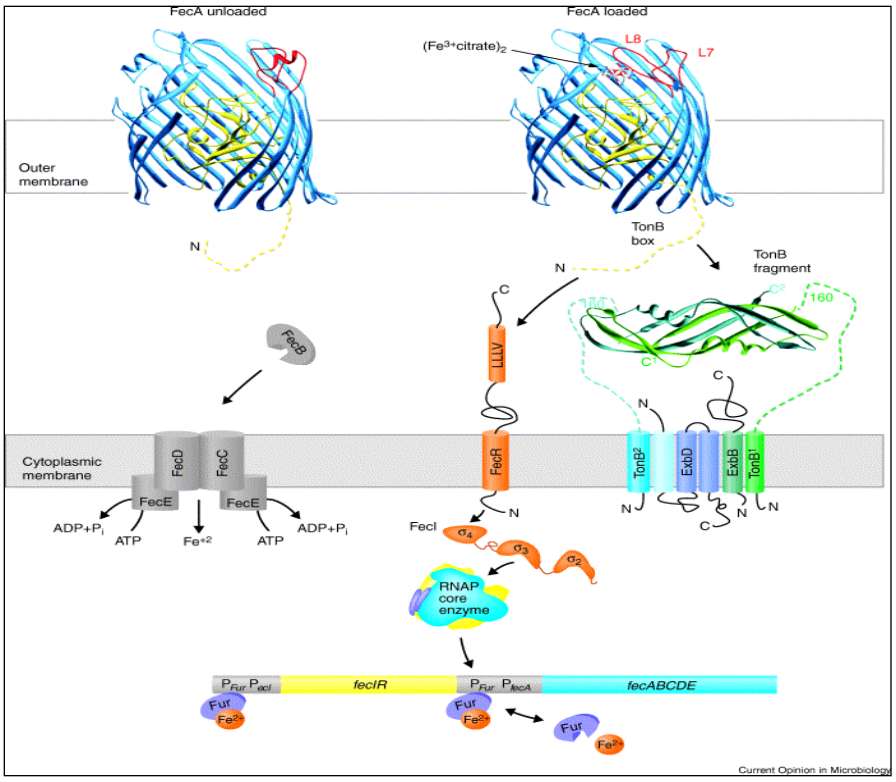


**Fig. 1.1. Model of signaling pathway as discovered in *E. coli* for the TonB-dependent transducer FecA (5).** Reprinted with permission from Elsevier.

**Regulation**

Genes coding for TBDTs that function as ferric-siderophore receptors are typically expressed preferentially under iron-limited conditions, and were therefore among the genes identified in microarrays evaluating iron-regulated gene expression by *P. aeruginosa* (42) and *P. syringae* (6). The ferric uptake regulator (Fur) protein plays a central role in iron-mediated regulation, serving as a transcriptional repressor under iron-replete conditions (41). Therefore, the presence of Fur binding sites was one criterion used to differentiate TBDPs functioning in iron-acquisition from those with other catabolic functions in *X. campestris* (4). A Fur-iron complex is formed when iron is present and binds to a DNA motif called the Fur box located upstream of the promoter, thus stopping transcription of the operon (15, 21, 50). Detailed analysis of the genomic regions surrounding each putative TBDP gene in Pf-5 for the presence of these conserved binding sites identified a Fur binding site upstream of *fpvA*, which encodes a ferric-pyoverdine receptor (Fig. 1.2) and in other genomic regions with TBDPs, but also identified six TBDP regions that lack Fur-binding sites and include genes with putative catabolic functions (Appendix 1).

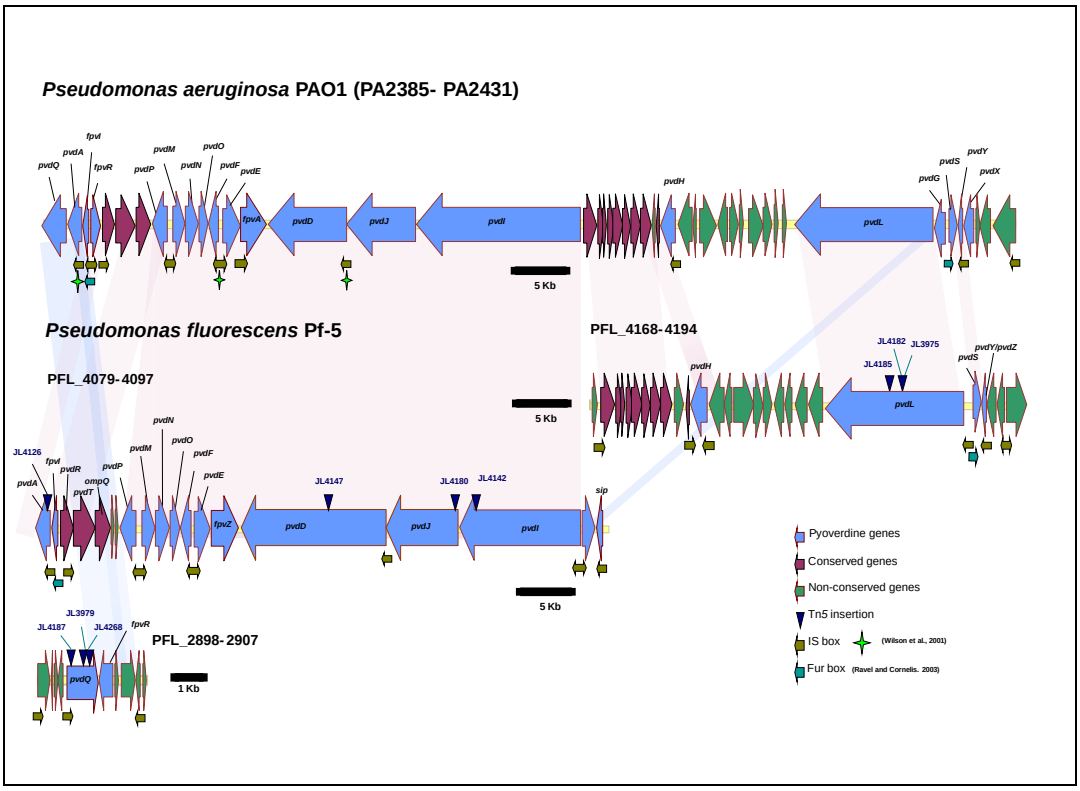


**Fig. 1.2. Comparison of *P. aeruginosa* PAO1 and *P. fluorescens* Pf-5 pyoverdine biosynthetic regions.** Illustration of the homologous genes, four Pf-5 gene regions, and Fur and PvdS binding sites.

Regulatory proteins, in addition to Fur, are involved in the expression of TBDPs. The ECF sigma factors, commonly encoded by genes located next to TBDP and anti-sigma factor genes, are integral in the regulation of TBDTs (11). The ECF sigma factor PvdS, called the iron starvation sigma factor, initiates pyoverdine siderophore production in low iron conditions by binding the iron starvation box (IS) motif to activate expression of siderophore biosynthetic genes (50). Two component regulatory protein pairs composed of a regulator and a sensor are also involved in the regulation of some TBDPs, specifically those involved in the uptake of enterobactin (13). AraC regulatory proteins are involved in the control of the pyochelin receptor in *P. aeruginosa* (37).

**Number of TonB-dependent outer-membrane proteins**

TonB-dependent outer-membrane proteins have long been known to function in ferric-siderophore uptake, vitamin B12 uptake, and phage recognition (45). Their broader functions in some bacteria, such as in uptake of sucrose (4), maltodextrins (28), nickel (48), sulfate (24), and other substrates, have been recognized more recently. Environmental bacterial tend to have more TBDPs than bacteria living in uniform environments, possibly because they occupy more diverse and dynamic habitats and need to adapt to continual changes in the nutrients available (4).

Gram-negative bacteria differ in the number of TonB-dependent outer-membrane proteins in their proteomes, with most strains having low numbers. In a survey of 226 Gram-negative bacterial genomes, 71% had less than 14 TBDPs and 16% had more than 30 (4). *Pseudomonas* species are among the bacteria with the largest numbers of TBDPs. Pf-5 has 45 TBDPs, which is on the high end of the range for *Pseudomonas* spp. (43). Of the 45 TonB-dependent outer-membrane proteins of Pf-5, 28 are similar to siderophore receptors of other *Pseudomonas* spp. (43). Eleven of the 36 TBDPs in the *P. aeruginosa* proteome are known to function in iron acquisition, with eight TBDPs having an established role in the uptake of microbial ferric-siderophore complexes (19). From a phylogenetic analysis of the TBDPs of Pf-5 and *P. aeruginosa* (43), candidate TBDPs

likely to function in iron-acquisition of Pf-5 have been identified, but there are many other TBDPs that are unique to Pf-5 which do not appear to function in iron acquisition. The TBDPs not linked to iron uptake may play important roles that enable Pf-5 to adapt to and colonize the changing environments of seed and root surfaces.

Why does Pf-5 have so many TBDPs? Pf-5 is able to colonize plant surfaces as well as live in the soil where TBDPs may play a role in enhancing access to limited resources, like iron (9). A study on rhizosphere populations of fluorescent *Pseudomonas* spp. found that siderophore production conferred the advantage of higher populations on roots (46). Strains able to take up a siderophore produced by another rhizosphere bacterium were better able to compete on the root surface (46). The ability of *Pseudomonas* spp. to use heterologous siderophores also was shown in *Pseudomonas putida* using an ice nucleation reporter gene fused to an iron regulated promoter (29). The presence of siderophore-producing strains of *P. putida* and *Enterobacter cloacae* increased iron availability to *P. putida* in the rhizosphere of cucumbers (29). The utilization of carbon substrates found on and around plant surfaces is a likely role for a subset of the TBDPs in Pf-5. The study conducted by Blanvillain et al. (4) described a TBDP for sucrose uptake, indicating that TBDPs have expanded roles beyond iron and B12 uptake. In the genome of Pf-5, there are genes for the catabolism and uptake of a wide range of organic compounds found in seed and root exudates (30).

**Pyoverdine Uptake**

The fluorescent pseudomonads are characterized by the production of pyoverdine siderophores, composed of a dihydroxyquinoline chromophore, which is responsible for diffusible green fluorescence, an acyl side chain (either dicarboxylic acid or amide) bound to the amino group of the chromophore, and a peptide chain of variable length and composition. The structures of more than 70 pyoverdines from different strains and species of *Pseudomonas* have now been determined (7, 35). Different strains of *P. aeruginosa* produce pyoverdines falling into three structural groups, and two TBDTs

(termed FpvA and FpvB for ferric-pyoverdine uptake) are responsible for the uptake of these ferric-pyoverdines. A far more complex structure-function relationship must exist in Pf-5, which produces two siderophores (a pyoverdine and enantio-pyochelin) and six putative ferric-pyoverdine transducers (here termed FpvU to FpvZ) (43), which are likely to be responsible for its capacity to utilize ferric-pyoverdines from many *Pseudomonas* strains as a source of iron.

**Ferric-pyoverdine TonB-dependent outer-membrane proteins**

TonB-dependent outer-membrane proteins for the uptake of pyoverdine were initially characterized in plant growth promoting *Pseudomonas* spp. (Table 1.1). *Pseudomonas* sp. B10 and *P. putida* WCS358 were investigated for the presence of receptor proteins required for the uptake of the cognate pyoverdines (then called pseudobactins) (31-32). Receptor proteins in both strains were identified as being in the size range for TBDPs and expressed in low iron conditions (31-32). Further research showed that WCS358 has multiple receptors for pyoverdine uptake with PupA for the uptake of the cognate pyoverdine and PupB for heterologous pyoverdines (3, 25). Subsequently, four TBDPs for the uptake of pyoverdines were found in *Pseudomonas* sp. strain M114, with PbuA identified for uptake of its own pyoverdine (39).

**Table 1.1. Characterized pyoverdine TonB-dependent outer-membrane proteins and associated pyoverdines**

| Pyoverdine TBDP | Producing strain and pyoverdine peptide chain | Reference |
|---|---|---|
| FpvA | *P. fluorescens* ATCC13525: Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (20, 36) |
| | *P. chlororaphis* ATCC9446: Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | |
| | *P. aeruginosa* PAO1: Ser–Arg–Ser–FOHOrn–(Lys–FOHOrn–Thr–Thr) | |
| | *P. fluorescens* DSM 50106: Ser–Lys–Gly–FOHOrn–Ser–Ser–Gly–(Orn–FOHOrn–Ser) | |
| | *P. fluorescens* Pfl 18.1: Ser–Lys–Gly–FOHOrn–Ser–Ser–Gly–(Lys–FOHOrn–Ser) | |
| FpvB | *P. aeruginosa* PA01: Ser–Arg–Ser–FOHOrn–(Lys–FOHOrn–Thr–Thr) (Type I pyoverdine) | (18) |
| FpvAII | *P. aeruginosa* ATCC27853: Ser-FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) | (34) |
| | *P. fluorescens* PL7: Ser-AcOHOrn-Ala-Gly-aThr-Ala-cOHOrn | |
| | *P. fluorescens* PL8:Lys-AcOHOrn-Ala-Gly-aThr-Ser-cOHOrn | |
| FpvAIII | *P. aeruginosa* 7NSK2: Ser-cDab-FOHOrn-Gln-Gln-FOHOrn-Gly (Type III pyoverdine) | (12) |
| PupB | Bn7: Unknown | (26) |
| PupA | WCS358: Asp-εLys-OHAsp-Ser-aThr-Ala-Thr-Lys-cOHOrn | (32) |
| WCS358 RF2 | *P. fluorescens* WCS374: Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (25) |
| | *P. fluorescens* PAO1: Ser–Arg–Ser–FOHOrn–(Lys–FOHOrn–Thr–Thr) | |
| WCS358 RF3 | *P. fluorescens* B10: εLys-OHAsp-Ala-aThr-Ala-cOHOrn | (25) |
| PbuA | *P. fluorescens* B10: εLys-OHAsp-Ala-aThr-Ala-cOHOrn | (39) |
| PupX | *P. fluorescens* B10: εLys-OHAsp-Ala-aThr-Ala-cOHOrn | (31, 39) |

Underline denotes D-amino acids. Parentheses define cyclic residues. cOHOrn is cyclo-hydroxy-ornithine. FOHOrn is δN-formyl- δN-hydroxy-ornithine. εLys is Lys linked by ε-NH2. OHAsp is threo-β-hydroxy-aspartic acid. Dab is diamino-butanoic acid. aThr is allo-Thr. AcOHOrn is δN-acetyl- δN-hydroxy-ornithine.

The opportunistic pathogen *P. aeruginosa* has the best studied Fpv outer-membrane proteins. Initial studies of the type strain and clinical strains identified two outer-membrane proteins with the potential for pyoverdine uptake (10). FpvA and FpvB from *P. aeruginosa* strains are present in different forms depending on the strain and are used for the uptake of the three pyoverdine types produced by *P. aeruginosa* strains and heterologous pyoverdines (12, 34, 44). The crystal structure of FpvA along with mechanistic analysis relating to pyoverdine binding and uptake have revealed how this receptor binds and moves ferric-pyoverdine across the outer membrane through interactions with both the plug and β-barrel (8, 40). A recent survey of fluorescent pseudomonads found variation in the number of the Fpv outer-membrane proteins among and within the species surveyed (9). As further research is done to characterize the TBDPs used for pyoverdine uptake, such as the work on Pf-5 presented here, variation of these proteins within the fluorescent pseudomonads will be elucidated.

**Research objectives**

TonB-dependent outer-membrane proteins of *P. fluorescens* Pf-5 are of interest due to their presence in high numbers within this strain. The characterization of TBDPs within Pf-5 and comparison to other fluorescent pseudomonads will aid in the understanding of how these environmental bacteria survive in complex conditions, particularly those relating to agricultural systems.

The first objective of my research was to analyze the 45 TBDPs in the proteome of *P. fluorescens* Pf-5, which consisted of categorizing the TBDPs as receptors or transducers based on the presence of characteristic domains. I then assigned putative functions based on amino acid similarity to characterized TBDPs and the functions and arrangement of associated genes. The putative functions of some TBDPs were determined by bioassays. A Pf-5 mutant deficient in the production of its own siderophores (pyoverdine and enantio-pyochelin) was derived and evaluated for growth in the presence of heterologous ferric-siderophores and iron-containing molecules.

Upon sequencing the genome of Pf-5, initial annotation identified six putative TonB-dependent outer-membrane proteins for the uptake of ferric-pyoverdines. The second objective of my research was to assess the roles of the six Fpv outer-membrane proteins in the uptake of a diverse set of ferric-pyoverdines. Pf-5 mutants deficient in *fpv* genes were tested for crossfeeding by pyoverdine-producing strains of *Pseudomonas* and uptake of purified pyoverdines. A survey of the literature indicates that some Fpv outer-membrane proteins recognize one pyoverdine structure (25, 31, 39) whereas other Fpv outer-membrane proteins recognize structurally-related pyoverdines (20, 34, 36). I hypothesize that the six Fpv outer-membrane proteins in Pf-5 could function in three ways; each Fpv recognizes a specific pyoverdine, each Fpv recognizes a distinct set of structurally-related pyoverdines, or two or more Fpv outer-membrane proteins recognize the same pyoverdine.

The results presented in Chapters 2 and 3 show that Pf-5 has TBDPs for the uptake of a variety of substrates primarily involved in the acquisition of iron. The roles of TBDPs in Pf-5 can be used as a platform for investigation of TBDPs in other fluorescent pseudomonads. Chapter 4 illustrates how, using Pf-5 as a template, a core set of five conserved TBDPs was identified for ten strains of *Pseudomonas fluorescens* and related species (termed the *P. fluorescens* group). The diversity of Fpv outer-membrane proteins present in strains of the *P. fluorescens* group also was assessed. The biological and genomic diversity of the ten strains of the *P. fluorescens* group is reflected in the diversity of the types of TBDPs present within the individual strains as well as those shared between the more related strains.

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# Chapter 2

## TonB-dependent Outer-membrane Proteins and Siderophore Utilization in *Pseudomonas fluorescens* Pf-5

Sierra L. Hartney, Sylvie Mazurier, Teresa A. Kidarsa, Maria Carolina Quecine, Philippe Lemanceau and Joyce E. Loper

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**Abstract**

The soil bacterium *Pseudomonas fluorescens* Pf-5 produces two siderophores, a pyoverdine and enantio-pyochelin, and its proteome includes 45 TonB-dependent outer-membrane proteins, which commonly function in uptake of siderophores and other substrates from the environment. The 45 proteins share the conserved β-barrel and plug domains of TonB-dependent outer-membrane proteins but only 18 of them have an N-terminal signaling domain characteristic of TonB-dependent transducers (TBDTs), which participate in cell-surface signaling systems. Phylogenetic analyses of the 18 TBDTs and 27 TonB-dependent receptors (TBDRs), which lack the N-terminal signaling domain, suggest a complex evolutionary history including horizontal transfer among different microbial lineages. Putative functions were assigned to certain TBDRs and TBDTs in clades including well-characterized orthologs from other *Pseudomonas* spp. A mutant of Pf-5 with deletions in pyoverdine and enantio-pyochelin biosynthesis genes was constructed and characterized for iron-limited growth and utilization of a spectrum of siderophores. The mutant could utilize as iron sources a large number of pyoverdines with diverse structures as well as ferric citrate, heme, and the siderophores ferrichrome, ferrioxamine B, enterobactin, and aerobactin. The diversity and complexity of the TBDTs and TBDRs with roles in iron uptake clearly indicate the importance of iron in the fitness and survival of Pf-5 in the environment.

## Introduction

TonB-dependent outer-membrane proteins are important components of the bacterial cellular machinery for the uptake of substrates from the environment. These proteins bind with high affinity to specific substrates external to the cell as the first step in the energy-dependent transport of the substrate into the periplasmic space. The energy for transport across the outer membrane is supplied by TonB proteins (74). TonB-dependent outer-membrane proteins are best known as receptors for siderophores, high-affinity iron-chelating compounds that are produced by microorganisms under iron-limiting conditions. Siderophores are exported from the cell, where they chelate ferric ions in the environment. Specific ferric-siderophore complexes are recognized by cognate TonB-dependent outer-membrane proteins, which initiate the process of iron transport into the cell where the iron becomes available for metabolic functions (38). The roles of TonB-dependent outer-membrane proteins as receptors for siderophores, vitamin B12, and certain phages have been recognized for decades (74) but their broader functions in the uptake of sucrose (7), maltodextrins (51), nickel (80), sulfate (44), and other substrates have been recognized only recently. Most bacteria have less than 14 TonB-dependent outer-membrane proteins in their proteomes but certain environmental bacteria, such as *Caulobacter crescentus* (27) and *Xanthomonas campestris* pv. *campestris* have very large numbers (7). This is also the case for *Pseudomonas fluorescens* Pf-5, a well-characterized soil bacterium that colonizes seed and root surfaces and protects plants from infection by certain soil-borne plant pathogens (52). The proteome of *P. fluorescens* Pf-5 includes 45 TonB-dependent outer-membrane proteins.

In environments in which iron is limited, fluorescent pseudomonads such as *P. fluorescens* produce pyoverdines. These siderophores are composed of a dihydroxyquinoline chromophore, an acyl side chain (either dicarboxylic acid or amide) bound to the amino group of the chromophore, and a peptide chain of variable length and composition. The structures of more than 70 pyoverdines from different strains and species of *Pseudomonas* have now been determined (64). Strains of *P. aeruginosa*

produce pyoverdines falling into three structural groups (66), and two of the 34 TonB-dependent outer-membrane proteins in the proteome of PAO1 are responsible for the uptake of these ferric-pyoverdines (49). Other *Pseudomonas* spp. differ in the range of pyoverdine structures that they can utilize as iron sources. *P. entomophila* L48 utilizes a wide range of pyoverdines, whereas the related species *P. putida* KT2440 can utilize relatively few of these siderophores to acquire iron from the environment (57). In addition to pyoverdine, a second siderophore having a lower affinity for iron than pyoverdine is produced by many strains of *Pseudomonas* spp. (14). For example, pyochelin is produced by *P. aeruginosa*, and its optical antipode enantio-pyochelin is produced by *P. fluorescens* Pf-5 (94). Furthermore, pseudomonads have a remarkable capacity to utilize heterologous siderophores produced by diverse taxa of bacteria and fungi (17). Of the 34 TonB-dependent outer-membrane proteins in the proteome of *P. aeruginosa* PAO1, eight serve as receptors for the heterologous siderophores enterobactin, aerobactin, ferrichrome, ferrioxamine B, heme or ferric-citrate (15-16). A more complex structure-function relationship likely exists in *P. fluorescens* Pf-5, with its 45 TonB-dependent outer-membrane proteins including six putative ferric-pyoverdine receptors (71).

In addition to their roles as outer membrane receptors, certain TonB-dependent outer-membrane proteins serve as components of cell-surface signaling (CSS) systems used by bacteria to sense signals from the extracellular medium and transmit them into the cytoplasm (28). Typically, CSS systems have three components: an alternative sigma factor of the extracytoplasmic function (ECF) family, a sigma factor regulator (anti-sigma factor) located in the cytoplasmic membrane, and a TonB-dependent outer-membrane protein having an N-terminal signaling domain. This signaling domain interacts with the C-terminus of the cognate anti-sigma factor, which releases the ECF sigma factor to function in transcription of specific target genes (28). Therefore, upon substrate binding, TonB-dependent outer-membrane proteins having the N-terminal signaling domain initiate a signaling pathway that controls the transcription of target genes. Genes encoding the three CSS components are typically clustered in the bacterial genome.

In this study, a combination of bioinformatic, phylogenetic and functional analyses were employed to characterize the 45 TonB-dependent outer-membrane proteins of *P. fluorescens* Pf-5. Motifs defining constituent domains were identified and the presence or absence of the N-terminal signaling domain was used to distinguish the 27 TonB-dependent receptors (TBDRs) from the 18 TonB-dependent transducers (TBDTs) in the Pf-5 proteome. Phylogenetic analyses of the TonB-dependent outer-membrane proteins from Pf-5 and characterized orthologs from other *Pseudomonas* spp. allowed the assignment of putative functions to certain Pf-5 TBDRs and TBDTs. A mutant of Pf-5 with deletions in pyoverdine and enantio-pyochelin biosynthesis genes was constructed and characterized for iron-limited growth and utilization of a spectrum of siderophores. Pf-5 exhibited a remarkable capacity to utilize pyoverdines with diverse structures produced by different *Pseudomonas* spp., as well as ferric citrate, heme, and the siderophores ferrichrome, ferrioxamine B, enterobactin, and aerobactin.

## Materials and methods

### Bacterial strains and growth conditions

*Pseudomonas* strains were grown on King's medium B (KMB) (45) at 27$^{o}$C. *Escherichia coli* and *E. cloacae* were grown on Luria-Bertani (LB) at 37$^{o}$C. Antibiotics were used at the following concentrations (μg/ml): gentamicin (Gm) 40 (*P. fluorescens*) and 12.5 (*E. coli*), kanamycin (Km) 50, streptomycin (Sm) 100, tetracycline (Tet) 200 (*P. fluorescens*) and 20 (*E. coli*).

### Pyoverdine peptide chain prediction

Pyoverdines produced by many strains of *Pseudomonas* spp. have unknown structures, but the amino acid composition of the peptide chain of these pyoverdines can be predicted bioinformatically from the nucleotide sequences of genes encoding the corresponding non-ribosomal peptide synthetases (NRPSs). Predicted amino acid sequences for the NRPSs for each strain were submitted to the NRPS/PKS predictor (2) and the NRPS predictor (http://www-

ab.informatik.uni-tuebingen.de/software/NRPSpredictor) which uses the methods of (84) and (76).

**Sequence compilation and domain analysis**

Alignments of amino acid sequences of the TonB-dependent outer-membrane proteins of Pf-5 were done using the multiple sequence alignment tool T-Coffee (69). Characteristic domains of TonB-dependent outer-membrane proteins were identified according to Pfam (31), using default settings with an E-value cutoff of 1.0. Additional domain analysis was done using the EMBL_EBI InterProScan domain search tool.

**Secondary structure prediction**

PSIPRED GenTHREADER (58) and a beta barrel prediction model (5) were used to predict secondary structure of the 45 TonB-dependent outer-membrane proteins in the Pf-5 genome.

**Phylogenetic analysis**

Amino acid sequences of TonB-dependent outer-membrane proteins were submitted to the NCBI database of non-redundant protein sequences to identify the five to ten best hits for each using the PSI-BLAST algorithm (1). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0.2 (87). The Clustal W (90) based alignment option with a gap open penalty of 15 and a gap extension penalty of 0.3 was used to align the amino acid sequences. The aligned sequences were masked to remove gaps. The masked sequences were then subjected to bootstrapped maximum parsimony analysis. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The maximum parsimony tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random

addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The % GC for each gene encoding a TonB-dependent outer membrane protein was compiled and those differing significantly from the Pf-5 genomic average of 63.3% were identified by chi square analysis.

**Construction of mutants of Pf-5**

Mutants of Pf-5 were constructed using overlap extension PCR methods modified from Choi and Schweizer (12). The *ofaA* (PFL_2145) mutant of Pf-5 is described in Hassan et al. (37). The Δ*pvdI* deletion mutant was made by the method described in Hassan et al. (2010) using the primers pyv UpF-Bam, pyv UpR-FRT, pyv DnF-FRT, and pyv DnR-Bam (Table 2.1). The *pchA* and *pchC* gene constructs were made by modified methods as described below. The *pchC* (PFL_3490) gene was amplified with primers PFL3490-Up and PFL3490-Low (Table 2.1) using iProof DNA polymerase (Bio-Rad, Hercules, CA) and cloned into pCR-blunt (Invitrogen, Carlsbad, CA). The GmR-*gfp* gene cassette was amplified from pPS858 (39) with primers Gm-F and Gm-R using KOD DNA polymerase (Novagen (Merk), Darmstadt, Germany). The GmR-gfp cassette was used to interrupt the *pchC* gene by cloning into a unique *PshAI* site. The interrupted *pchC* gene was re-amplified with PFL3490-Up and PFL3490-Low primers using KOD DNA polymerase and ligated into the *SmaI* site of pEX18Tc (39). This construct was introduced into Pf-5 as described in Hassan et al. (37). The *pchA* (PFL_3488) gene construct was made by PCR amplification of 5' and 3' regions of *pchA* with the primers 3488 UpFHind, 3488 UpR, 3488 DnF, and 3488 DnRHind (Table 2.1). The resulting PCR products were combined in a second round of PCR with the primers 3488 UpFHind and 3488 DnRHind added during the 3$^{rd}$-cycle extension, yielding a product consisting of the 5' and 3' regions of the *pchA* gene with the middle portion of the gene deleted. The final PCR product was digested with *HindIII* and cloned into pEX18Tc (39). The *pchA* deletion construct was transformed into One Shot TOP10 Chemically Competent *E. coli* (Invitrogen) and then into the mobilizing strain *E. coli* S17-1 (83). Pf-5 transconjugants

were selected on KMB (45) with streptomycin (100 µg/ml, innate resistance of Pf-5) and tetracycline (200 µg/ml). Resulting colonies were grown for 3 h without selection in LB broth and plated on LB with 5% sucrose to favor growth of resolved merodiploids. Colonies growing on sucrose were patched onto KMB containing tetracycline (200 µg/ml) to confirm resolution of merodiploids. Tetracyline-sensitive clones were screened for presence of the *pchA* deletion by PCR and the PCR product sequenced to confirm correct incorporation of the deleted allele.

**Table 2.1. Primers used in the construction of mutants of *P. fluorescens* Pf-5**

| Primer | Sequence 5'-3' |
|---|---|
| *pchA* | |
| 3488 UpFHind | GACGAAGACGAAGCTTTTCTACCTGCGCGAGCAACA |
| 3488 UpR | TGCTCGCGGATAACAGGCAGGATTCACTCATC |
| 3488 DnF | AATCCTGCCTGTTATCCGCGAGCATGAGCAA |
| 3488 DnRHind | GTGGTTGTGGAAGCTTATTCCTTCGCCATAAACCGC |
| *pvdI* | |
| pyv UpF-Bam | CTCTGCTTCTGGATCCTCGGTTTCTTCGTCAACACC |
| pyv UpR-FRT | TCAGAGCGCTTTTGAAGCTAATTCGGAGGTGTAGATCGAATAGGC |
| pyv DnF-FRT | AGGAACTTCAAGATCCCCAATTCGTGCTGGATGCATCCTTGCAA |
| pyv DnR-Bam | CACACCATCAGGATCCATCTGCCAGAACAGCCATTG |
| *pchC* | |
| PFL3490-Up | CGGCCAGGCTGTACACCAC |
| PFL3490-Low | TACCTGAGCACCGAGCAGC |
| Gm-F | CGAATTAGCTTCAAAAGCGCTCTGA |
| Gm-R | CGAATTGGGGATCTTGAAGTTCCT |

**Arbitrary PCR**

Tn*5* insertions in an extant set of pyoverdine-deficient mutants (48) were mapped by using arbitrary PCR. Genomic DNA flanking the Tn*5* insertion was amplified in two rounds of PCR reactions. In the first round, Primer 1 was complementary to sequences of Tn*5* and Primer 2 was a degenerate primer. The 5' end of the degenerate primer was 5'-GGTCCG, a sequence that occurs 350 times, at an average of every 600 bp, in the pyoverdine region of Pf-5. This primer also contained 10 random nucleotides and a previously-described 20-nucleotide sequence (21). Round 2, Primer 1 was composed of the 3' 20 nucleotides from the round one degenerate primer. Round 2, Primer 2 was complementary to Tn*5* at a location internal to the Round 1, Primer 1 sequence. The final product was sequenced to identify the DNA flanking the Tn*5* insertion.

Round 1, Primer 1: 5'-GGGCAGTACGGCGAGGAT-3'

Round 1, Primer 2: 5'-GGTCCGNNNNNNNNNNACTGATCAGCTGCGCACCGG-3'

Round 2, Primer 1: 5'-ACTGATCAGCTGCGCACCGG-3'

Round 2, Primer 2: 5'-CCTTTCTGATCGCCTCGG-3'

**Iron limited growth**

Pf-5 and derivative strains were tested for iron limited growth on KMB containing the iron chelator 2,2'-dipyridyl (Sigma-Aldrich, St Louis, MO) at 0, 100, 200, 400, 600, and 800 µM. Bacterial cells from overnight cultures grown in KMB broth were collected by centrifugation and suspended in water to 0.1 $OD_{600}$. This suspension was diluted to $10^{-2}$, 5 µl of the diluted cell suspension was placed on the agar surface, and bacterial growth was observed following 24 hrs incubation at 27$^{o}$C. Each strain was tested in at least two experiments, each evaluating two replicate plates.

**Enantio-pyochelin extraction and detection**

Production of enantio-pyochelin in Pf-5 and derivative strains was analyzed using the following method: for each treatment, four tubes each containing 5 ml M9 minimal medium (78) broth were inoculated with 5µl of overnight culture and incubated at 27$^{o}$C for 48 hrs at 200 rpm. Two cultures were combined for each of two replicates and centrifuged at 7000 rpm for 10 min. Supernatants were decanted into 50 ml polypropylene conical screw-cap centrifuge tubes and adjusted to pH 2.0 with 1M HCl. The enantio-pyochelin was extracted by adding 0.5 volumes ethyl acetate and vortexing. The organic and aqueous phases were separated by centrifugation at 7000 rpm for 10 min. The organic top layer was transferred to 5 ml glass tubes and dried under vacuum. Dried samples were resuspended in 100 µl methanol and stored at -20 $^{o}$C. Enantio-pyochelin extracts were separated on thin layer chromatography plates (silica gel 60 $F_{254}$ on aluminum, EM Science, Gibbstown, NJ) using n-butyl alcohol/water/acetic acid 4:1:1 (v/v/v) as the mobile phase (94). Compounds were viewed by fluorescence at 365 nm and by spraying with 2 M $FeCl_3$ in 0.1 M HCl.

**CAS agar assay**

Pf-5 and mutants were tested for siderophore production by observing zones surrounding colonies grown on CAS (Chrome azurol S) agar for pseudomonads (81). Ten µL of a 0.1 $OD_{600}$ cell suspension was spotted on the agar surface; plates were incubated at 27°C, and observed for zone formation. Each mutant was tested in at least two experiments, each evaluating two replicate plates. In some experiments, CAS agar was amended with $FeCl_3$ to a final concentration of 1 mM.

**Crossfeeding assays**

*Pseudomonas* spp. producing diverse pyoverdines (test strains presented in Table 2.2) were evaluated for their capacities to provide iron to the Δ*pvdI-pchC* mutant of Pf-5 (indicator strain) in crossfeeding experiments. Cells from test strains and the indicator

strain were collected from overnight cultures grown in KMB broth and suspended in water to 0.1 $OD_{600}$. Cell suspensions of the indicator strain were further diluted to $10^{-2}$ in sterile water. Ten µL each test strain suspension was placed on the surface of KMB amended with 2,2'-dipyridyl at 400 µM or 600 µM. Five µL of the diluted cell suspension of the indicator strain was spotted on the agar surface at a distance of 1 cm from each test strain. An alternative method was used for those test strains that did not grow on KMB amended with 2,2'-dipyridyl at 400 µM or 600 µM. For those strains, an agar plug (6mm) obtained from a 48 hr culture on KMB was substituted for the cell suspension on the surface of the test plate. Plates were incubated at 27$^{o}$C, and growth of the indicator strain was observed at 24 hr and 36-48 hr. Each test strain was evaluated in at least two experiments, each evaluating two replicate plates.

**Siderophore utilization assays**

The capacity of Pf-5 to utilize specific ferric-siderophore complexes as sources of iron was evaluated. Cells of a Δ*pvdI-pchC* mutant of Pf-5 were collected from overnight cultures grown in KMB broth, suspended in water to 0.1 $OD_{600}$, diluted to $10^{-2}$ in sterile water, and 100 µL of the diluted sample was spread on the surface of KMB amended with 400 µM or 600 µM 2,2'-dipyridyl. Filter paper disks (5 mm diameter) were placed at the center of the agar surface, and 10 µL of a purified siderophore solution or water (negative control) was placed on the filter paper disk. Plates were incubated at 27°C for 24 hr and then scored for the presence of bacterial growth in a halo surrounding the disk. The following compounds were tested: 20 mM ferric citrate in water, 7.7 mM hemin chloride in 10 mM NaOH, 5 mg hemoglobin in 1 ml PBS (phosphate-buffered saline), 20mM desferrioxamine in 10 mM Tris-HCl pH 8.8, and 10 mM ferrichrome in 0.5 M Tris-HCl, pH 8.8. All of the compounds were obtained from Sigma-Aldrich. Each assay was done twice, with each experiment evaluating two replicate plates.

## Results

### Identification of conserved domains within the TonB-dependent outer-membrane proteins of *P. fluorescens* Pf-5

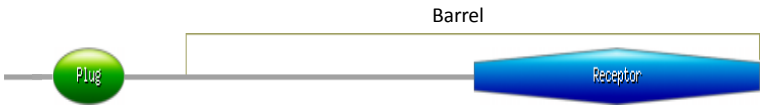
Analysis of the amino acid sequences of each of the 45 TonB-dependent outer-membrane proteins in the Pf-5 proteome revealed the conserved transmembrane pore and receptor domains of this protein family. Sequences characteristic of an outer membrane-spanning pore, formed by a β-barrel made up of repeated β-strands (Interpro: IPR000531) were identified in all 45 deduced peptide sequences (Fig. 2.1). Two domains involved in substrate binding, a receptor domain (Pfam: PF00593) comprising a highly conserved region of the pore, and a plug domain (Pfam: PF07715) (13, 72, 82), were also identified in 43 TonB-dependent outer-membrane proteins. The receptor domain was not identified in the proteins PFL_2919 and PFL_3612. A TonB box, defined as the five to seven amino acids required for interaction with TonB (73), was not identified consistently in the 45 proteins following analysis of sequence alignments with known TonB boxes in other *Pseudomonas* spp. The lack of conservation of this motif across the TonB-dependent outer-membrane proteins of Pf-5 may be related to the presence of four putative TonB proteins in the Pf-5 genome (71). Multiple copies of TonB are also present in other species of *Pseudomonas* (42, 95).

a. Location of transducer domains (amino acid)


| Locus Tag | STN | Plug | Receptor | Barrel | Length |
|---|---|---|---|---|---|
| PFL_4092 | 67-116 | 159-263 | 570-821 | 274-822, 278-822 | 822 |
| PFL_2391 | 74-125 | 168-272 | 579-822 | 283-823, 286-823 | 823 |
| PFL_2527 | 73-124 | 166-267 | 584-808 | 276-809, 282-809 | 809 |
| PFL_3315 | 66-116 | 159-262 | 576-823 | 273-824, 277-824 | 824 |
| PFL_3485 | 66-115 | 157-260 | 562-804 | 270-806, 274-806 | 806 |
| PFL_0125 | 98-149 | 191-298 | 609-855 | 311-856 | 856 |
| PFL_0147 | 68-117 | 161-267 | 579-820 | 280-821 | 821 |
| PFL_0982 | 111-162 | 190-305 | 586-834 | 317-835 | 835 |
| PFL_0995 | 71-122 | 160-259 | 567-807 | 278-811 | 811 |
| PFL_1371 | 55-105 | 128-236 | 566-850 | 319-853 | 853 |
| PFL_2293 | 65-116 | 158-263 | 582-823 | 277-824 | 824 |
| PFL_2365 | 64-107 | 127-235 | 662-942 | 361-944 | 944 |
| PFL_3154 | 77-128 | 151-255 | 571-827 | 280-828 | 828 |
| PFL_3612 | 62-113 | 131-258 | N/A | 354-931 | 931 |
| PFL_4039 | 70-120 | 149-264 | 557-819 | 273-821 | 821 |
| PFL_4627 | 76-127 | 148-259 | 598-871 | 305-872 | 872 |
| PFL_5378 | 74-122 | 152-261 | 591-900 | 355-901 | 901 |
| PFL_5706 | 61-112 | 154-255 | 565-808 | 269-809 | 809 |

**Fig. 2.1. Schematic representation of TonB-dependent outer-membrane proteins in the Pf-5 genome.** **a**. The TonB-dependent Transducers (TBDTs) have an additional N-terminal signaling domain (STN) (Pf07660). **b**. Conserved domains identified in the 28 TonB-dependent Receptors (TBDRs) include an outer membrane-spanning pore formed by a β-barrel made up of repeated β-strands (Interpro: IPR000531), and a receptor (Pfam: PF00593) and plug (Pf07715) involved in substrate binding.

b. Location of receptor domains (amino acid)




| Locus Tag | Plug | Receptor | Barrel | Length |
|---|---|---|---|---|
| PFL_5511 | 71-178 | 424-655 | 186-654 | 654 |
| PFL_4912 | 45-151 | 420-679 | 238-679 | 679 |
| PFL_5169 | 81-194 | 459-712 | 205-712 | 712 |
| PFL_4063 | 28-130 | 450-691 | 144-691 | 691 |
| PFL_3620 | 57-173 | 447-696 | 203-696 | 696 |
| PFL_2240 | 96-205 | 459-697 | 217-695 | 697 |
| PFL_0310 | 60-156 | 462-699 | 171-699 | 699 |
| PFL_0932 | 74-174 | 477-711 | 187-711 | 711 |
| PFL_2772 | 52-163 | 442-707 | 195-707 | 708 |
| PFL_1740 | 46-156 | 563-708 | 186-708 | 708 |
| PFL_0646 | 58-163 | 468-709 | 180-709 | 709 |
| PFL_0648 | 64-173 | 449-710 | 179-710 | 710 |
| PFL_2604 | 57-157 | 477-711 | 173-711 | 711 |
| PFL_1417 | 71-171 | 474-718 | 184-718 | 718 |
| PFL_3835 | 69-169 | 485-727 | 180-727 | 727 |
| PFL_3498 | 97-199 | 503-743 | 212-743 | 743 |
| PFL_2663 | 50-170 | 465-746 | 181-746 | 746 |
| PFL_0992 | 47-158 | 487-746 | 196-746 | 746 |
| PFL_0255 | 101-210 | 523-784 | 244-784 | 784 |
| PFL_3176 | 58-167 | 487-756 | 201-756 | 756 |
| PFL_0864 | 78-178 | 512-761 | 191-761 | 761 |
| PFL_2970 | 122-227 | 530-770 | 243-770 | 770 |
| PFL_0213 | 73-181 | 518-790 | 186-790 | 790 |
| PFL_1386 | 50-172 | 507-788 | 265-822 | 822 |
| PFL_3177 | 53-177 | 510-823 | 272-823 | 823 |
| PFL_3715 | 52-158 | 567-839 | 254-839 | 840 |
| PFL_2919 | 65-161 | N/A | 189-859 | 859 |

**Fig. 2.1., Continued**

An N-terminal signaling domain (Pfam:PF07660), which is known to interact with regulatory proteins controlling the expression of ECF sigma factors (28), was identified in 18 of the 45 TonB-dependent outer-membrane proteins (Fig. 2.1). Seventeen of the genes encoding these proteins are immediately adjacent to or clustered with genes encoding ECF sigma factors and associated regulatory proteins (anti-sigma factors) in the Pf-5 genome (Fig. 2.2). One gene (PFL_4092) is located in a pyoverdine biosynthesis gene cluster also containing the corresponding ECF sigma factor gene FpvI (PFL_4080), but the corresponding anti-sigma factor encoding gene FpvR (PFL_2903) is distal in the genome.

The 27 TonB-dependent outer-membrane proteins lacking an N-terminal signaling domain range in length from 654 to 859 amino acids (72.9-93.8 kDa) whereas the 18 proteins having an N-terminal signaling domain are typically larger, ranging from 806 to 944 amino acids (88.05-104.48 kDa) (Fig. 2.1). Alignment of all 45 proteins showed a lack of conservation over much of the sequence between the groups, which is due partially to differences in protein length. Therefore, our phylogenetic analyses considered the TBDRs and TBDTs separately, revealing differences that could, in some cases, be assigned to distinct substrates.

**a**

| | Linked anti-sigma | Linked ECF sigma factor | GC content of TBDR |
|---|---|---|---|
| **Pyoverdine** | | | |
| PFL_4092 | PFL_2903 | PFL_4080 | 62 |
| PFL_2391 | PFL_2392 | PFL_2393 | 64 |
| PFL_2527 | PFL_2528 | PFL_2529 | 64 |
| PFL_3315 | PFL_3314 | PFL_3313 | 66* |
| PFL_3485 | PFL_3485 | PFL_3483 | 63 |
| PFL_2293 | PFL_2292 | PFL_2291 | 66* |
| **Ferrioxamine** | | | |
| PFL_0125 | PFL_0126 | PFL_0127 | 62 |
| **Ferrichrome** | | | |
| PFL_0147 | PFL_0146 | PFL_0147 | 64 |
| PFL_5706 | PFL_5705 | PFL_5704 | 61* |
| **Ferric citrate** | | | |
| PFL_0982 | PFL_0983 | PFL_0984 | 65 |
| PFL_4039 | PFL_4040 | PFL_4041 | 66* |
| **Heme** | | | |
| PFL_1371 | PFL_1372 | PFL_1373 | 64 |
| PFL_2365 | PFL_2364 | PFL_2363 | 65 |
| PFL_5378 | PFL_5379 | PFL_5380 | 66* |
| PFL_4627 | PFL_4626 | PFL_4625 | 63 |
| **Aerobactin** | | | |
| PFL_3154 | PFL_3155 | PFL_3156 | 66* |
| **Unknown** | | | |
| PFL_3612 | PFL_3611 | PFL_3610 | 63 |
| PFL_0995 | PFL_0989 | PFL_0988 | 65 |

**Fig. 2.2. GC content of TBDTs and TBDRs. a.** GC content of genes encoding TBDTs in the Pf-5 genome. **b**. GC content of genes encoding TBDRs in the Pf-5 genome. * %GC differs significantly from the genomic mean of 63% as determined by chi-square test.

**b**

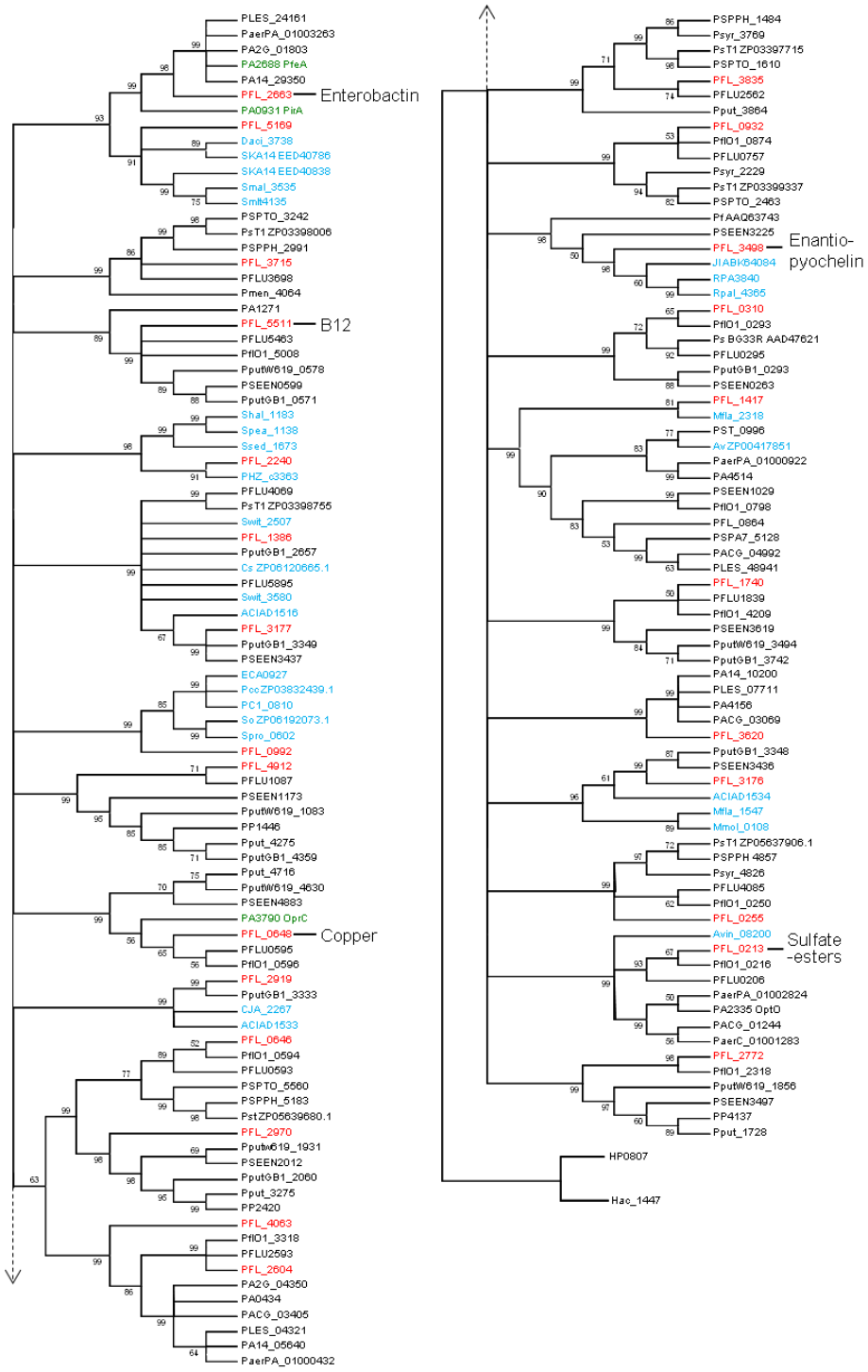
| Locus Tag | GC content |
|---|---|
| PFL_5511 | 61* |
| PFL_4912 | 66* |
| PFL_5169 | 62 |
| PFL_4063 | 66* |
| PFL_3620 | 64 |
| PFL_2240 | 65 |
| PFL_0310 | 65 |
| PFL_0932 | 64 |
| PFL_2772 | 67* |
| PFL_1740 | 64 |
| PFL_0646 | 66* |
| PFL_0648 | 67* |
| PFL_2604 | 66* |
| PFL_1417 | 64 |
| PFL_3835 | 64 |
| PFL_3498 | 66* |
| PFL_2663 | 66* |
| PFL_0992 | 61* |
| PFL_0255 | 63 |
| PFL_3176 | 65* |
| PFL_0864 | 61* |
| PFL_2970 | 65 |
| PFL_0213 | 66* |
| PFL_1386 | 66* |
| PFL_3177 | 67* |
| PFL_3715 | 65 |
| PFL_2919 | 67* |

**Fig. 2.2., Continued**

**Phylogenetic analysis of TonB-Dependent Receptors (TBDRs)**

The compiled best hits from PSI_BLAST of the 27 TBDRs were aligned and subjected to maximum parsimony analysis, using two TBDRs from *Helicobacter* spp. as an outgroup. A tree with 22 distinct clades was generated (Fig. 2.3). The majority of the clades are composed exclusively of TBDRs from *Pseudomonas* spp., but nine of the 22 clades include TBDRs present in proteomes of diverse genera representing the alpha-, beta- and gamma-proteobacteria. For eight of the nine TBDR genes corresponding to the proteins in clades having a member from genera other than *Pseudomonas* spp., the % GC differs significantly from the Pf-5 genomic mean of 63.3% (Fig. 2.2). The diversity of genera with orthologous TBDRs implies that horizontal gene transfer of TBDR genes is a possible mode for acquisition.

**Fig. 2.3. Phylogenetic analysis of TonB-dependent receptors.** Phylogenetic analysis of the 27 TBDRs of *P. fluorescens* Pf-5 (PFL_) and orthologs was done using the Maximum Parsimony method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the proteins analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The tree is rooted with two *Helicobacter* spp. TBDRs. Pf-5 proteins are shown in red font; proteins with known functions are shown in green font, and proteins from genera other than *Pseudomonas* are shown in blue font. Putative functions assigned to Pf-5 TBDRs are labeled. The tree has been divided into two portions to improve visualization, and positions where the tree is joined are indicated with dotted lines. Abbreviations for species represented in the tree are as follows: *Acinetobacter* sp. ADP1 (ACIAD), *Azotobacter vinelandii* DJ (Avin), *Azotobacter vinelandii* AvOP (Av), *Caulobacter segnis* ATCC 21756 (Cs), *Cellvibrio japonicus* Ueda107 (CJA), *Delftia acidovorans* SPH-1 (Daci), *Helicobacter acinonychis* str. Sheeba (Hac), *Helicobacter pylori* 26695 (HP), *Janthinobacterium lividum* (Jl), *Methylobacillus flagellatus* KT (Mfla), *Methylotenera mobilis* JLW8 (Mmol), *P. aeruginosa* 2192 (PA2G), *P. aeruginosa* C3719 (PACG), *P. aeruginosa* LESB58 (PLES), *P. aeruginosa* PA14 (PA14), *P. aeruginosa* PACS2 (PaerPA), *P. aeruginosa* PAO1 (PA), *P. entomophila* (PSEEN), *P. fluorescens* Pf0-1 (Pfl01), *P. fluorescens* SBW25 (PFLU), *P. mendocina* ymp (Pmen), *P. putida* F1 (Pput), *P. putida* GB1 (PputGB1), *P. putida* KT2440 (PP), *P. putida* W619 (PputW619), *P. stutzeri* A1501 (PST), *P. syringae* pv. phaseolicola 1448A (PSPPH), *P. syringae* pv. syringae B728a (Psyr), *P. syringae* pv. tomato T1 (PsT1), *Pectobacterium atrosepticum* SCRI1043 (ECA), *Pectobacterium carotovorum* subsp. *carotovorum* PC1 (PC1), *Pectobacterium carotovorum* subsp. *carotovorum* WPP14 (Pcc), *Phenylobacterium zucineum* HLK1 (PHZ), *Pseudomonas filiscindens* (Pf), *Pseudomonas* sp. BG33R (Ps BG33R), *Pseudomonas syringae* pv. tomato DC3000 (PSPTO), *Rhodopseudomonas palustris* CGA009 (RPA), *Rhodopseudomonas palustris TIE-1* (Rpa1), *Serratia odorifera* 4Rx13 (So), *Serratia proteamaculans* 568 (Spro), *Shewanella halifaxensis* HAW-EB4 (Shal), *Shewanella pealeana* ATCC 700345 (Spea), *Shewanella sediminis* HAW-EB3 (Ssed), *Sphingomonas wittichii* RW1 (Swit), *Stenotrophomonas maltophilia* K279a (Smlt), *Stenotrophomonas maltophilia* R551-3 (Smal), *Stenotrophomonas* sp. SKA14 (SKA14).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Daci_3738
SKA14 EED40786
SKA14 EED40838
Smal_3535
Smlt4135
PSPTO_3242
PsT1 ZP03398006
PSPPH_2991
PFL_3715
PFLU3698
Pmen_4064
PA1271
PFL_5511
B12
PFLU5463
PflO1_5008
PputW619_0578
PSEEN0599
PputGB1_0571
Shal_1183
Spea_1138
Ssed_1673
PFL_2240
PHZ_c3363
PFLU4069
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ACIAD1533
PFL_0646
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PFLU0593
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PSEEN2012
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Pput_3275
PP2420
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PA0434
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PLES_04321
PA14_05640
PaerPA_01000432
PSPPH_1484
Psyr_3769
PsT1 ZP03397715
PSPTO_1610
PFL_3835
PFLU2562
Pput_3864
PFL_0932
PflO1_0874
PFLU0757
Psyr_2229
PsT1 ZP03399337
PSPTO_2463
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PFL_3498
Enantio-
pyochelin
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RPA3840
Rpal_4365
PFL_0310
PflO1_0293
Ps BG33R AAD47621
PFLU0295
PputGB1_0293
PSEEN0263
PFL_1417
Mfla_2318
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PFL_1740
PFLU1839
PflO1_4209
PSEEN3619
PputW619_3494
PputGB1_3742
PA14_10200
PLES_07711
PA4156
PACG_03069
PFL_3620
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PFL_0255
Avin_08200
PFL_0213
Sulfate
-esters
PflO1_0216
PFLU0206
PaerPA_01002824
PA2335 OptO
PACG_01244
PaerC_01001283
PFL_2772
PflO1_2318
PputW619_1856
PSEEN3497
PP4137
Pput_1728
HP0807
Hac_1447

**Fig. 2.3.**

Of the 27 TBDRs, only PFL_3498 (*fetA*) has a demonstrated function in *P. fluorescens*, serving as the receptor for enantio-pyochelin (40). Putative functions were assigned to four other TBDRs (PFL_2663, PFL_0648, PFL_5511, PFL_0213) (Fig. 2.3) based on clustering with and similarity to sequences of functionally characterized TBDRs in other bacteria, as well as the identity of adjacent genes in the Pf-5 genome. PFL_2663 is 82% identical at the amino acid level to PfeA of PAO1 (PA2688), which functions as a receptor for the ferric complex of enterobactin, a catecholate siderophore produced by *E. coli* and other species of the Enterobacteriaceae (23). In the Pf-5 genome, PFL_2663 is clustered with orthologs of *pfeS* and *pfeR* (Fig. 2.4c), involved in the regulation of *pfeA* (22), and *pfeE*, which functions in esterification of enterobactin prior to transport across the cytoplasmic membrane (96). Amino acid sequences of each pair of orthologs in the syntenic *pfe* clusters of Pf-5 and PAO1 have 66% to 82% identity. Therefore, evidence for the role of PFL_2663 as a ferric-enterobactin receptor is provided both by sequence similarity to *pfeA* and conservation of the *pfe* gene cluster.

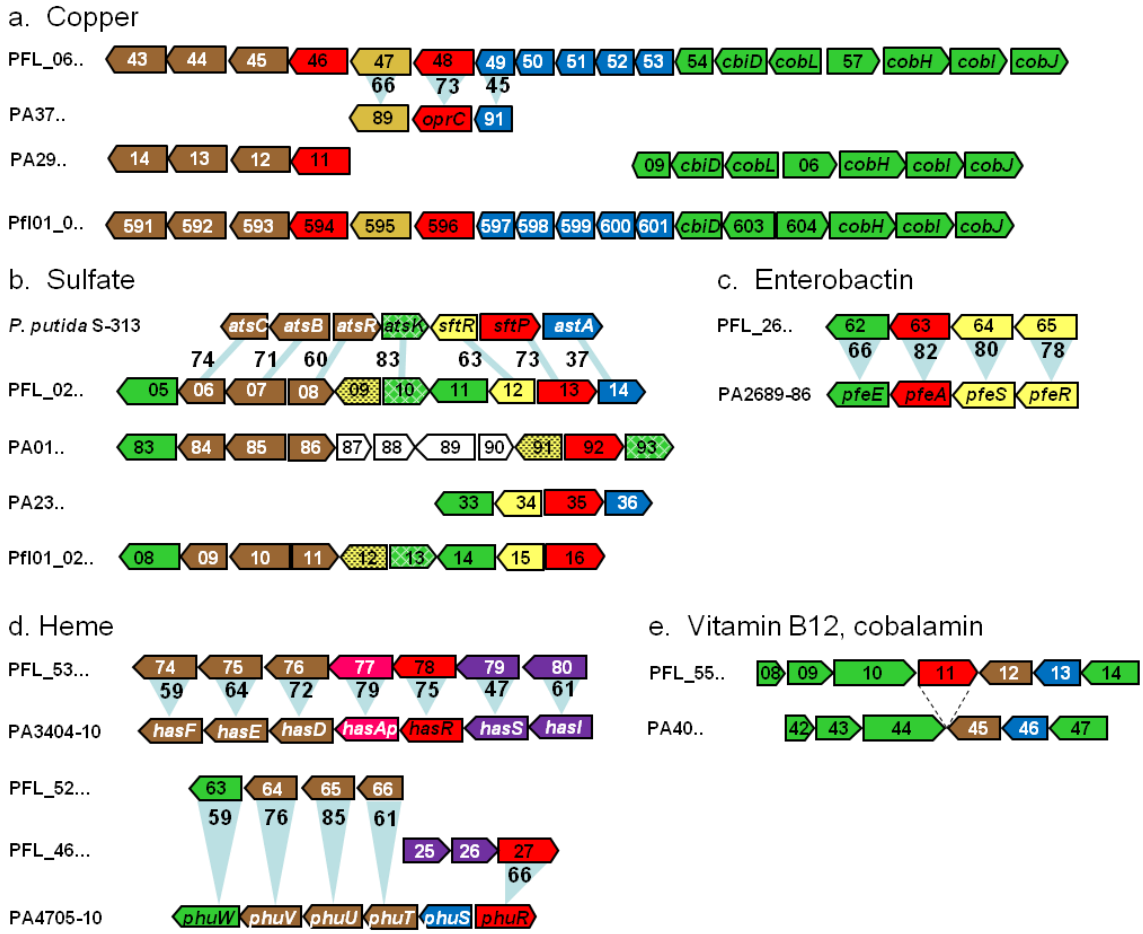


**Fig. 2.4. Gene clusters with TBDRs and TBDTs of known function.** Gene clusters in *P. fluorescens* Pf-5 (PFL_), *P. aeruginosa* PAO1 (PA), *P. putida* S-313, and *P. fluorescens* Pf0-1 (Pfl01_) with characterized or putative functions in the uptake of **a**. Copper, **b**. Sulfate, **c**. Enterobactin, **d**. Heme or, **e**. Cobalamin (B12). Predicted gene functions are denoted by color: red, TBDR or TBDT; brown, ABC transport; gold, membrane protein (other than ABC transport); green, biosynthesis; purple, ECF sigma factor and anti-sigma factor; yellow, regulatory (other than ECF sigma factor); pink, hemophore; blue, hypothetical. Genes whose functions appear unrelated to that of the TBDR/TBDT are shown in white. Orthologs not readily identifiable by their position in the gene cluster are indicated by identical patterns. Light blue lines and triangles connect orthologs, and accompanying numbers indicate the percent identity of amino acid sequences.

PFL_0648 is a putative copper receptor, having 73% identity at the amino acid level to PA3790 (*oprC*), which encodes a TBDR that binds copper and is thought to function in copper utilization in PAO1 (93). PFL_0648 is in a three-gene cluster that is conserved in *Pseudomonas* spp. but located in different genomic regions in *P. fluorescens* and *P. aeruginosa* (Fig. 2.4a). PFL_5511 is a putative receptor for vitamin B12 (cobalamin), exhibiting 29% identity at the amino acid level to BtuB, the characterized B12 receptor of *E. coli*. Protein structure analysis using PSIPRED GenTHREADER matched the TBDR encoded by PFL_5511 ($2e^{-17}$) to BtuB from *E. coli*. In Pf-5, this TBDR is adjacent to a gene encoding a putative periplasmic binding protein for cobalamin (PFL_5512) (Fig. 2.4e), whereas the ortholog in PAO1 (PA1271) is adjacent to a cobalamin biosynthesis gene cluster. PFL_0213 is a putative receptor for sulfate esters, exhibiting 73% identity at the amino acid level to SftP, a TBDR required for growth of *P. putida* strain S-313 on aryl- or alkylsulfate esters (44). Contiguous to PFL_0213 are homologs for the sulfate ester/sulfonate transporter (*atsRBC*), a LysR-type regulator (*sftR*), an oxygenolytic alkylsulfatase (*atsK*), and an arylsulfotransferase (*astA*) clustered with *sftP* in *P. putida* S-313 (Fig. 2.4b), providing further evidence for the putative function of PFL_0213 as a sulfate ester receptor. Analysis of the sequenced *Pseudomonas* genomes indicates conservation of the gene cluster across the genus, with duplications of genes having metabolic functions evident in the genomes of *P. fluorescens* and those with metabolic and regulatory functions evident in *P. aeruginosa*.

**Phylogenetic analysis of TonB-dependent transducers (TBDTs)**

The compiled best PSI-BLAST hits for the 18 TBDTs were aligned and subjected to maximum parsimony analysis generating a tree with ten distinct, well-supported clades (Fig. 2.5). Close orthologs having known functions in *P. aeruginosa* PAO1 were also included. Two sequences from *Caulobacter* spp. were used as an outgroup to root the tree. Of the ten clades, two include TBDTs from bacteria other than *Pseudomonas* spp. PFL_3612 clusters with TBDTs from *Yersinia* spp., *Stenotrophomonas* spp., and *Pectobacterium wasabiae*, gamma-proteobacteria found in terrestrial or aquatic

environments. PFL_2527, which falls in the pyoverdine clade, clusters with TBDTs from the beta-proteobacteria *Achromobacter piechaudii* ATCC 43553, a human pathogen, and *Janthinobacterium* sp. and *Methylovorus* sp. SIP3-4, which are found in soil and aquatic environments, respectively. The capacity to utilize pyoverdines as iron sources has not been observed outside of *Pseudomonas* spp. and *Azotobacter vinelandii* to date (15), but these results highlight the possibility that such capacity exists in other bacteria. For six of the 18 TBDT genes, the % GC differs statistically from the Pf-5 genomic mean of 63.3% (Fig. 2.2), but none of the six corresponding proteins are in clades with genera other than *Pseudomonas* spp. Therefore, while horizontal gene transfer of the TBDTs provides the most plausible explanation for the presence of diverse genera of proteobacteria in certain clades, we did not uncover convincing evidence for recent horizontal acquisition as a mechanism of inheritance of these genes by Pf-5.

**Fig. 2.5. Maximum Parsimony analysis of TonB-dependent transducers.** A phylogenetic analysis of the 18 TBDTs of *P. fluorescens* Pf-5 (PFL_) and orthologous transducers was done using the Maximum Parsimony method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The tree is rooted with two transducers from *Caulobacter* sp. K31 as an outgroup. Pf-5 proteins are shown in red font; proteins with known functions are shown in green font; and proteins from genera other than *Pseudomonas* are shown in blue. Putative substrates assigned to Pf-5 TBDTs are labeled on the periphery of the circle. Abbreviations for species represented in the tree are as follows: *Achromobacter piechaudii* ATCC 43553 (Ap), *Caulobacter* sp. K31 (Caul), *Janthinobacterium* sp. *Marseille* (mma), *Methylovorus* sp. SIP3-4 (Msip34), *P. aeruginosa* (Pa), *P. aeruginosa* 2192 (PA2G), *P. aeruginosa* PA14 (PA14), *P. aeruginosa* PA7 (PSPA7), *P. aeruginosa* PACS2 (PaerPA), *P. aeruginosa* PAO1 (PA), *P. entomophila* (PSEEN), *P. fluorescens* Pf0-1 (Pfl01), *P. fluorescens* SBW25 (PFLU), *P. putida* F1 (Pput), *P. putida* GB1 (PputGB1), *P. putida* KT2440 (PP), *P. putida* W619 (PputW619), *P. syringae* pv. oryzae str. 1_6 (Pso), *P. syringae* pv. phaseolicola 1448A (PSPPH), *P. syringae* pv. tabaci ATCC 11528 (Pst), *Pectobacterium wasabiae* WPP163 (Pecwa), *Stenotrophomonas* sp. SKA14 (St), *Yersinia bercovieri* ATCC 43970 (Yb), *Yersinia kristensenii* ATCC 33638 (Yk), *Yersinia mollaretii* ATCC 43969 (Ym).

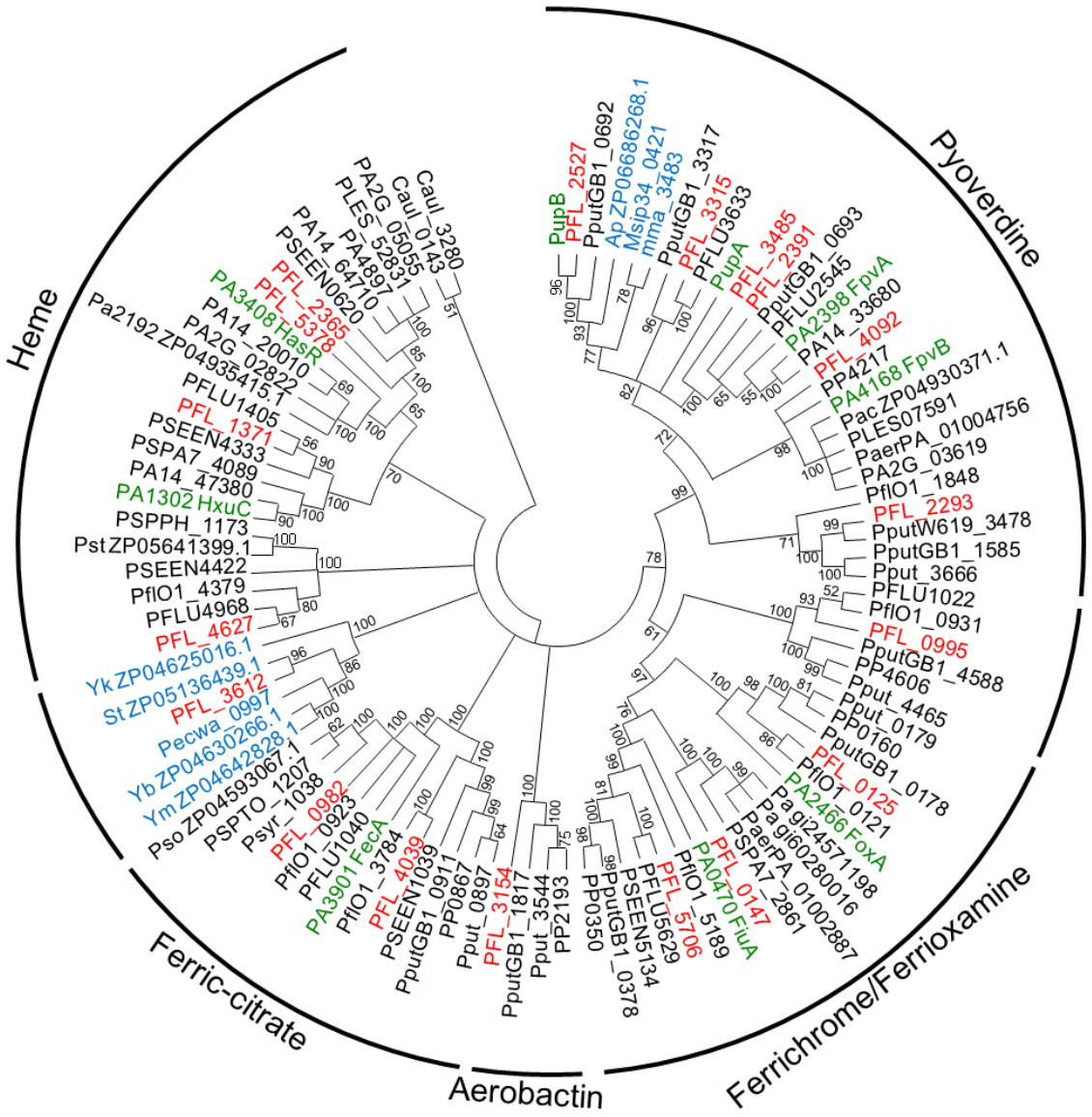
Heme
Pyoverdine
Ferric-citrate
Aerobactin
Ferrichrome/Ferrioxamine
Caul_3280
Caul_01143
Caul_05055
PLES_52831
PA4897
PA14_64710
PSEEN0620
PFL_2365
PA3408 HasR
PFL_5378
Pa2192 ZP04935415.1
PA14_20010
PA2G_02822
PFLU1405
PFL_1371
PSEEN4333
PSPA7_4089
PA14_47380
PA1302 HxuC
PSPPH_1173
PstZP05641399.1
PSEEN4422
PflO1_4379
PFLU4968
PFL_4627
YkZP04625016.1
StZP05136439.1
PFL_3612
Pecwa_0997
YbZP04630266.1
YmZP04642828.1
PsoZP04593067.1
PSPTO_1207
Psyr_1038
PFL_0982
PflO1_0923
PFLU1040
PA3901 FecA
PFL_4039
PSEEN1039
PputGB1_0911
PP0867
Put_0897
PFL_3154
PputGB1_1817
Put_3544
PP2193
PputGB1_0378
PP0350
PFL_5706
PflO1_5189
PFLU5629
PSEEN5134
PA0470 FiuA
PFL_0147
PSPA7_2861
PaerPA_01002887
Pagi60280016
Pagi24571198
PA2466 FoxA
PFL_0125
PflO1_0121
PputGB1_0178
PP0160
Put_0179
Put_4465
PP4606
PputGB1_4588
PFL_0995
PflO1_0931
PFLU1022
Put_3666
PputGB1_1585
PputW619_3478
PFL_2293
PflO1_1848
PA2G_03619
PaerPA_01004756
PLES07591
PacZP04930371.1
PA4168 FpvB
PP4217
PFL_4092
PA14_33680
PA2398 FpvA
PFLU2545
PputGB1_0693
PFL_2391
PFL_3485
PupA
PFLU3633
PputGB1_3317
PFL_3315
Msip34_0421
mma_3483
Ap ZP06686268.1
PputGB1_0692
PFL_2527
PupB

**Fig. 2.5.**

Five of the 10 TBDT clades include characterized proteins known to function in iron uptake in other *Pseudomonas* spp. (Fig. 2.5). Four TBDTs (PFL_1371, PFL_2365, PFL_4627, and PFL_5378) are in a large clade also containing HasR and HxuC, which function in heme uptake in *P. aeruginosa* PAO1 (16, 70). PFL_5378 is 75% identical to PA3408 (HasR), the hemophore receptor in *P. aeruginosa* PAO1, and is clustered with orthologs of genes functioning in hemophore production and uptake (Fig. 2.4d). PFL_1371 is 61% identical to PA1302 (HxuC) with no conservation of contiguous genes beyond the sigma factors and anti-sigma factors adjacent to the transducers. The deduced amino acid sequence of PFL_4627 is 66% identical to PA4710 (PhuR), a heme receptor (18), but PFL_4627 is clustered with an ECF sigma factor/anti-sigma factor gene pair whereas PA4710 is clustered with other genes having a demonstrated role in heme uptake in *P. aeruginosa* (Fig. 2.4d). PA4710 does not have an N-terminal signaling domain so it was not included in the phylogenetic analysis of TBDTs in the Pf-5 genome. This large clade also includes PFL_2365, which is 69% identical to PA4897 (OptI), a TBDT that is iron regulated in *P. aeruginosa* (18).

Two of the 10 TBDT clades include proteins with known or putative roles in the uptake of ferric-complexes of citrate or aerobactin. PFL_0982 and PFL_4039 fall in a clade with PA3901 (FecA) (Fig. 2.5), which functions in ferric citrate uptake in *P. aeruginosa* PAO1 (55). PFL_0982 is clustered with an ECF sigma factor and anti-sigma factor pair orthologous to PA3900 (*fecR*) and PA3899 (*fecI*), as determined by reciprocal best-hit analysis, suggesting that the PFL_0982-PFL_0984 cluster is likely to function in ferric-citrate uptake. Another clade includes PFL_3154, which is similar (49% identity) to the TBDR PA4675 (ChtA) involved in aerobactin, rhizobactin 1021 and schizokinen uptake by *P. aeruginosa* (20). ChtA lacks a signaling domain so was not included in the phylogenetic analysis.

Three TBDTs (PFL_0125, PFL_0147, and PFL_5706) are in a large clade that also contains TBDTs functioning in the uptake of the hydroxamate siderophores ferrioxamine and ferrichrome in *P. aeruginosa*. PFL_0125 is 66% identical to FoxA

(PA2466), which is a ferrioxamine uptake receptor in PAO1 (36). PFL_0125 and *foxA* are components of syntenous clusters with orthologous genes encoding an ECF sigma factor, anti-sigma factor, and putative transmembrane protein. PFL_5706 is 66% identical to PA0470 (FiuA), the ferrichrome receptor of *P. aeruginosa* PAO1 (36). PFL_5706 is also clustered with an ECF sigma factor and anti-sigma factor pair orthologous to PA0471 and PA0472, as determined by reciprocal best-hit analysis, suggesting that the PFL_5704-PFL_5706 cluster is likely to function in ferrichrome uptake. Recently, Hannauer et al. reported that, in *P. aeruginosa*, both FiuA and FoxA transport ferrichrome, which suggests that the Pf-5 TBDTs in this clade may also exhibit relaxed specificities in the transport of these hydroxamate siderophores (36). The three Pf-5 TBDTs, PFL_0125, PFL_5706 and PFL_0147, are all contained within a well-supported clade, suggesting that PFL_0147 may also function in uptake of ferrichrome, ferrioxamine, or both siderophores. PFL_0995 and orthologs from other *Pseudomonas* spp. form a clade related to the ferrichrome/ferrioxamine clade with a bootstrap of 61, indicating a possible role for these proteins in the uptake of hydroxamate siderophores.

Another large clade includes characterized pyoverdine receptors FpvA and FpvB from *P. aeruginosa* PAO1 (13, 33) and PupA and PupB from *P. putida* WCS358 (6, 47). Pf-5 has six TBDTs falling within this clade (Fig. 2.5), whose sequences are 35% to 68% identical to FpvA or FpvB of *P. aeruginosa* PAO1 at the amino acid level. PFL_4092 is present within one of the four pyoverdine gene clusters in the Pf-5 genome (Fig. 2.6) whereas the other five TBDTs in this clade are clustered with ECF sigma factor and anti-sigma factor gene pairs at dispersed locations in the Pf-5 genome. In this clade, PFL_2293 appears to be ancestral, and PFL_4092 forms its own sub-clade with FpvB from *P. aeruginosa* PAO1. The other four TBDTs in this clade (PFL_2391, PFL_3315, PFL_2527, and PFL_3485) are more closely related to each other and to FpvA, PupA and PupB.

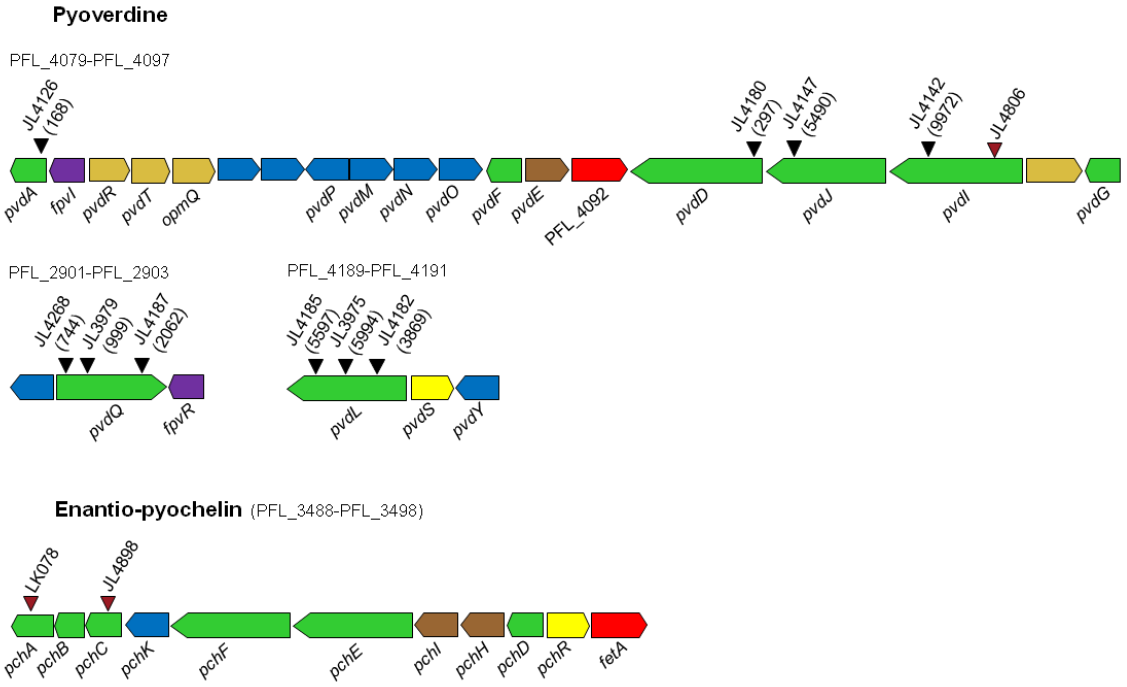


**Fig. 2.6. Locations of mutations in the pyoverdine or enantio-pyochelin gene clusters of Pf-5.** Arrows denote genes functioning in siderophore biosynthesis (green), ABC transport (brown), ECF sigma factor and anti-sigma factor (purple), membrane proteins (other than ABC transport) (gold), regulatory (other than ECF sigma factor) (yellow), unknown function and hypothetical (blue). The TonB-dependent outer-membrane proteins are in red. Black triangles denote sites of Tn*5* insertions eliminating pyoverdine production, and red triangles denote sites of deletions eliminating pyoverdine or enantio-pyochelin production by Pf-5. Strain numbers of mutants having the designated mutations are shown above the triangles. In parentheses below strain number is the nucleic acid position of the Tn*5* insertion.

**Characterization of siderophore-biosynthesis mutants of *P. fluorescens* Pf-5**

Arbitrary polymerase chain reaction (PCR) was used to map Tn*5* insertions in nine Pf-5 mutants deficient in pyoverdine production (Pvd$^-$) (48). Three insertions were mapped to Δ*pvdL*, a non-ribosomal peptide synthetase involved in the biosynthesis of the pyoverdine chromophore (Fig. 2.6). Three Tn*5* insertions mapped to the non-ribosomal peptide synthetases involved in biosynthesis of the pyoverdine peptide chain: one each in Δ*pvdD*, Δ*pvdI*, and Δ*pvdJ*. Three insertions mapped to Δ*pvdQ*, an acylase functioning in maturation of the pyoverdine (46). Therefore, the Tn*5* insertions were mapped to three of the four pyoverdine gene clusters predicted from bioinformatic analysis of the Pf-5 genome, providing functional support for these predictions (Fig. 2.6).

To further characterize siderophore biosynthesis and uptake in Pf-5, we made unmarked deletions in the pyoverdine and enantio-pyochelin gene clusters of Pf-5. A pyoverdine deficient mutant, constructed by deletion of a sequence internal to Δ*pvdI* (PFL_4095), lacked the characteristic fluorescence of the pyoverdine siderophore when cultures grown on KMB were viewed under UV light. Mutants in enantio-pyochelin biosynthesis were constructed by deletion of a sequence internal to *pchA* (PFL_3488), or *pchC* (PFL_3490) (Fig. 2.6). PchA catalyses the first step in the synthesis of salicylate from chorismate (32) whereas PchC is a thioesterase involved in subsequent conversion of salicylate to pyochelin (77). Enantio-pyochelin was detected by TLC in culture extracts of Pf-5 but not the *pchA* mutant. Less than wildtype levels were detected in extracts of the *pchC* mutant (data not shown). A *pchC* mutant of *P. aeruginosa* also produces low levels of pyochelin compared to wild type (32).

Double mutants were created by stacking deletions in Δ*pvdI* with Δ*pchA* or Δ*pchC*. These mutants were evaluated for growth under iron-limited conditions imposed by amending KMB with varying concentrations of the iron chelator 2,2'-dipyridyl. The wildtype Pf-5 grew on KMB amended with up to 800 μM 2,2'-dipyridyl whereas the Pvd$^-$ Tn*5* mutants and the Δ*pvdI* deletion mutant grew only on KMB containing 600 μM or

less of the chelator, as expected due to the known role of pyoverdine production in iron-limited growth of *Pseudomonas* spp. The *pchC* and *pchA* mutants grew on KMB containing up to 800 µM 2,2'-dipyridyl, indicating that enantio-pyochelin is not required for iron-limited growth of pyoverdine-producing strains. In contrast, the double Δ*pvdI-pchC* and Δ*pvdI-pchA* mutants did not grow on KMB containing 400 µM to 800 µM 2,2'-dipyridyl, demonstrating the role of both siderophores in iron-limited growth of Pf-5.

The mutants were also characterized by observing their phenotypes on CAS agar, the universal siderophore detection medium (81). This medium contains a blue dye (CAS) that turns orange when iron is removed. Typically, siderophore production results in an orange zone surrounding a colony. In preliminary experiments, we found that Pf-5 also caused a cleared halo with a deep blue margin (Fig. 2.7), whereas this type of halo was not generated by an *ofa*A mutant of Pf-5 deficient in the production of orfamide A, an anionic biosurfactant (35). The clearing zone was also observed surrounding colonies of Pf-5, but not the *ofaA* mutant, on CAS agar amended with 1 mM $FeCl_3$ (data not shown). This clearing could be related to the formation of micelles around the CAS dye, which has been reported for anionic surfactants (11). Clear zones with blue margins were seen on CAS agar plates spotted with 10 µl of 1 mg/ml orfamide A or 1% sodium dodecyl sulfate (SDS) (data not shown), an anionic surfactant known to form micelles with CAS (11). Therefore, the *pchA*, *pchC*, and Δ*pvdI* mutations were introduced into an *ofaA* mutant of Pf-5, which lacks orfamide A production, so that siderophore production could be assessed on CAS agar without interference from the biosurfactant. By visualizing halos surrounding mutant colonies on CAS agar, we confirmed that both siderophores chelate iron and observed no additional siderophore produced by Pf-5 on this medium (Fig. 2.7).

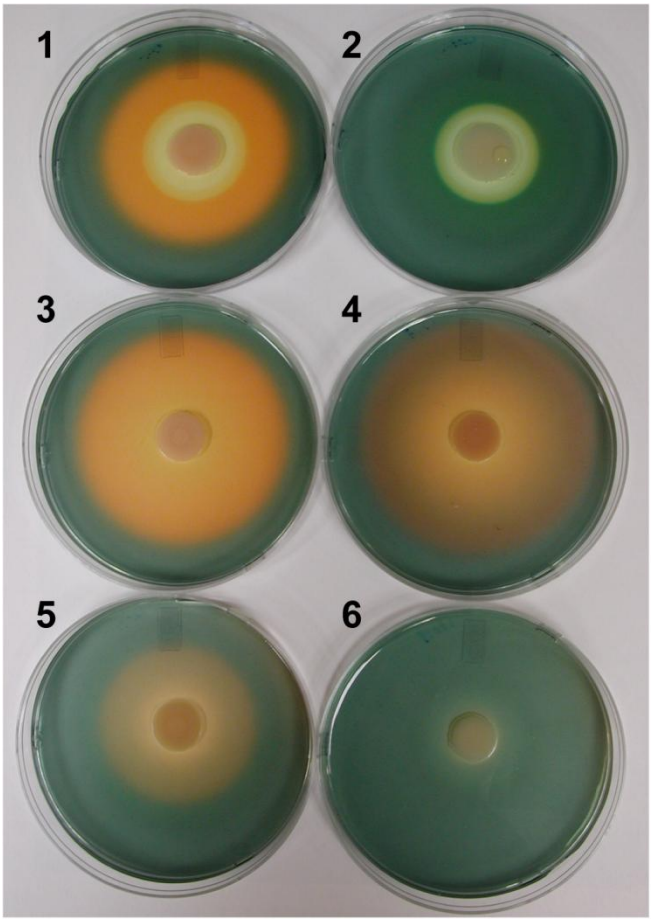


**Fig. 2.7. Siderophore and orfamide A production detected as halos surrounding colonies of Pf-5 or derivative strains on the universal siderophore detection medium, CAS.** On CAS agar, an orange halo surrounding a colony of strain Pf-5 (1) indicates siderophore production, and a smaller clear halo is due to orfamide production. The clear zone is also evident surrounding a colony of a Δ*pvdI-pchA* mutant of Pf-5 (2). Pyoverdine production is visualized as an orange halo surrounding the Δ*pchA-ofaA* mutant (3) whereas enantio-pyochelin production is visualized as the halo surrounding colonies of the Δ*pvdI-ofaA* mutant (4). A smaller halo surrounds colonies of the Δ*pvdI-pchC-ofaA* mutant (5), which is attributed to residual levels of enantio-pyochelin or salicylate biosynthesis. No halo surrounds colonies of Δ*pvdI-pchA-ofaA* mutant (6), indicating the lack of detectable siderophore production by this mutant. Plates were incubated at room temperature for 12 days.

**Utilization of diverse siderophores by *P. fluorescens* Pf-5**

The ability of Pf-5 to utilize a diverse set of pyoverdines as iron sources was assessed in crossfeeding experiments. Sixty-one strains of *Pseudomonas* spp. were tested, 34 of which produce pyoverdines of known amino acid composition (Table 2.2). Nine strains produce pyoverdines representing distinct siderotypes, although their structures are not known. The length and amino acid composition of the pyoverdine peptide chain was predicted bioinformatically from genomic sequence data for four strains (Pf-5, *P. syringae* B728A, *P. syringae* pv. *tomato* DC3000, and *P. aeruginosa* PA14) (Fig. 2.8). As stated above, the Δ*pvdI-pchC* mutant of Pf-5 did not grow on KMB amended with 400 μM 2,2'-dipyridyl under the conditions of this assay. When grown in proximity to 32 of the 61 test strains of *Pseudomonas* spp., however, the Δ*pvdI-pchC* mutant grew on this iron-limited medium, indicating its capacity to utilize siderophores produced by the test strains as iron sources. $Pvd^-$ mutants were available for four of the crossfeeding strains, and these mutants did not crossfeed the Δ*pvdI-pchC* mutant of Pf-5 (Table 2.2), indicating that the pyoverdine was responsible for crossfeeding. The 32 strains of *Pseudomonas* spp. that crossfed the Δ*pvdI-pchC* mutant represent 17 pyoverdine structures. Therefore, Pf-5 can utilize a diverse set of pyoverdines as iron sources.

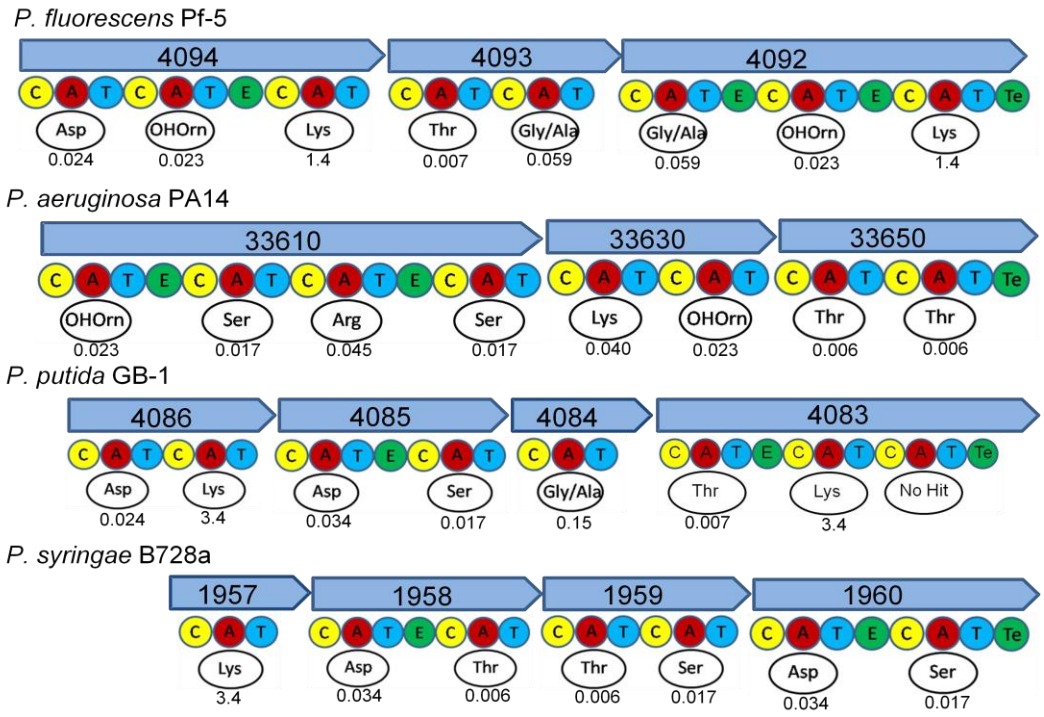


**Fig. 2.8. Predicted length and composition of pyoverdine peptide chains.** The length and composition of pyoverdine peptide chains were predicted bioinformatically for four *Pseudomonas* strains with sequenced genomes but uncharacterized pyoverdines. Genes encoding non-ribosomal peptide synthetases (NRPSs) for the biosynthesis of the pyoverdine peptide chain in *P. fluorescens* Pf-5 (PFL_4095, PFL_4094, PFL_4093), *P. aeruginosa* PA14 (PA14_33610, PA14_33630, PA14_33650), *P. putida* GB-1 (PputGB1_4086, PputGB1_4085, PputGB1_4084, PputGB1_4083), and *P. syringae* B728a (Psyr_1957, Psyr_1958, Psyr_1959, Psyr_1960). The domain organization of the NRPSs (C, condensation; A, Adenylation, T, thiolation; E, epimerization; and Te, thioesterification) and the specific amino acid predicted from the sequence of each A domain is shown with its E-value.

**Table 2.2. Crossfeeding of the Pf-5 siderophore mutant by strains of *Pseudomonas***

| Test Strains | Cross-feeding[a] | Composition of Peptide Chain or Siderotype | Reference or source |
|---|---|---|---|
| **Six amino acids** | | | |
| *P. fluorescens* B10 | + | εLys-OHAsp-Ala-aThr-Ala-cOHOrn | (89) |
| *P. lini* DLE411J | + | *Lys-OHAsp-Ala-Thr-Ala-OHOrn* | (65) |
| *P. putida* CS111 syn SB8.3 | + | *Ala-Lys-Thr-Ser-OHOrn-OHOrn* | (65) |
| *P. putida* CFML90-40 | + | Asp–Ala–Asp–AOHOrn–Ser–cOHOrn | (60) |
| *P. putida* ATCC17470 | + | Ser-εLys-OHHis-aThr-Ser-cOHOrn | (85) |
| **Seven amino acids** | | | |
| *P. aeruginosa* ATCC 27853 | + | Ser- FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) | (88) |
| *P. aeruginosa* Pa6 | + | (Ser-Dab)-FOHOrn-Gln-Gln –FOHOrn-Gly (Type III pyoverdine) | (34) |
| *P. fluorescens* CLR711 syn PL7 | + | Ser-AOHOrn-Ala-Gly-aThr-Ala-cOHOrn | (3) |
| *P. chlororaphis* ATCC 9446 | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (59) |
| *P. fluorescens* ATCC 13525 | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (59) |
| *P. fluorescens* SBW25 | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (68) |
| *P. fluorescens* WCS374 | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (26) |
| *P. fluorescens* WCS374 Pvd- | - | | (56) |
| *P. fluorescens* CTRp112 syn PL8 | + | Lys-AOHOrn-Ala-Gly-aThr-Ser-cOHOrn | (3) |
| *P. putida* DSM3601 syn CFML90-33 | - | Asp-Lys-Thr-OHAsp-Thr-aThr-cOHOrn | (86) |
| *P. syringae* ATCC 19310 | - | εLys-OHAsp-Thr-(Thr-Ser-OHAsp-Ser) | (43) |
| *P. syringae pv.syringae* B728A | - | Lys-Asp-Thr-Thr-Ser-Asp-Ser | This study, predicted |
| *P. syringae pv. tomato* DC3000 | - | εLys-OHAsp-Thr-(Thr-Ser-OHAsp-Ser) | (43) |
| *P. cichorii* | - | εLys-OHAsp-Thr-(Thr-Gly-OHAsp-Ser) | (10) |
| *P. libanensis* CFBP4841 | - | Ala–Orn–OHAsp–Ser–Orn–Ser–cOHOrn | (64) |
| **Eight amino acids** | | | |
| *P. chlororaphis* DTR133 | + | Asp-FOHOrn-Lys-(Thr-Ala-Ala-FOHOrn-Ala) | (3) |

**Table 2.2. (Continued)**

| Test Strains | Cross-feeding[a] | Composition of Peptide Chain or Siderotype | Reference or source |
|---|---|---|---|
| *P. aeruginosa* PA14 | + | Ser-Arg-Ser-FOHOrn-Lys-FOHOrn-Thr-Thr (Type I pyoverdine) | This study, predicted |
| *P. aeruginosa* PA14 Pvd- | - | | (50) |
| *P. aeruginosa* PAO1 | + | Ser-Arg-Ser-FOHOrn-(Lys-FOHOrn-Thr-Thr) (Type I pyoverdine) | (25) |
| *P. fluorescens* CHA0 | + | Asp-FOHOrn-Lys-(Thr-Ala-Ala-FOHOrn-Lys) | (92) |
| *P. fluorescens* Pf-5 | + | Asp-OHOrn-Lys-Thr-Ala/Gly-Ala/Gly-OHOrn-Lys | This study, predicted |
| *P. fluorescens* Pf-5 Pvd- | - | | |
| *P. salomonii* CFBP2022 | + | *Ser–Orn–FOHOrn–Ser–Ser–Lys–FOHOrn–Ser* | (64) |
| *Pseudomonas sp.* 7SR1 | - | (Ser-aOHOrn-Ala-Gly-(Ser-Ser-OHAsp-Thr) | (29) |
| *P. fluorescens* CTR1015 syn PL9 | - | Ser-AOHOrn-Ala-Gly-(Ser-Ser-OHAsp-Thr) | (60) |
| *P. putida* GB-1 | - | Asp-Lys-Asp-Ser-Gly-Thr-Lys-? | This study, predicted |
| **Nine amino acids** | | | |
| *P. costantinii* CFBP5705 | + | Ser-AcOHOrn-Gly-aThr-Thr-Gln-Gly-Ser-cOHOrn | (30) |
| *P. fluorescens* A6 | + | Lys-AcOHOrn-Gly-aThr-Thr-Gln-Gly-Ser-cOHOrn | (4) |
| *P. putida* ATCC 12633 | - | Asp-Lys-OHAsp-Ser-Thr-Ala-Glu-Ser-cOHOrn | (65) |
| *P. putida* WCS358 | - | Asp-εLys-OHAsp-Ser-Thr-Ala-Thr-Lys-OHOrn | (9) |
| *P. putida* CFBP2461 | - | Asp-εLys-OHAsp-Ser-aThr-Ala-Thr-Lys-OHOrn | (91) |
| *P. monteilii* DSM14164 | - | Asp–Lys–AcOHOrn–Ala–Ser–Ser–Gly–Ser–cOHOrn | (64) |
| *P. fluorescens* Pf0-1 | + | Ala-AcOHOrn-Orn-Ser-Ser-Ser-Arg-OHAsp-Thr | (64) |
| *P. fluorescens* Pf0-1 Pvd- | - | | M. Silby |
| **Ten amino acids** | | | |
| *P. fluorescens* DSM50106 | + | Ser–Lys–Gly–FOHOrn–Ser–Ser–Gly–(Orn–FOHOrn–Ser) | (64) |
| *P. rhodesiae* DSM14020 | + | *Ser–Lys–FOHOrn–Ser–Ser–Gly–(Lys–FOHOrn–Ser–Ser)* | (62, 65) |
| *P. fluorescens* Pfl 17400 | - | Ala-Lys-Gly-Gly-OHAsp-Gln/Dab-Ser-Ala-cOHOrn | (60) |
| *P. tolaasii* NCPPB 2192 | - | Ser-Lys-Ser-Ser-Thr-Ser-AcOHOrn-Thr-Ser-cOHOrn | (24) |

**Table 2.2. (Continued)**

| Test Strains | Cross-feeding[a] | Composition of Peptide Chain or Siderotype | Reference or source |
|---|---|---|---|
| **Unknown structures** | | | |
| *P. flectens* CFBP3281 | + | Unknown | http://www.straininfo.net/strains/621587 |
| *P. fluorescens* ATCC17513 | + | Unknown | (85) |
| *P. fluorescens* ATCC17518 | + | Unknown | (85) |
| *P. fluorescens* CFBP2130 | + | Unknown | http://www.straininfo.net/strains/757032 |
| *P. marginalis* pv. alfalfae CFBP2039 | + | Unknown | http://www.straininfo.net/strains/544626 |
| *P. marginalis* pv. marginalis CFBP2037 | + | Unknown | http://www.straininfo.net/strains/17707 |
| *P. marginalis* pv. pastinacae CFBP2038 | + | Unknown | http://www.straininfo.net/strains/544628 |
| *P. reactans* NCPPB387 | + | Unknown | http://www.straininfo.net/strains/53319 |
| *P. blatfordae* CFBP3280 | - | Unknown | http://www.straininfo.net/strains/757233 |
| *P. fluorescens* ATCC17467 | - | Unknown | (85) |
| *P. fluorescens* ATCC17559 | - | Unknown | (85) |
| *P. mosselii* MFY161 | - | Unknown | Isolated from a blood culture in Evreux, France |
| *P. viridiflava* CFBP2107 | - | Unknown | http://www.straininfo.net/strains/270228 |
| *P. corrugata* CFBP2431 | - | Corr | (62); (60) |
| *P. fluorescens* C7R12 | - | PL1 | J.M. Meyer, personal communication |

**Table 2.2. (Continued)**

| Test Strains | Cross-feeding[a] | Composition of Peptide Chain or Siderotype | Reference or source |
|---|---|---|---|
| *P. frederiksbergensis* DSM13022 | - | Fred | (60, 62) |
| *P. fuscovaginae* CFBP2065 | - | G17 | (60, 62) |
| *P. gessardii* CIP105469 | - | Gess-bren | (61) |
| *P. graminis* DSM11363 | - | Gram | (60, 62) |
| *P. kilonensis* CFBP5372 | - | Kilo | (61) |
| *P. plecoglossicida* DSM15088 | - | Plec | (60, 63) |
| *P. thivervalensis* CFBP5754 | - | Thiv/ML45 | (60) |

Underline denotes D-amino acids. Parentheses define cyclic residues. cOHOrn is cyclo-hydroxy-ornithine. FOHOrn is δN-formyl- δN-hydroxy-ornithine. εLys is Lys linked by ε-NH2. OHAsp is threo-β-hydroxy-aspartic acid. Dab is diamino-butanoic acid. OHH is threo- β-hydroxy-histidine. aThr is allo-Thr. AcOHOrn is δN-acetyl- δN-hydroxy-ornithine. Italicized peptide chains are inferred from siderotyping analysis (64). These pyoverdines are in the same siderotype as a pyoverdine having the structure provided. [a] + indicated growth of the Δ*pvdI-pchC* mutant in the presence of the test strain, - indicates no growth of the Δ*pvdI-pchC* mutant in the presence of the test strain.

**Table 2.3. Crossfeeding of the Δ*pvdI-pchC* mutant of Pf-5 by *Enterobacter cloacae***

| ***E. cloacae* strain** | **Genotype** | **Siderophores produced** | **Iron limited growth of JL4900** |
|---|---|---|---|
| EcCT-501 | Field isolate | enterobactin & aerobactin | + |
| LA122 | Δ*iuc* | enterobactin | + |
| LA266 | Δ*ent* | aerobactin | + |
| LA235 | Δ*iuc* Δ*ent* | None | - |

Genotype abbreviations: Aerobactin (iuc). Enterobactin (ent) (19)

**Discussion**

The 45 TonB-dependent outer-membrane proteins in the proteome of *P. fluorescens* Pf-5 (71) comprise 27 TBDRs and 18 TBDTs that share conserved β-barrel and plug domains but differ in the presence of an N-terminal signaling domain. Phylogenetic and bioinformatic analyses suggest a complex evolutionary history for the TonB-dependent outer-membrane proteins in Pf-5 including horizontal transfer among different microbial lineages. In a recent phylogenetic analysis of ~4,600 TonB-dependent outer-membrane proteins, Mirus et al. reported that, with few exceptions, the proteins cluster according to their substrate rather than taxonomy (67). The results of our study also provide convincing evidence of lateral transmission of these proteins among diverse groups of bacteria.

Iron is a limiting factor for many soil microorganisms including Pf-5, which uses pyoverdine and enantio-pyochelin to retrieve iron from its surroundings (40, 94). Here, we showed that Pf-5 can utilize a broad spectrum of exogenous siderophores as sources of iron. Phylogenetic analysis of the TBDTs in the Pf-5 genome indicated a high level of redundancy for the uptake of certain compounds, notably ferrioxamine, ferric-citrate, heme, and pyoverdines. The number of TBDTs in certain phylogenetic clades, such as those with putative functions in heme and pyoverdine acquisition, exceeds the number found in other bacteria such as *P. aeruginosa* PAO1, which also has multiple TonB-dependent outer-membrane proteins functioning in the uptake of ferrioxamine, enterobactin, heme and pyoverdines (15, 17). The diversity and complexity of the TBDTs with roles in iron uptake clearly indicate the importance of iron in the biology of Pf-5.

*P. fluorescens* Pf-5 was isolated from soil (41) and establishes populations in the rhizosphere when inoculated onto seed or root surfaces (8, 48-49, 79). The roles of TonB-dependent outer-membrane proteins in enhancing the access of bacteria to limited resources in the rhizosphere or bulk soil has been demonstrated only for iron and sulfur to

date. Siderophore-mediated competition for iron is a major determinant in interactions between certain strains of *Pseudomonas* spp., and the capacity to utilize a pyoverdine produced by a competing strain was shown to enhance the fitness of *P. fluorescens* living on root surfaces (75). Furthermore, levels of iron available to *Pseudomonas* spp. in the rhizosphere are known to be enhanced by siderophores produced by other rhizosphere bacteria. For example, a pyoverdine-producing strain of *Pseudomonas* spp. and enterobactin- and aerobactin- producing strains of *E. cloacae* enhanced the levels of iron available to *P. putida* in the rhizosphere, assessed using an iron biosensor (53-54). The results from these studies indicate that TonB-dependent outer-membrane proteins confer an advantage to *Pseudomonas* spp. in the rhizosphere due to enhanced iron uptake. Similarly, the capacity to utilize sulfur esters is necessary for optimal survival of *P. putida* in agricultural and grassland soils (44), and the sulfur-inducible TonB-dependent receptor SftP appears to function in sulfate ester metabolism. In addition to the SftP ortholog PFL_0213, several other genes encoding TonB-dependent receptors are linked to transport proteins with putative functions in sulfur transport in the Pf-5 genome (data not shown), and their role in sulfur metabolism is an intriguing area for future study. In addition to their roles in iron and sulfur uptake, TonB-dependent outer-membrane proteins are likely to function more broadly in the acquisition of resources by environmental prokaryotes like *P. fluorescens*, and future investigations should reveal novel roles of these transport systems in the ecology of soil and rhizosphere bacteria.

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## Chapter 3: *Pseudomonas fluorescens* Pf-5: The Pyoverdine Pirate

### Abstract

The soil bacterium *Pseudomonas fluorescens* Pf-5 produces two siderophores, a pyoverdine and enantio-pyochelin, and also utilizes ferric-complexes of pyoverdines produced by other strains of *Pseudomonas* spp. as sources of iron. Previously, phylogenetic analysis of the 45 TonB-dependent outer-membrane proteins in Pf-5 indicated that six are related to ferric-pyoverdine (Fpv) receptors from other *Pseudomonas* spp. Here, we introduced mutations in each of the six *fpv* genes to investigate their roles in heterologous pyoverdine uptake by Pf-5. Uptake of ferric-complexes of 14 pyoverdine structures was eliminated in Pf-5 by deleting a single *fpv*. We identified at least one ferric-pyoverdine that was taken up by each of the six Fpv outer-membrane proteins of Pf-5 and determined that phylogenetically-related Fpv proteins take up structurally-related pyoverdines. Functional redundancy of the Pf-5 Fpv outer-membrane proteins also was apparent, with several ferric-pyoverdines taken up by all single *fpv* mutants but not by mutants having deletions in two *fpv* genes, and eight ferric-pyoverdines taken up by all of the double *fpv* mutants of Pf-5. We speculate that the combined low-affinity uptake of ferric-pyoverdines by the six Fpv outer membrane proteins of Pf-5 provides access to iron sequestered by a broad set of structurally-diverse pyoverdines that are not taken up with high affinity by any single Fpv. Both high-affinity and low-affinity uptake mechanisms may operate in natural habitats of Pf-5, allowing the bacterium to access iron from the structurally-diverse pyoverdines produced by its co-inhabitants in soil and the rhizosphere.

## Introduction

*Pseudomonas* is a genus of γ proteobacteria known for its ubiquity in natural habitats and striking ecological, metabolic and biochemical diversity. *Pseudomonas fluorescens* are common inhabitants of soil and plant surfaces, and certain strains function in the biological control of plant disease, protecting plants from infection by soilborne and aerial plant pathogens. The soil bacterium *P. fluorescens* Pf-5 is a well-characterized biological control strain, distinguished by its prolific production of secondary metabolites including a spectrum of antibiotics that suppress plant pathogenic fungi (29, 35). Among the secondary metabolites produced by Pf-5 are two siderophores, enantio-pyochelin (66) and a pyoverdine, which function in iron acquisition by the bacterium.

Biologically-available iron is limited in aerobic environments at neutral pH. This lack of readily available iron stimulates the fluorescent pseudomonads to produce and release into the environment pyoverdine siderophores capable of binding ferric iron with high affinity (1, 19). Pyoverdines are a diverse group of siderophores with over 70 structures identified (10, 43). They are composed of a dihydroxyquinoline chromophore, which is responsible for diffusible green fluorescence; an acyl side chain (either dicarboxylic acid or amide) bound to the amino group of the chromophore; and a peptide chain of variable length (6-14 amino acids) and composition (43). The structural differences are primarily in the peptide chain, but differences in the chromophore and acyl side chains also occur (10). Iron is bound through interactions with the catechol unit of the chromophore and hydroxamate- or hydroxy acid-containing amino acids of the peptide chain (10).

Like other siderophores, the ferric-pyoverdines are bound and transported into the bacterial cell by TonB-dependent outer-membrane proteins (TBDPs) called ferric-pyoverdine (Fpv) outer-membrane proteins. The structure of Fpv outer-membrane proteins include a 22-stranded β-barrel, which forms a channel for transport of the ferric-

pyoverdine complex through the outer-membrane, extra-cellular loops, and a plug domain to block the channel formed by the β-barrel (13, 19, 50). Ferric-pyoverdines are moved via Fpv outer-membrane proteins across the outer-membrane into the periplasm, where the iron is released from the pyoverdine (22). Transport of ferric-siderophore complexes by Fpv outer-membrane proteins requires energy (18), which is provided by proton motive force by means of TonB-ExbB-ExbD complexes in the inner membrane. In FpvAI, a well-characterized Fpv in *P. aeruginosa*, a six amino acid motif called the TonB box is required for the interaction with TonB (50). To date, all Fpv outer-membrane proteins also have an N-terminal signaling domain that interacts with a regulatory protein (anti-sigma factor), which controls the expression of an ECF sigma factor (18, 25). Together, the TBDP, ECF sigma factor, and anti-sigma factor constitute a cell surface signaling system that functions in environmental sensing and signal relay into the cytoplasm.

The ferric-pyoverdine/Fpv interaction is best understood in *P. aeruginosa* PAO1 where the structural components and key binding residues of FpvAI have been characterized (49, 56). Collectively, strains of *P. aeruginosa* produce pyoverdines having three distinct structures (type I, II or III), with each strain producing one pyoverdine and the corresponding variant of FpvA (FpvAI, FpvAII, and FpvAIII). The ferric complex of the type I pyoverdine produced by PAO1 is bound by specific amino acids located in the plug, extra-cellular loops, and the β-barrel of FpvAI. These amino acid residues interact primarily with the pyoverdine chromophore and the hydroxamate-containing amino acids of the peptide chain (23). The specificity of Fpv outer-membrane proteins in binding and transport of cognate pyoverdines is well established (23, 50), but Fpv outer-membrane proteins also can function in the uptake of ferric complexes of heterologous pyoverdines having similar peptide chain sequences (23, 42, 45). For example, FpvAI can bind and take up ferric-pyoverdines produced by several *Pseudomonas* spp., albeit with varied affinities. Not all ferric-pyoverdines are bound by FpvAI, so the affinity determinants must lie outside of the regions common to all pyoverdines (i.e., the chromophore and iron-chelating hydroxamates) (23). The

conformation of the first three residues of the pyoverdine peptide chain are critical determinants of affinity, but other factors such as isomerization also play a role in binding and transport by FpvAI (23).

The capacity to utilize siderophores produced by other microorganisms provides a selective advantage to bacteria, providing a mechanism to acquire iron without investing in siderophore biosynthesis (27). Gram-negative bacteria commonly have multiple TBDPs in their outer membranes, some of which function in uptake of ferric complexes of siderophores produced by other organisms (28). For example, the soil bacterium *Pseudomonas fluorescens* Pf-5 has 45 TBDPs, many of which have predicted functions in the uptake of ferric-complexes of heterologous siderophores such as enterobactin, aerobactin, citrate, ferrioxamine, or ferrichrome (25). Phylogenetic analysis of the 45 TBDPs in the Pf-5 proteome indicated that six TBDPs are related to Fpv outer-membrane proteins from *Pseudomonas* spp. (25). Like many other *Pseudomonas* spp. (23, 31, 36, 46, 52), Pf-5 can utilize the ferric-complexes of many structurally-distinct pyoverdines as iron sources (25). The goal of this study was to characterize the six putative Fpv outer-membrane proteins in the proteome of Pf-5. First, homology modeling provided further evidence for the functions of the six putative Fpv outer-membrane proteins in uptake of ferric-pyoverdine complexes. We confirmed these functions by constructing six mutants, each having a deletion in one *fpv* gene, and assessing the mutants for utilization of specific heterologous pyoverdines produced by 37 strains of *Pseudomonas* spp. in crossfeeding assays. Each of the six mutants lacked the capacity to utilize ferric-complexes of one or more structurally-related pyoverdines, enabling the assignment of specific pyoverdines to each of the Fpv outer-membrane proteins in the Pf-5 genome. These results demonstrate that the capacity of *P. fluorescens* Pf-5 to utilize a diverse spectrum of ferric-pyoverdines as iron sources is achieved both through the capacity of individual Fpv outer-membrane proteins to take up pyoverdines with similar structures and the possession of six Fpv outer-membrane proteins, each recognizing structurally-distinct pyoverdines.

## Materials and methods

### Bacterial growth conditions

*Pseudomonas* strains were grown on King's medium B (KMB) (30) at 27$^{o}$C. *Escherichia coli* was grown on solidified Luria-Bertani at 37$^{o}$C. Antibiotics were used at the following concentrations (µg/ml): gentamicin 40 (*P. fluorescens*) and 12.5 (*E. coli*), kanamycin 50, streptomycin 100, tetracycline 200 (*P. fluorescens*) and 20 (*E. coli*) for generation of mutants.

### Pyoverdine peptide chain prediction

The amino acid composition of the peptide chain of pyoverdines with unknown structures was predicted from the nucleotide sequences of genes encoding the corresponding non-ribosomal peptide synthetases (NRPSs) using the NRPS/PKS predictor (3) and the NRPS predictor (http://www-ab.informatik.uni-tuebingen.de/software/NRPSpredictor) (53, 58).

### Sequence alignment and structure prediction

The multiple sequence alignment tool T-Coffee was used to align the amino acid sequences of the Fpv outer-membrane proteins of Pf-5 (51). PSIPRED GenTHREADER (37) and a β-barrel prediction model (7) were used to predict the secondary structure of the six Fpv outer-membrane proteins in the Pf-5 proteome. Homology modeling of the six Fpv outer-membrane proteins was done using the SWISS-MODEL server and Deepview (2, 9). The homology models were constructed using a structure-based sequence alignment with the crystal structure of FpvAI from *P. aeruginosa* PAO1 as a template.

### Construction of Pf-5 mutants

Individual deletion constructs for the six *fpv* genes and PFL_2772 were made as described in Hassan et al. (26) using overlap-extension PCR methods modified from Choi

and Schweizer (12) with primers specific to each gene (Table 1). Deletions were introduced into Pf-5 and into mutants deficient in pyoverdine and enantio-pyochelin production (i.e., Δ*pvdI-pchC* and Δ*pvdI-pchA* mutants), which were derived previously (25). Combinations of *fpv* deletions were introduced into the Δ*pvdI-pchC* mutant background as described previously (25).

**Table 3.1. Primers used in the construction of mutants of *P. fluorescens* Pf-5**

| Target gene and primers | Sequence 5'-3' |
|---|---|
| **FpvZ (PFL_4092)** | |
| 4092UpFBam | CACACCATCAGGATCCACAACACCGACTGACCCCTTT |
| 4092DnFFRT-1 | AGGAACTTCAAGATCCCCAATTCGATGCCGGAGCCATCTATGA |
| 4092UpRFRT | TCAGAGCGCTTTTGAAGCTAATTCGATGTTCTGGTCATCCATGCGC |
| 4092DnRBam-1 | CTCTGCTTCTGGATCCTGTAGATGGTGTTCTGGCCA |
| **FpvW (PFL_2293)** | |
| 2293UpFHind | GTGGTTGTGGAAGCTTTTCACAAGTCGAAGTTGGCC |
| 2293DnFFRT | AGGAACTTCAAGATCCCCAATTCGCCGACGACAGCTACTACGAAA |
| 2293UpRFRT | TCAGAGCGCTTTTGAAGCTAATTCGTCTCCATCACCTGGTCAATG |
| 2293DnRHind | GACGAAGACGAAGCTTAGTTGTCACTCTGGGCGTTGA |
| **FpvX (PFL_3315)** | |
| 3315UpFBam | GTTGTGCTGAGGATCCCAAACGGTGACGGTGATCA |
| 3315DnFFRT-1 | AGGAACTTCAAGATCCCCAATTCGTGGTCTACGACCTCAACGACA |
| 3315UpRFRT | TCAGAGCGCTTTTGAAGCTAATTCGTGAAGTACATCGGGAAGCC |
| 3315DnRBam-1 | GAGAAGGAGAGGATCCGAAGCCGGTGCTGAAATTG |
| **FpvV (PFL_2527)** | |
| 2527UpFBam | GTGTGGTAGTGGATCCTGCACCGTAGTTACCGTAGGA |
| 2527DnFFRT | AGGAACTTCAAGATCCCCAATTCGTTGCGGATCAGGTTGATGGT |
| 2527UpRFRT | TCAGAGCGCTTTTGAAGCTAATTCGTACACCAGCATCTTCAACCCC |
| 2527DnRBam | CACACCATCAGGATCCGCGCACATTTTGCTGTCCTA |
| **FpvU (PFL_2391)** | |
| 2391UpFBam | CTCTGCTTCTGGATCCACAGGTTCTGGGTGATCTGGT |
| 2391DnFFRT | AGGAACTTCAAGATCCCCAATTCGTTCTTGCGCACCAGGTTGAT |
| 2391UpRFRT | TCAGAGCGCTTTTGAAGCTAATTCGAAGGACAGCAAGCTGCTCAAC |
| 2391DnRBam | GAGAAGGAGAGGATCCAACTGAGTACCCAGAGCGGTT |
| **FpvY (PFL_3485)** | |
| 3485UpFBam | GAGAAGGAGAGGATCCCGGGCTATCGGGGTAATACA |
| 3485DnFFRT1 | AGGAACTTCAAGATCCCCAATTCGCCTACTTCGAGGTGCATGA |
| 3485UpRFRT | TCAGAGCGCTTTTGAAGCTAATTCGTCGAGGATGCCGTAGTAGA |
| 3485DnRBam | GTGAGTTGCTGGATCCCCTTGCCGTAGTTGCTGAGTA |
| **PFL_2772** | |
| 2772UpFHind | CAGCACGAAGCTTCGGTTTTCACCGCCAGCTTC |
| 2772UpR | GGCGTGATGGCGCTCCAGCAATTCATAGGGC |
| 2772DnF | TTGCTGGAGCGCCATCACGCCTTACGAACT |
| 2772DnRHind | CTCCTCGAAGCTTAGGAGTACCTGGTGATACGC |

**Iron-limited growth**

The six *fpv* mutants of Pf-5 were tested for iron-limited growth as described in Hartney et al. (25). Briefly, bacterial cells from overnight cultures grown in KMB broth were suspended in water to 0.1 $OD_{600}$. 5 µl of 100 fold diluted cell suspension was placed on KMB agar containing the iron chelator 2, 2'-dipyridyl (Sigma-Aldrich, St Louis, MO, USA) at 0, 100, 200, 400, 600, and 800 µM. Bacterial growth was observed following 24 h incubation at 27$^{o}$C. Each strain was tested in at least two experiments, each evaluating two replicate plates.

**Crossfeeding assays**

Crossfeeding assays were performed as described in Hartney et al. (25). Briefly, cells from test strains and indicator strains were suspended in water to 0.1 $OD_{600.}$ Indicator strain cell suspensions were diluted 100 fold in sterile water. Ten µl of the test strain suspension and 5 µl of the diluted cell suspension of the indicator strains were placed 10 mm apart from one another on the surface of KMB amended with 2,2'-dipyridyl at 400 µM or 600 µM. For test strains that did not grow on KMB amended with 2,2'-dipyridyl at 400 µM or 600 µM, an agar plug (6 mm) obtained from a 48 h culture on KMB was substituted for the cell suspension. Plates were incubated at 27°C, and growth of the indicator strain was observed at 24 h and 36 to48 h. Growth of each test strain was evaluated on two replicate plates in at least two experiments.

**Pyoverdine purification**

Pyoverdines from *Pseudomonas* spp. were obtained from cultures grown in succinate medium (41) at 25°C with shaking at 200 rpm. After 72 h incubation, bacterial cells were harvested by centrifugation. The supernatant was adjusted to pH 6.0 and centrifuged again (5200 x g, 30 min) before ion exchange chromatography onto a column of Amberlite XAD-4 (11). The pyoverdines were eluted with 100% methanol.

Pyoverdine eluate was evaporated in a rotary evaporator, suspended in 5 ml of milliQ water and placed at 4°C overnight. A second ion exchange chromatography was performed on a column of LiChroprep RP-18 (40-63 µm) (Merck, Whitehouse Station, NJ). After loading, the column was rinsed with EDTA (0.1M) followed by pH 4.0 acidified water (formic acid 1%) and pyoverdines were eluted with 50% methanol. After use, the column was washed with methanol, regenerated with 1% HCl, rinsed with deionized water, and stored at 4°C. Pyoverdines were concentrated and lyophilized prior to storage at 4°C in the dark.

**Utilization of purified pyoverdines as iron sources**

*Fpv* mutants of Pf-5 were evaluated for utilization of pyoverdines produced by selected strains of *Pseudomonas* spp. One hundred µl of a 100 fold dilution of a 0.1 $OD_{600}$ suspension of a Pf-5 mutant was spread onto KMB agar plates amended with 400µM 2,2'-dypyridyl . Five µl of an aqueous pyoverdine solution (8 mM) was applied to a 5mm filter paper disk placed on the agar surface. Plates were incubated at 27°C for 24 h prior to evaluation of growth of the mutant strain.

**Phylogenetic analysis**

A phylogenetic analysis of the six Fpv outer-membrane proteins from Pf-5 and orthologous proteins was performed using the Neighbor Joining method available through MEGA version 4.0.2 (60). Alignment of the amino acid sequences was done using Clustal W with a gap open penalty of 15 and a gap extension penalty of 0.3. The aligned and masked sequences were subjected to Neighbor Joining analysis. The bootstrap consensus tree inferred from 1000 replicates represented the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 30% bootstrap replicates were collapsed. Phylogenetic analysis of the plug domains from the 45 TBDPs in Pf-5 was preformed as above, except using the Maximum Parsimony analysis method.

## Results and discussion

### Structural analysis of the pyoverdine TonB-dependent outer-membrane proteins

Protein structure analysis using PSIPRED GenTHREADER matched all six of the putative Pf-5 Fpv outer-membrane proteins to FpvAI (PA2398) from *P. aeruginosa* PAO1. Due to their similarities to FpvAI, we adopted the naming convention established for *P. aeruginosa*, and the six pyoverdine uptake proteins in Pf-5 are hereafter called FpvU (PFL_2391), FpvV (PFL_2527), FpvW (PFL_2293), FpvX (PFL_3315), FpvY (PFL_3485), and FpvZ (PFL_4092).

Homology modeling of the six Pf-5 Fpv outer-membrane proteins was done using the known crystal structure of FpvAI (PDB: 2w16A) as a template (Fig. 3.1a). A model of FpvW could not be generated due to its divergence from FpvAI, but root mean squared (RMS) values were calculated for backbone residues of the other five Fpv outer-membrane proteins in Pf-5: FpvU, 0.173Å; FpvY, 0.40Å; FpvX, 0.55Å; FpvZ, 0.626Å; and FpvV, 1.51Å. Comparison of the six Fpv outer-membrane proteins of Pf-5 to FpvAI indicated secondary structural similarities of the proteins, which is especially evident in the β-strands of the β-barrel, the plug domain involved in binding and movement of substrate (50), and the N-terminal signaling domain involved in the signaling cascade to regulate pyoverdine biosynthesis (56) (Fig. 3.1a, Fig. 3.2). Differences in the extra-cellular loops and the position of the connecting loop between the plug and the N-terminal signaling domain can be seen between the models of FpvAI and FpvV, Y, X, and Z (Fig. 3.1a1, a3-a6).

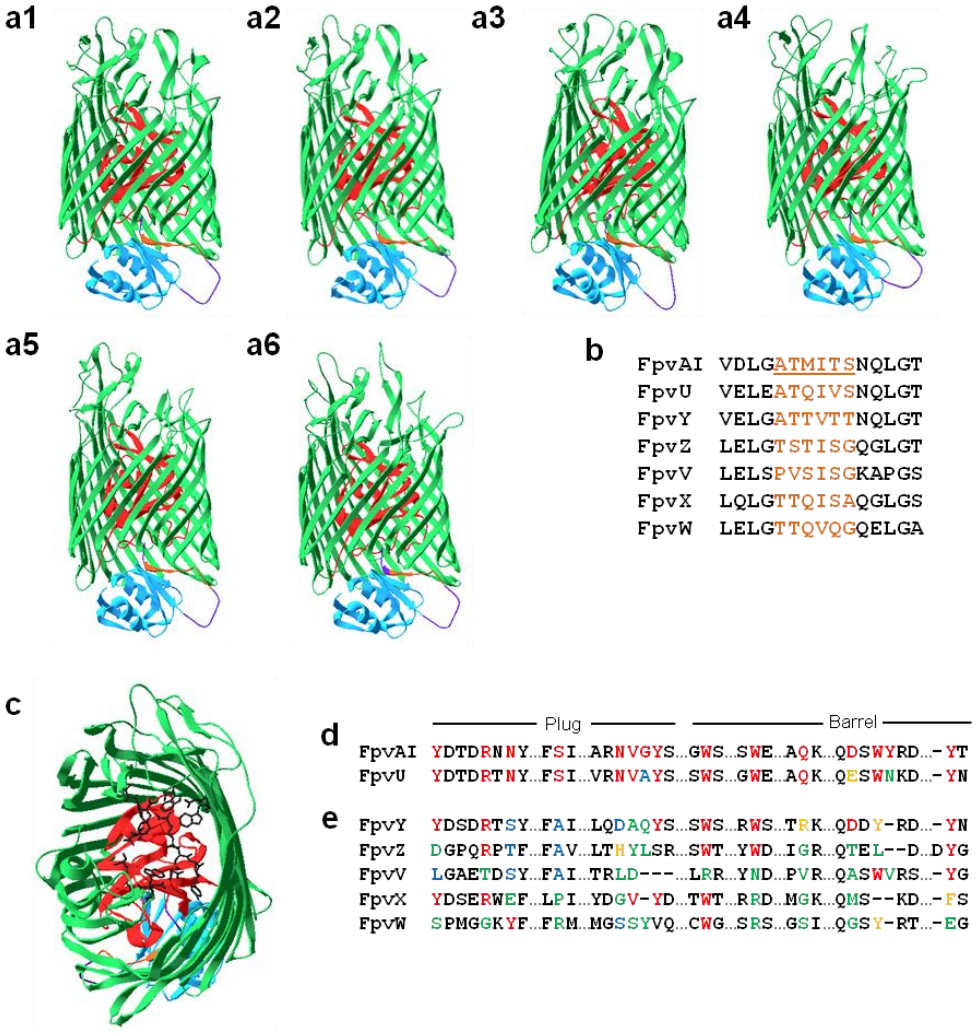


**Fig. 3.1. Homology models of the Pf-5 Fpv outer-membrane proteins**. Models of the Fpv outer-membrane proteins in Pf-5 and FpvAI showing the structural components with the β-barrel in green, plug in red, N-terminal signaling domain in blue, connecting loop in purple, and TonB box in brown. **a1**, FpvAI; **a2**, FpvU; **a3**, FpvY; **a4**, FpvZ; **a5**, FpvV; **a6**, FpvX. **b**. Alignment of the TonB box region for FpvAI and the six Fpv outer-membrane proteins from Pf-5. The characterized amino acid residues from FpvAI are underlined. The TonB box residues located within the sheet structure are in brown. **c**. Position of amino acid residues in FpvU from the plug and β-barrel with homology to the residues of FpvAI that are involved in the binding of pyoverdine. Amino acid side chain structures are shown in black. **d & e**. Alignment of FpvAI and the six Pf-5 Fpv outer-membrane proteins showing amino acid residues of FpvAI involved in pyoverdine binding in the plug and β-barrel domains (red). Identical residues are in red, conservative substitutions are in blue, semi-conservative substitutions are in yellow and non-conservative substitutions are in green.

The RMS values and sequence alignments of the Pf-5 proteins indicate that FpvU is the most closely related to FpvAI (68% identical at the amino acid level). Of the 15 amino acids located in the binding pocket of FpvAI that interact with amino acids in the peptide chain of the ferric pyoverdine complex of PAO1 (Fig. 3.1d) (23, 56), 12 amino acids also are present in the binding pocket of FpvU (Fig. 3.1d). Homology modeling between FpvAI and FpvU indicated that the 15 binding residues are in identical locations in both proteins: the channel of the β-barrels, the extra-cellular loops, and the plug domains (Fig. 3.1c). Two of the altered binding residues are conservative substitutions (G230 to A237 in the plug domain, and D597 to E606 in the β-barrel) whereas one is a non-conservative substitution (Y600 to N609 in the β-barrel). Based upon this high level of conservation in the binding residues of FpvU and FpvAI, we expect that FpvU and FpvAI would recognize similar pyoverdines. Putative pyoverdine binding residues of FpvZ, FpvV, FpvW, FpvX, and FpvY could not be identified from homology models or by alignment due to their divergence from FpvAI (Fig. 3.1e).

The amino acid sequences of the TonB boxes of the Pf-5 Fpv outer-membrane proteins are divergent, but nevertheless, could be identified based on their locations in corresponding structural models and the presence of bordering leucine residues (Fig. 3.1a and 3.1b). FpvU shares four of the six residues with the well-characterized TonB box of FpvAI (13, 57), whereas the TonB boxes of the other five Fpv outer-membrane proteins of Pf-5 share only one to three of the six residues. The lack of sequence conservation in TonB boxes was noted previously for the 45 TonB-dependent outer membrane proteins of Pf-5 (25) and for an extensive set of characterized TonB-dependent outer-membrane proteins from other bacteria (33). The latter study concluded that divergence in the amino acid sequence of the TonB box has little effect on substrate binding as long as the surrounding residues forming a β-strand are conserved. Secondary protein structure rather than primary amino acid sequence is thought to determine binding to TonB; specifically the interaction of a β-strand encompassing the TonB box motif of the TBDP with the β-sheet structure of TonB (33). Homology models of five of the Pf-5 Fpv outer-membrane proteins clearly indicate the presence of a β-strand structure overlapping the

TonB box (Fig. 3.1a), providing evidence for the secondary structure being required for interaction with TonB.

**Fig. 3.2. Alignment of PA2398 (FpvAI) from *P. aeruginosa* PAO1 with the six Fpv outer-membrane proteins from Pf-5.** The colored lines above the alignment delineate domains and conserved regions based on the characterized residues of FpvAI. The N-terminal signaling domain is blue. The connecting loop is purple. The TonB box is brown. The plug domain is red and the β-strands of the β-barrel are in dark green. Amino acid residues of FpvAI (PA2398) involved in pyoverdine binding are indicated with an asterisk. Residues are highlighted to show levels of similarity. Similar residues are green, identical residues are pink and black residues are globally conserved.

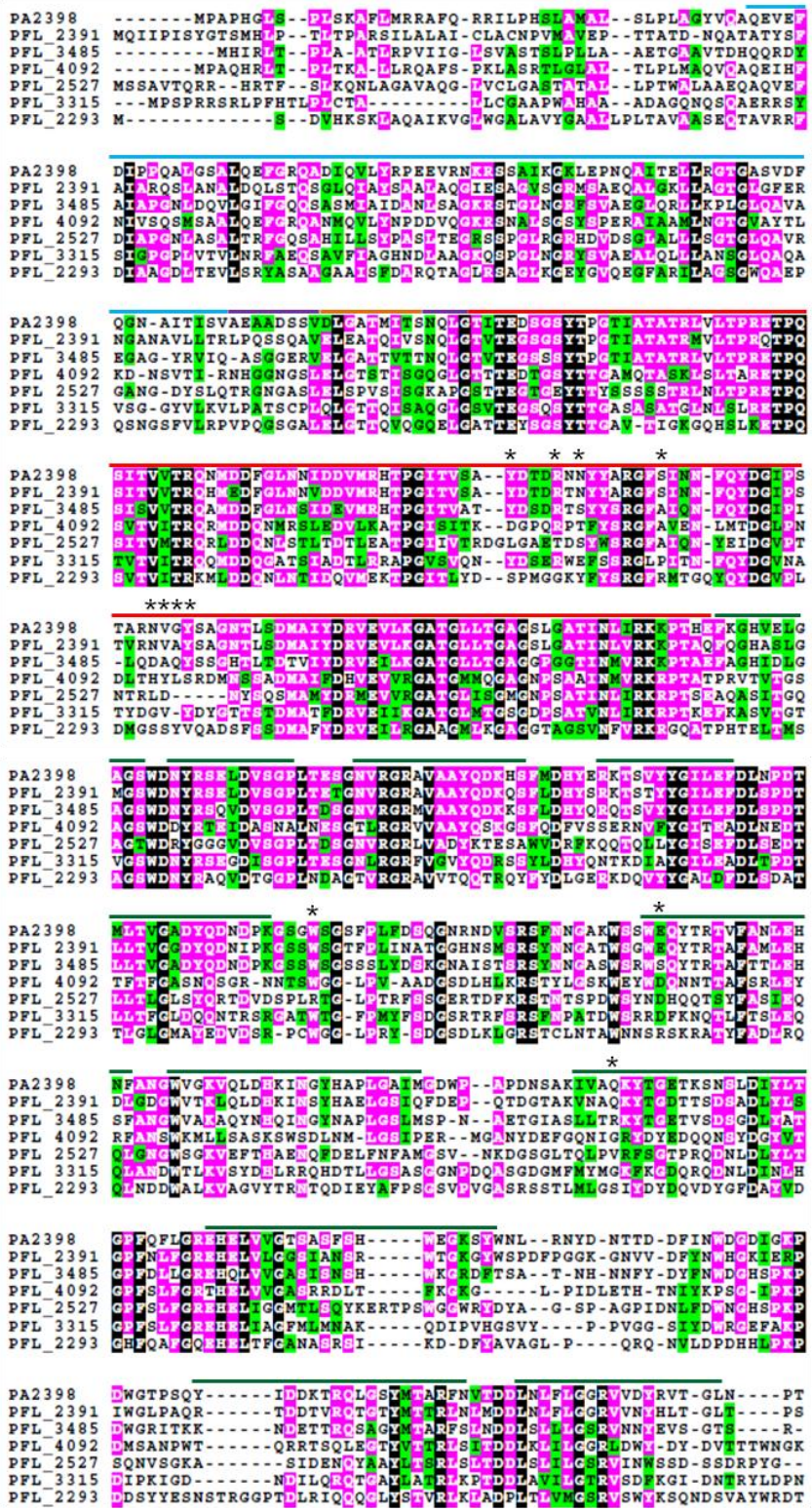

**Fig. 3.2.**

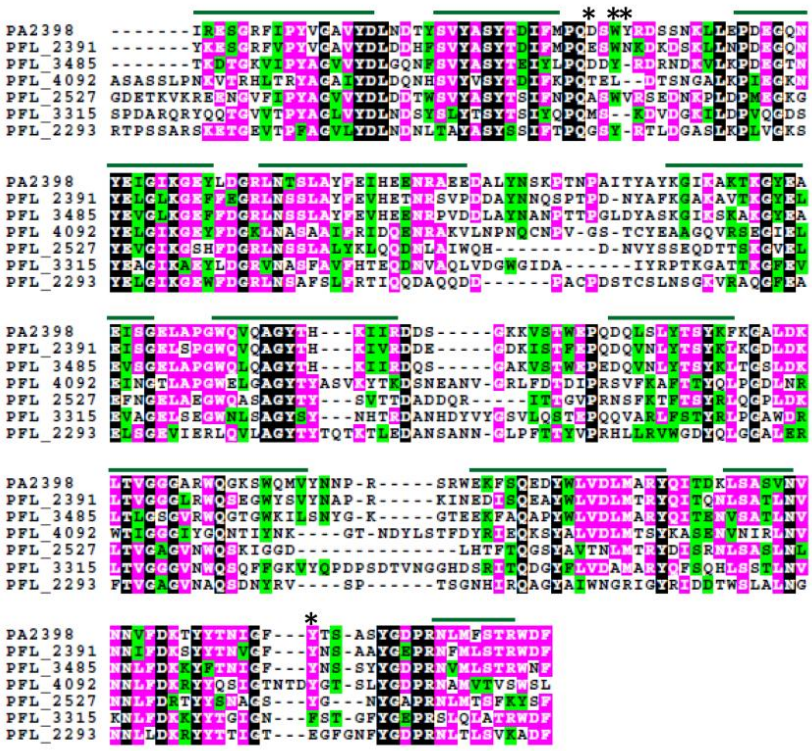

**Fig. 3.2., Continued**

**Iron-limited growth of *fpv* mutants of Pf-5**

To determine the functions of the six Fpv outer-membrane proteins in ferric-pyoverdine uptake, a deletion in each *fpv* gene was introduced into wild type Pf-5 and its Δ*pvdI-pchC* and Δ*pvdI-pchA* mutant derivatives, which are deficient in producing pyoverdine and enantio-pyochelin siderophores. Deletions in five of the *fpv* genes had no detectable effect on iron-limited growth when introduced into the wild type background (Table 3.2). These five mutants (*fpvU, fpvV, fpvW, fpvX,* and *fpvY*) exhibited wild type levels of fluorescence and, like Pf-5, grew on KMB amended with up to 800 μM of the chelator 2,2'-dipyridyl. In contrast, a mutation in *fpvZ,* which is located in a pyoverdine biosynthesis gene cluster, reduced the capacity of Pf-5 for iron-limited growth. This mutant, like a Δ*pvdI* mutant of Pf-5, grew only on KMB containing ≤ 600 μM 2,2'-dipyridyl (Table 3.2). In *P. aeruginosa* the coordinate regulation of pyoverdine biosynthesis and transport genes has been described, where the FpvA mutant exhibits loss of pyoverdine production (54-55). We attribute the phenotype of the *fpvZ* mutant to the loss of pyoverdine production due to altered regulation of the pyoverdine biosynthesis genes related to the deleted *fpvZ* gene.

**Table 3.2. Iron limited growth of Pf-5 mutants**

| Strain # | Siderophore biosynthesis mutations | Deleted *fpv* | | | | | | Fluorescence[a] | 2,2'-dipyridyl Concentration (µM)[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 200 | 400 | 600 | 800 |
| JL4585 | $Pvd^+$, $Pch^+$ (Pf-5 Wt) | | | | | | | + | + | + | + | + |
| JL4806 | *pvdI* (PFL_4095) | | | | | | | - | + | + | * | - |
| JL4898 | *pchC* (PFL_3490) | | | | | | | + | + | + | + | + |
| LK078 | *pchA* (PFL_3488) | | | | | | | + | + | + | + | * |
| JL4900 | *pvdI, pchC* | | | | | | | - | + | * | - | - |
| LK032 | *pvdI, pchA* | | | | | | | - | + | * | - | - |
| JL4992 | $Pvd^+$, $Pch^+$ | *fpvZ* | | | | | | + | + | + | * | - |
| JL4993 | *pvdI, pchC* | *fpvZ* | | | | | | - | + | * | - | - |
| LK155 | *pvdI, pchA* | *fpvZ* | | | | | | - | + | * | - | - |
| LK157 | $Pvd^+$, $Pch^+$ | | *fpvV* | | | | | + | + | + | + | + |
| JL4994 | *pvdI, pchC* | | *fpvV* | | | | | - | + | * | - | - |
| LK151 | *pvdI, pchA* | | *fpvV* | | | | | - | + | * | - | - |
| LK149 | $Pvd^+$, $Pch^+$ | | | *fpvU* | | | | + | + | + | + | + |
| JL4997 | *pvdI, pchC* | | | *fpvU* | | | | - | + | * | - | - |
| LK154 | *pvdI, pchA* | | | *fpvU* | | | | - | + | * | - | - |
| LK176 | $Pvd^+$, $Pch^+$ | | | | *fpvY* | | | + | + | + | + | + |
| JL4996 | *pvdI, pchC* | | | | *fpvY* | | | - | + | * | - | - |
| LK148 | *pvdI, pchA* | | | | *fpvY* | | | - | + | * | - | - |
| LK177 | $Pvd^+$, $Pch^+$ | | | | | *fpvX* | | + | + | + | + | + |
| JL4995 | *pvdI, pchC* | | | | | *fpvX* | | - | + | * | - | - |
| LK150 | *pvdI, pchA* | | | | | *fpvX* | | - | + | * | - | - |
| LK156 | $Pvd^+$, $Pch^+$ | | | | | | *fpvW* | + | + | + | + | + |
| LK036 | *pvdI, pchC* | | | | | | *fpvW* | - | + | * | - | - |
| LK153 | *pvdI, pchA* | | | | | | *fpvW* | - | + | * | - | - |
| LK124 | *pvdI, pchC* | *fpvZ* | | | | | *fpvW* | - | + | * | - | - |
| LK072 | *pvdI, pchC* | *fpvZ* | | | *fpvY* | | | - | + | * | - | - |
| LK054 | *pvdI, pchC* | *fpvZ* | | *fpvU* | | | | - | + | * | - | - |
| LK074 | *pvdI, pchC* | *fpvZ* | | | | *fpvX* | | - | + | * | - | - |
| JL4999 | *pvdI, pchC* | *fpvZ* | *fpvV* | | | | | - | + | * | - | - |
| LK000 | *pvdI, pchC* | | *fpvV* | | | *fpvX* | | - | + | * | - | - |
| LK125 | *pvdI, pchC* | | *fpvV* | | | | *fpvW* | - | + | * | - | - |
| LK073 | *pvdI, pchC* | | *fpvV* | | *fpvY* | | | - | + | * | - | - |

**Table 3.2. (Continued)**

| **Strain #** | **Siderophore biosynthesis mutations** | **Deleted *fpv*** | | | | | | **Fluorescence[a]** | **2,2'-dipyridyl Concentration (µM)[b]** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | **200** | **400** | **600** | **800** |
| LK076 | *pvdI, pchC* | | *fpvV* | *fpvU* | | | | - | + | * | - | - |
| LK128 | *pvdI, pchC* | | | *fpvU* | | | *fpvW* | - | + | * | - | - |
| JL4998 | *pvdI, pchC* | | | *fpvU* | *fpvY* | | | - | + | * | - | - |
| LK075 | *pvdI, pchC* | | | *fpvU* | | *fpvX* | | - | + | * | - | - |
| LK126 | *pvdI, pchC* | | | | | *fpvX* | *fpvW* | - | + | * | - | - |
| LK127 | *pvdI, pchC* | | | | *fpvY* | | *fpvW* | - | + | * | - | - |
| LK071 | *pvdI, pchC* | | | | *fpvY* | *fpvX* | | - | + | * | - | - |

[a]+ indicates fluorescence of colonies under UV light. – indicates no fluorescence of colonies under UV light. [b]+ indicates growth, - indicates no growth, * indicates limited growth

**Utilization of heterologous ferric-pyoverdines by Pf-5 and *fpv* mutants**

Thirty-one strains of *Pseudomonas* spp. known to crossfeed the Δ*pvdI-pchC* mutant of Pf-5 (25) and six additional strains were tested for crossfeeding of the six *fpv* mutants (*fpvU, fpvV, fpvW, fpvX, fpvY, and fpvZ*) in the Δ*pvdI-pchC* mutant background (Table 3.3). A subset of strains was tested for crossfeeding of *fpv* mutants in a Δ*pvdI-pchA* background to rule out the influence of residual enantio-pyochelin production by the Δ*pvdI-pchC* mutant (25). There was no detectable difference between a given *fpv* mutant in the two mutant backgrounds in crossfeeding assays (Table 3.3 and Table 3.4).

As predicted from its location within a pyoverdine biosynthesis gene cluster (25), FpvZ was necessary for uptake of the pyoverdine produced by wildtype Pf-5. FpvZ also was necessary for crossfeeding by *P. chlororaphis* DTR133, *P. fluorescens* CFBP2130, *P. fluorescens* CHA0, and *P. chlororaphis* subsp. *aureofaciens* ATCC13985 (Table 3.3). Three of these strains with known structures produce pyoverdines having eight amino acids in the peptide chain with the first four amino acids (Asp-FOHOrn-Lys-Thr) in common (Table 3.3).

**Table 3.3. Crossfeeding of single *fpv* mutants in a Δ*pvdI-pchC* mutant background of Pf-5**

| Pyoverdine-Producing Strain | Pf-5 Deletion Mutant[a] | | | | | | Composition of Peptide Chain[b] | Reference or Source |
|---|---|---|---|---|---|---|---|---|
| | *fpvZ* | *fpvU* | *fpvX* | *fpvW* | *fpvY* | *fpvV* | | |
| *P. fluorescens* Pf-5 | - | + | + | + | + | + | Asp-FOHOrn-Lys-Thr-Ala-Ala-FOHOrn-Lys | Harald Gross, University of Bonn |
| *P. fluorescens* CHA0 | - | + | + | + | + | + | Asp-FOHOrn-Lys-(Thr-Ala-Ala-FOHOrn-Lys) | (63) |
| *P. chlororaphis* D-TR133 | - | + | + | + | + | + | Asp-FOHOrn-Lys-(Thr-Ala-Ala-FOHOrn-Ala) | (43) |
| *P. fluorescens* CFBP2130 | - | + | + | + | + | + | Unknown | http://www.straininfo.net/strains/757032 |
| *P. chlororaphis* subsp. *aureofaciens* ATCC 13985 | - | + | + | + | + | + | Unknown | (59) |
| *P. aeruginosa* PAO1 | + | - | + | + | + | + | Ser-Arg-Ser-FOHOrn-(Lys-FOHOrn-Thr-Thr) (Type I pyoverdine) | (16) |
| *P. aeruginosa* PA14 | + | - | + | + | + | + | Ser-Arg-Ser-FOHOrn-Lys-FOHOrn-Thr-Thr | (25) |
| *P. fluorescens* SBW25 | + | - | + | + | + | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (47) |
| *P. fluorescens* ATCC 13525 | + | - | + | + | + | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (39) |
| *P. chlororaphis* ATCC 9446 | + | - | + | + | + | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (39) |
| *P. fluorescens* ATCC 17518 | + | - | + | + | + | + | Unknown | (59) |
| *P. putida* CS111 syn SB8.3 | + | + | - | + | + | + | *Ala-Lys-Thr-Ser-OHOrn-OHOrn* | (44) |

**Table 3.3. (Continued)**

| Pyoverdine-Producing Strain | Pf-5 Deletion Mutant[a] | | | | | | Composition of Peptide Chain[b] | Reference or Source |
|---|---|---|---|---|---|---|---|---|
| | *fpvZ* | *fpvU* | *fpvX* | *fpvW* | *fpvY* | *fpvV* | | |
| *P. putida* ATCC 17470 | + | + | - | + | + | + | Unknown | (59) |
| *P. fluorescens* B10 | + | + | + | - | + | + | εLys-OHAsp-Ala-aThr-Ala-cOHOrn | (62) |
| *P. lini* DLE411J | + | + | + | - | + | + | *Lys-OHAsp-Ala-Thr-Ala-OHOrn* | (44) |
| *P. fluorescens* ATCC 17513 | + | + | + | - | + | + | Unknown | (59) |
| *P. putida* W4P63 | + | + | + | - | + | + | Unknown | (64) |
| *P. rhodesiae* CFML92-104 | + | + | + | + | - | + | Ser–Lys–FOHOrn–Ser–Ser–Gly–(Lys–FOHOrn–Ser–Ser) | (10) |
| *P. rhodesiae* DSM14020 | + | + | + | + | - | + | *Ser–Lys–FOHOrn–Ser–Ser–Gly–(Lys–FOHOrn–Ser–Ser)* | (43-44) |
| *P. salomonii* CFBP2022 | + | + | + | + | - | + | *Ser-Orn-FOHOrn-Ser-Ser-Lys-FOHOrn-Ser* | (38) |
| *P. marginalis* pv. *alfalfa* NCPPB 2644 | + | + | + | + | - | + | Unknown | (65) |
| *P. marginalis* pv. *marginalis* NCPPB 667 | + | + | + | + | - | + | Unknown | (65) |
| *P. marginalis* pv. *pastinacae* NCPPB 806 | + | + | + | + | - | + | Unknown | (65) |
| *P. reactans* NCPPB387 | + | + | + | + | - | + | Unknown | http://www.straininfo.net/strains/53319 |
| *P. putida* Bn7 | + | + | + | + | + | - | Unknown | (32) |
| *P. fluorescens* WCS374 | + | + | + | + | + | + | Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser) | (17) |
| *P. fluorescens* DSM50106 | + | + | + | + | + | + | Ser–Lys–Gly–FOHOrn–Ser–Ser–Gly–(Orn–FOHOrn–Ser) | (43) |
| *P. fluorescens* CLR711 syn PL7 | + | + | + | + | + | + | Ser-AcOHOrn-Ala-Gly-aThr-Ala-cOHOrn | (5) |

**Table 3.3. (Continued)**

| Pyoverdine-Producing Strain | Pf-5 Deletion Mutant[a] | | | | | | Composition of Peptide Chain[b] | Reference or Source |
|---|---|---|---|---|---|---|---|---|
| | *fpvZ* | *fpvU* | *fpvX* | *fpvW* | *fpvY* | *fpvV* | | |
| *P. costantinii* CFBP5705 | + | + | + | + | + | + | Ser-AcOHOrn-Gly- aThr-Thr-Gln-Gly-Ser-cOHOrn | (40) |
| *P. fluorescens* CTRp112 syn PL8 | + | + | + | + | + | + | Lys-AcOHOrn-Ala-Gly-aThr-Ser-cOHOrn | (5) |
| *P. fluorescens* A6 | + | + | + | + | + | + | Lys-AcOHOrn-Gly-aThr-Thr-Gln-Gly-Ser-cOHOrn | (6) |
| *P. putida* CFML90-40 | + | + | + | + | + | + | Asp-Ala-Asp-AcOHOrn-Ser-cOHOrn | (44) |
| *P. fluorescens* Pf0-1 | + | + | + | + | + | + | Ala-AcOHOrn-Orn-Ser-Ser-Ser-Arg-OHAsp-Thr | (43) |
| *P. aeruginosa* ATCC 27853 | + | + | + | + | + | + | Ser-FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) | (61) |
| *P. aeruginosa* 7NSK2 | + | + | + | + | + | + | Ser-FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) | (14) |
| *P. aeruginosa* Pa6 | + | + | + | + | + | + | Ser-cDab-FOHOrn-Gln-Gln-FOHOrn-Gly (Type III pyoverdine) | (21) |
| *P. aeruginosa* LESB58 | + | + | + | + | + | + | Ser-?-OHOrn-Gln-Gln-OHOrn-Gly (Type III pyoverdine) | This study, predicted structure |

[a]+ indicates growth of the Pf-5 mutant on an iron-limited medium in the presence of the pyoverdine-producing strain. – indicates no growth of the mutant in the presence of the pyoverdine-producing strain.

[b]Underline denotes D-amino acids. Parentheses define cyclic residues. cOhOrn is cyclo-hydroxy-ornithine. FOHOrn is δN-formyl- δN-hydroxy-ornithine. εLys is Lys linked by its ε-NH2. OHAsp is threo-β-hydroxy-aspartic acid. Dab is diamino-butanoic acid. OHHis is threo- β-hydroxy-histidine. aThr is allo-Thr. AcOHOrn is δN-acetyl- δN-hydroxy-ornithine. Italicized peptide chains are inferred from siderotyping analysis (43). These pyoverdines are in the same siderotype as a pyoverdine having the structure provided. [a] + indicates growth of the mutant in the presence of the donor strain. – indicates no growth of the mutant in the presence of the donor strain.

As described above, FpvU is similar to FpvAI, which recognizes the type I pyoverdine produced by *P. aeruginosa*. As expected, a deletion in *fpvU* eliminated crossfeeding of Pf-5 by the type I-pyoverdine-producing strains *P. aeruginosa* PAO1 and *P. aeruginosa* PA14. FpvU also was required for crossfeeding by *P. fluorescens* SBW25, *P. fluorescens* ATCC 13525, *P. chlororaphis* ATCC 9446, and *P. fluorescens* ATCC 17518. Of these four strains, three make pyoverdines with known structures (Table 3.3). The pyoverdines recognized by FpvU have seven or eight amino acids in the peptide chain, with D-Ser in the first position, Arg or Lys in the second position, a small residue (Ser or Gly) in the third position, followed by FOHOrn-Lys-FOHOrn (Table 3.3). Thus, the first four amino acids of the peptide chains recognized by FpvU are identical to those of the pyoverdines found by Greenwald et al. (23) to bind with high affinity to FpvAI.

FpvY is similar in sequence to FpvU of Pf-5 and FpvAI of *P. aeruginosa* PAO1, with 60% amino acid identity to both proteins. FpvY and FpvU also share six of the fifteen substrate binding residues in the plug and receptor domains, as determined through comparison to FpvAI (Fig. 3.1d & e; Fig. 3.2). Despite the sequence similarity between FpvY and FpvU, deletion of *fpvY* indicated its requirement for crossfeeding by a different set of *Pseudomonas* spp. than FpvU (*P. rhodesiae* DSM14020, *P. rhodesiae* CFML92-104, *P. salomonii* CFBP2022, *P. reactans* NCPPB387, and three pathovars of *P. marginalis*). Three of the strains produce pyoverdines with peptide chains identified chemically or by siderotyping (Table 3.3). Pyoverdines of these three strains have peptide chains with eight or ten residues, and share similar amino acid sequences that vary in a Lys in the second position, and insertions of Gly in the sixth position and a Ser residue at the C-terminus (two strains of *P. rhodesiae*).

**Table 3.4. Crossfeeding assays with *fpv* deletion mutants in a Δ*pvdI-pchA* background of Pf-5**

| | **Pf-5 *fpv* mutants in Δ*pvdI-pchA* background[a]** | | | | | |
|---|---|---|---|---|---|---|
| **Feeding Strains** | *fpvZ* | *fpvU* | *fpvV* | *fpvX* | *fpvY* | *fpvW* |
| *P. fluorescens* Pf-5 | - | ND | ND | ND | ND | ND |
| *P. fluorescens* CHA0 | - | ND | ND | ND | ND | ND |
| *P. chlororaphis* subsp. *aureofaciens* ATCC 13985 | - | ND | ND | ND | ND | ND |
| *P. fluorescens* SBW25 | ND | - | ND | ND | ND | ND |
| *P. aeruginosa* PA14 | ND | - | ND | ND | ND | ND |
| *P. aeruginosa* PAO1 | ND | - | ND | ND | ND | ND |
| *P. fluorescens* ATCC 13525 | ND | - | ND | ND | ND | ND |
| *P. chlororaphis* ATCC 9446 | ND | - | ND | ND | ND | ND |
| *P. fluorescens* ATCC 17518 | ND | - | ND | ND | ND | ND |
| *P. putida* Bn7 | + | + | - | + | + | + |
| *P. putida* ATCC 17470 | + | + | + | - | + | + |
| *P. putida* CS111 syn SB8.3 | + | + | + | - | + | + |
| *P. reactans* NCPPB387 | ND | ND | ND | ND | - | ND |
| *P. fluorescens* B10 | ND | ND | ND | ND | ND | - |
| *P. putida* W4P63 | ND | ND | ND | ND | ND | - |
| *P. aeruginosa* LESB58 | + | + | + | + | + | + |

[a]– indicates lack of growth promotion by the *Pseudomonas* strain, + indicated growth promotion and ND indicates that this combination was not tested.

FpvW is 75.3% identical to PbuA from *Pseudomonas* sp. strain M114, which recognizes the pyoverdine produced by *P. fluorescens* B10 (48). FpvW was required for crossfeeding by *P. fluorescens* B10, *P. lini* DLE411J, *P. putida* W4P63, and *P. fluorescens* ATCC17513. Of these strains, only B10 and DLE411J have characterized pyoverdines, which share the peptide chain of εLys-OHAsp-Ala-aThr-Ala-cOHOrn (Table 3.3).

Three strains of *Pseudomonas putida* crossfed Pf-5 via FpvX or FpvV. FpvX was required by Pf-5 for crossfeeding by *P. putida* ATCC 17470 and *P. putida* CS111, and FpvV was required for crossfeeding by *P. putida* Bn7 (Table 3.3). FpvV is 79% identical to PupB from *P. putida* WCS358, which also recognizes the pyoverdine from *P. putida* Bn7 (32).

In summary, each of the six Fpv outer-membrane proteins was required by Pf-5 for crossfeeding by one to seven strains of *Pseudomonas* spp. Crossfeeding by a total of 25 strains was eliminated by knocking out a single *fpv* gene. Of the 25 strains, 14 produce pyoverdines having peptide chains with known or bioinformatically-predicted amino acid composition (Table 3.2). Samples of representative pyoverdines, which were partially purified from culture supernatants of *Pseudomonas* sp., were tested to verify the crossfeeding assays. Recognition of specific purified pyoverdines by the six Fpv outer-membrane proteins was confirmed (Table 3.3). Therefore, specific pyoverdines were associated with each of the Fpv outer-membrane proteins of Pf-5, and purified pyoverdines of known structure could be matched to five of the Fpv outer-membrane proteins.

**Table 3.5. Purified pyoverdines stimulated iron-limited growth of derivatives of Pf-5 with deletions in specific *fpv* genes in a Δ*pvdI-pchA* mutant background**

| Strain isolated from | Peptide chain structure[a] | *fpv* mutant[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *pvdI-pchA* | *fpvZ* | *fpvU* | *fpvX* | *fpvW* | *fpvY* | *fpvV* |
| *P. fluorescens* Pf-5 | Asp-FOHOrn-Lys-Thr-Ala-Ala-FOHOrn-Lys | + | - | + | + | + | + | + |
| *P. aeruginosa* PAO1 | Ser-Arg-Ser-FOHOrn-(Lys-FOHOrn-Thr-Thr) (Type I pyoverdine) | + | + | - | + | + | + | + |
| *P. putida* SB8.3 | Ala-Lys-Thr-Ser-AOHOrn-cOHOrn | + | + | + | - | + | + | + |
| *P. putida* CS111 | *Ala-Lys-Thr-Ser-OHOrn-OHOrn* | + | + | + | - | + | + | + |
| *P. fluorescens* B10 | εLys-OHAsp-Ala-aThr-Ala-cOHOrn | + | + | + | + | - | + | + |
| *P. rhodeisiae* CFML92-104 | Ser–Lys–FOHOrn–Ser–Ser–Gly–(Lys–FOHOrn–Ser–Ser) | + | + | + | + | + | - | + |
| *P. putida* Bn7 | Unknown | + | + | + | + | + | + | - |
| *P. aeruginosa* ATCC 27853 | Ser-FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) | + | + | + | + | + | + | + |
| *P. aeruginosa* 7NSK2 | Ser-FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) | + | + | + | + | + | + | + |
| *P. aeruginosa* Pa6 | Ser-cDab-FOHOrn-Gln-Gln-FOHOrn-Gly (Type III pyoverdine) | + | + | + | + | + | + | + |

[a]Underline denotes D-amino acids. Parentheses define cyclic residues. cOHOrn is cyclo-hydroxy-ornithine. FOHOrn is δN-formyl- δN-hydroxy-ornithine. εLys is Lys linked by its ε-NH2. OHAsp is threo-β-hydroxy-aspartic acid. Dab is diamino-butanoic acid. OHH is threo- β-hydroxy-histidine. Italicized structures were predicted by siderotyping.

[b]+ indicates growth of the mutant in the presence of the purified pyoverdine. – indicates no growth of the mutant in the presence of the purified pyoverdine.

**Phylogenetic analysis of Fpv outer-membrane proteins**

The six Fpv outer-membrane proteins of Pf-5, their closest orthologs, and characterized Fpv outer-membrane proteins with known substrates from other *Pseudomonas* spp. were aligned and subjected to phylogenetic analysis. Of the six Fpv outer-membrane proteins in Pf-5, three are closely related to characterized Fpv outer-membrane proteins. FpvV is in the same clade as PupB from *P. putida* WCS358 (32) and, both recognize the pyoverdine produced by *P. putida* Bn7 (Fig. 3.3). FpvY and FpvU are related to FpvAI from *P. aeruginosa* PA01 as well as Fpv outer-membrane proteins from *P. fluorescens* SBW25 and *P. putida* GB1. FpvY and FpvU of Pf-5 exhibited similarities in substrate recognition, taking up ferric-complexes of pyoverdines having similar, but not identical, peptide chains. For the pyoverdines taken up by FpvY and FpvU, the N-terminal amino acids of the peptide chain have high levels of conservation. The first four amino acids of these peptide chains are identical to those found in pyoverdines that bind with high affinity to FpvAI (23), which is in the same clade as FpvY and FpvU in our phylogenetic analysis (Fig. 3.3). FpvW from Pf-5 is in a sub-clade with PbuA, the Fpv from *Pseudomonas* sp. M114, and Fpv outer-membrane proteins from *P. brassicacearum* and *P. putida* W619. PbuA is reported to recognize the pyoverdine produced by *P. fluorescens* B10 (48), which agree with the role of FpvW in the uptake of the B10 pyoverdine by Pf-5 (Fig. 3.3). Pf-5 does not have an ortholog to PupA, which recognizes the pyoverdine from *P. putida* WCS358 (8). These data are consistent with our earlier observation that the WCS358 pyoverdine is not recognized by Pf-5 (25). FpvZ, the TBDP for the uptake of Pf-5s own pyoverdine, appears to be distantly related to FpvB, the secondary Fpv of *P. aeruginosa* spp., as they form a clade with their related orthologs, despite sharing only 47% amino acid identity. The clade with FpvZ is underrepresented as there is a lack of similar Fpv outer-membrane proteins currently sequenced, contributing to low resolution of the phylogenetic relationships of FpvZ to other Fpv outer-membrane proteins (Fig. 3.3). FpvX is unique in that it is the only characterized TBDP in the clade it forms with Fpv outer-membrane proteins from *P.*

*fluorescens* SBW25 and *P. putida* GB-1. FpvX recognizes the pyoverdines produced by *P. putida* strains CS111, SB8.3, and ATCC 17470, but few orthologous sequences are available. Further sequencing of Fpv outer-membrane proteins will aid in resolving the distribution and evolution of this Fpv in *Pseudomonas* spp..

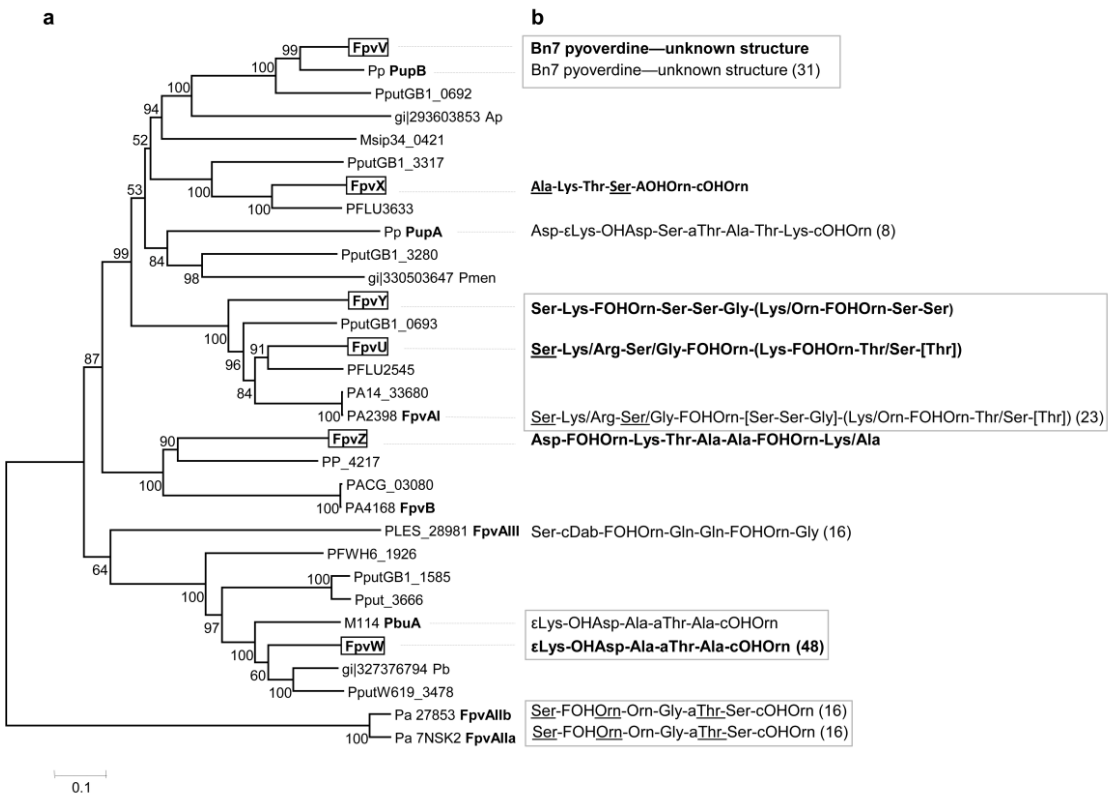


**Fig. 3.3. Phylogenetic analysis of Fpv outer-membrane proteins and association with pyoverdine structures. a**. Neighbor Joining analysis of Fpv outer-membrane proteins. Pf-5 Fpv outer-membrane proteins are shown in bold boxed font and Fpv outer-membrane proteins having known substrates are shown in bold font. Abbreviations for species represented in the tree are as follows: *P. fluorescens* Pf-5 (Fpv), *Achromobacter piechaudii* ATCC 43553 (AP), *P. putida* GB-1 (PputGB1), *P. putida* F1 (Pput), *P. putida* W619 (W619), *Methylovorus* sp. SIP3-4 (Msip34), *P. fluorescens* SBW25 (PFLU), *P. fluorescens* WH6 (PFWH6), *P. aeruginosa* LESB58 (PLES), *P. aeruginosa* PA14 (PA14), *P.* sp. M114 (M114), *P. putida* (Pp), *P. brassicacearum* subsp. brassicacearum NFM421 (Pb), *P. aeruginosa* C3717 (PACG), *P. aeruginosa* (Pa), *P. mendocina* NK-01 (Pmen), *P. aeruginosa* 7NSK2 (Pa 7NSK2) and *P. aeruginosa* 27853 (Pa 27853). **b**. Pyoverdine peptide chain sequences recognized by the adjacent Fpv receptors. Pyoverdines associated with Fpv outer-membrane proteins of Pf-5 in this study are in bold font. Peptide chains that are a consensus of multiple peptide chains contain amino acids in brackets indicating an addition, or a / indicating either of two residues, being present at that position. References are in parentheses after peptide chains.

**Functional redundancy of Fpv outer-membrane proteins in *P. fluorescens* Pf-5**

Twelve of the 37 pyoverdine-producing strains of *Pseudomonas* spp. crossfed all of the single *fpv* mutants, indicating that crossfeeding by these strains was not mediated by a single Fpv outer-membrane protein in Pf-5. *P. fluorescens* WCS374 was among the twelve strains that crossfed all of the single *fpv* mutants of Pf-5. This result was unexpected because the pyoverdine produced by WCS374 (17) is reported to be identical to the pyoverdines produced by other strains (*P. fluorescens* SBW25, *P. fluorescens* ATCC 13525, and *P. chlororaphis* ATCC 9446), which crossfeed Pf-5 via FpvU. WCS374 produces the secondary siderophore pseudomomine (17), which could mask the role of FpvU in crossfeeding if the ferric-complex of pseudomomine also is utilized as an iron source by Pf-5. A pyoverdine-deficient mutant of WCS374 did not crossfeed the Pf-5 Δ*pvdI-pchC* mutant (25), indicating that the pyoverdine, rather than pseudomomine or any other secondary siderophore that may be produced by this strain, was responsible for the crossfeeding. Because the first four amino acids (Ser-Lys-Gly-FOHOrn) of the peptide chain of the WCS374 pyoverdine conform to those produced by strains that crossfed Pf-5 via FpvU and FpvY (Table 3.3), we reasoned that there may be overlap in pyoverdine uptake by these two closely-related Pf-5 proteins. To explore this possibility, we generated and tested a double *fpvU-fpvY* mutant in the Δ*pvdI-pchC* background and found that it was not crossfed by strain WCS374. Therefore, strain WCS374 crossfed Pf-5 via both FpvU and FpvY whereas three other strains that produce pyoverdines having peptide sequences with identical amino acid composition crossfed Pf-5 via FpvU alone. This apparent discrepancy could be due to unknown differences between the chemical structures of the pyoverdines, such as amino acid modification or isomerization. For example, FpvAI recognizes with high affinity only one of the possible stereoisomers of ferric-pyoverdine complexes, and has low affinity for pyoverdines that exist primarily in the incorrect conformation (23). It also is possible that FpvU and FpvY differ from one another with respect to the efficiency of their uptake of the various derivatives of a given pyoverdine, which could explain their differential roles in the crossfeeding tests of this study. A given strain of *Pseudomonas* spp. commonly produces derivatives (or analogs)

of a pyoverdine due to somewhat relaxed specificity of the adenylation domains of an NRPS or incomplete amino acid modifications (10).

Due to the redundancy of FpvU and FpvY in uptake of the WCS374 pyoverdine, we tested strain DSM50106, which produces a similar pyoverdine, for crossfeeding of the *fpvU-fpvY* mutant in a Δ*pvdI-pchC* background. DSM50106 failed to promote growth of this mutant, indicating that, like WCS374, DSM50106 crossfed Pf-5 via FpvU and the closely-related FpvY (Table 3.6). Pyoverdines produced by strains WCS374 and DSM50106 have the same amino acids in the first four and the last two positions of their peptide chains (Table 3.3). We submit that the conserved amino acid motifs common to these pyoverdines are involved in their interactions with both FpvU and FpvY, allowing uptake of the ferric-pyoverdine complexes by either receptor. Overlapping functions of TBDPs are not uncommon. For example, *P. aeruginosa* has two TonB-dependent receptors for enterobactin (15, 20) and two for ferrichrome (24). In Pf-5, the closely-related FpvU and FpvY provide similar redundancy for the uptake of certain pyoverdines. The capacity to utilize these pyoverdines as iron sources may contribute to the fitness of the bacterium in some habitats that functional redundancy developed in Pf-5.

**Table 3.6. Crossfeeding of double *fpv* mutants of Pf-5 by *P. fluorescens* WCS374 (shaded squares) or DSM50106 (open squares)**.

| | *fpvZ* | *fpvU* | *fpvX* | *fpvW* | *fpvY* | *fpvV* |
|---|---|---|---|---|---|---|
| *fpvZ* | | + | + | + | + | + |
| *fpvU* | + | | + | + | - | + |
| *fpvX* | + | + | | + | + | + |
| *fpvW* | + | + | + | | + | + |
| *fpvY* | + | - | + | + | | + |
| *fpvV* | + | + | + | + | + | |

+ indicates growth of derivatives of Pf-5 with deletions in two *fpv* genes in a Δ*pvdI-pchC* background on an iron-limited medium in the presence of the strains. – indicates no growth on the iron-limited medium in the presence of the strains.

Due to the overlapping roles of FpvU and FpvY in pyoverdine uptake, we tested the possibility that other Pf-5 Fpv outer-membrane proteins also have overlapping functionalities. The remaining ten strains of *Pseudomonas* spp. that crossfed all single *fpv* mutants were tested for crossfeeding of mutants lacking two of the six Fpv outer-membrane proteins in all combinations (data not shown). One strain, *P. aeruginosa* LESB58, crossfed Pf-5 via FpvZ and FpvY (Table 3.7), which was unexpected given the structural difference between its pyoverdine (type III) and those that crossfed Pf-5 via FpvZ or FpvY exclusively (Table 3.3). The type III pyoverdine purified from *P. aeruginosa* strain Pa6 also crossfed Pf-5 via both FpvZ and FpvY, confirming the role of FpvZ and Y in uptake of the type III pyoverdine (Table 3.7). Although beyond the scope of the present study, we speculate that the signaling and regulatory roles of FpvZ could provide an explanation for this result. The *fpvZ* mutant of Pf-5 was deficient in pyoverdine production and may lack other aspects of iron homeostasis, including altered expression of other Fpv outer-membrane proteins. When the primary Fpv (FpvZ) is non-functional, other Fpv outer-membrane proteins, such as FpvY, may be over-expressed, thereby facilitating iron acquisition through low affinity binding of a heterologous ferric-pyoverdine. A similar pattern was observed by Mirleau et al. (46), who evaluated heterologous ferric-pyoverdine uptake by *P. fluorescens* C7R12. In that study, quantitative differences in the levels of iron incorporated from ferric-pyoverdines by C7R12 and a pyoverdine-biosynthesis mutant were attributed to alterations in the expression of the iron-regulated genes caused by the mutation.

**Table 3.7. Crossfeeding of double *fpv* mutants of Pf-5 by *P. aeruginosa* LESB58 (type III pyoverdine-producing strain) (open squares) and utilization of ferric-complexes of pyoverdines purified from *P. aeruginosa* Pa6 (type III) (shaded squares)**.

| | *fpvZ* | *fpvU* | *fpvX* | *fpvW* | *fpvY* | *fpvV* |
|---|---|---|---|---|---|---|
| *fpvZ* | | + | + | + | - | + |
| *fpvU* | + | | + | + | + | + |
| *fpvX* | + | + | | + | + | + |
| *fpvW* | + | + | + | | + | + |
| *fpvY* | - | + | + | + | | + |
| *fpvV* | + | + | + | + | + | |

+ indicates growth of derivatives of Pf-5 with deletions in two *fpv* genes in a Δ*pvdI-pchC* background on an iron-limited medium in the presence of the purified pyoverdine from *P. aeruginosa* Pa6 or a colony of *P. aeruginosa* LESB58. – indicates no growth on the iron-limited medium.

Eight of the remaining nine strains crossfed all of the double *fpv* mutants. To verify the results for two strains, we isolated the type II pyoverdines from culture supernatants of *P. aeruginosa* strains 7NSK2 and ATCC 27853 and tested them in crossfeeding experiments. The pyoverdines, like the producing strains, crossfed all of the double *fpv* mutants of Pf-5 (Appendix 8). As described above, secondary siderophores produced by the strains could be responsible for crossfeeding Pf-5, but this possibility could be excluded for two strains (Pf0-1 and 7NSK2) for which a mutant deficient in pyoverdine production was available. The pyoverdine-deficient mutants did not crossfeed the Δ*pvdI-pchC* mutant of Pf-5, indicating that the pyoverdines produced by Pf0-1 and 7NSK2 were responsible for crossfeeding. We also considered the possibility that TBDPs other than the six identified Fpv outer-membrane proteins could function in crossfeeding of Pf-5 by the eight strains. To test that possibility, we identified PFL_2772 as the protein most closely related to the Fpv outer-membrane proteins from a phylogenetic tree constructed from the plug domains of all TBDPs in the Pf-5 proteome (Fig. 3.4). We derived a mutant in PFL_2772 in the Δ*pvdI-pchC* background and tested it for crossfeeding. All nine strains crossfed the PFL_2772-*pvdI-pchC* mutant (data not shown), indicating that this TBDP plays no role in uptake of pyoverdines produced by the nine strains. Based on these results, we conclude that functional redundancy of the Pf-5 Fpv outer-membrane proteins is the most likely explanation for our observations that eight strains crossfed all of the double *fpv* mutants. Pyoverdines produced by the strains differ structurally from those associated with the Fpv outer-membrane proteins, as determined through the single *fpv* mutant analysis (Table 3.3) nevertheless; they may be recognized by these Fpv outer-membrane proteins. Although Fpv outer-membrane proteins exhibit strict specificity in high affinity pyoverdine uptake, previous studies have shown that pyoverdines can be transported into the cell with lower affinity by Fpv outer-membrane proteins lacking strict specificity (23). Differences between high and low-affinity uptake cannot be distinguished in cross-feeding experiments such as those done in this study, but low-affinity uptake of pyoverdines by multiple Fpv outer-membrane

proteins is a likely explanation for our observation that one third of the strains of *Pseudomonas* spp. tested crossfed Pf-5 via more than one Fpv outer-membrane protein.

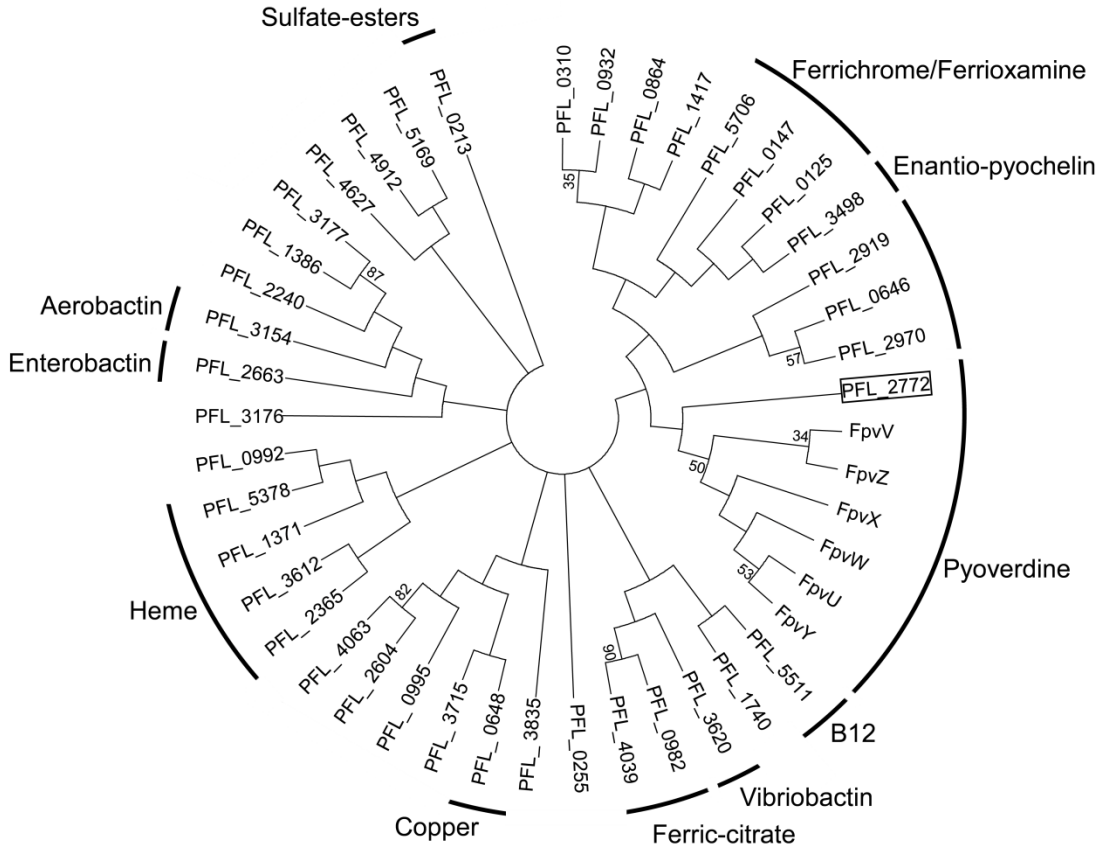


**Fig. 3.4. Maximum Parsimony analysis of the plug domains from the 45 Pf-5 TonB-dependent outer-membrane proteins.** Bootstrap values greater than 30 are shown. PFL_2772 is boxed to indicate that is receptor was deleted and tested in crossfeeding assays. TBDPs with putative functions are labeled with the substrate as characterized in Hartney et al. (25).

## Conclusion

For a soil bacterium like *P. fluorescens* Pf-5, which can establish in the rhizosphere where biologically-available iron is limited, the ability to use heterologous pyoverdines can provide a competitive advantage (4, 34). Utilizing heterologous pyoverdines transfers the cost of pyoverdine production to neighboring bacterial cells while sequestering iron away from competitors (27). Piracy is a good way to describe the behavior of *P. fluorescens* Pf-5, which is able to produce and utilize the high affinity siderophore pyoverdine and the lower affinity siderophore enantio-pyochelin to provide itself with iron, yet maintains an arsenal of TonB-dependent outer-membrane proteins for the uptake of heterologous siderophores. In this study, we demonstrated that Pf-5 uses its six Fpv outer-membrane proteins to utilize a variety of pyoverdines produced by other pseudomonads as iron sources. We employed a combination of phylogenetics, bioinformatics, mutagenesis, and crossfeeding bioassays to assign functionalities to each of the six Fpv outer-membrane proteins in the Pf-5 proteome. We demonstrated that phylogenetically-related Fpv outer-membrane proteins take up ferric complexes of structurally-related pyoverdines, thereby establishing structure-function relationships that can be employed in the future to predict the pyoverdine substrates of Fpv outer-membrane proteins in other *Pseudomonas* spp.

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## Chapter 4: TonB-dependent Outer-membrane Proteins of the *Pseudomonas fluorescens* Group

### Abstract

The *Pseudomonas fluorescens* group is made up of environmental bacteria including strains associated with soil or plant surfaces that have biological control capabilities. This study identifies the TonB-dependent outer-membrane proteins (TBDP) in the predicted proteomes of ten strains of the *P. fluorescens* group. Among the ten strains, the number of TBDPs ranges from 14 to 45. Collectively, the ten strains have 317 putative TBDPs, which phylogenetic analysis places into 84 groups. Of the 84 TBDP types, 28 have putative roles in the uptake of vitamin B12, sulfur-esters, or iron, including 11 groups for the uptake of pyoverdine siderophores. In 54 TBDP types, no putative functions could be assigned. The five TBDP types conserved in all ten strains have putative functions in uptake of vitamin B12, heme, and the siderophore ferrichrome, with the remaining two of unknown function. Each strain has three to six ferric-pyoverdine outer-membrane proteins (Fpvs). Using a strategy developed for *P. fluorescens* Pf-5 (Chapter 3), I assigned putative pyoverdine substrates to many Fpv outer-membrane proteins in strains of the *P. fluorescens* group.

## Introduction

The bacterial genus *Pseudomonas* is made up of diverse species (35), including *P. fluorescens, P. chlororaphis* and 50 other related species that fall within the *P. fluorescens* group. Among the heterogeneous bacteria in the *P. fluorescens* group are plant-associated strains that suppress plant disease. Multi-locus sequence analysis of ten conserved genes in the *Pseudomonas* group by Bayesian analysis placed ten non-pathogenic plant- or soil-associated strains into a single clade (Loper et al. unpublished) (Fig. 4.1), which corresponds to the *P. fluorescens* group identified by Mulet et al. (35). This clade has three sub-clades. Two strains of *P. chlororaphis* (30-84 and O-6) and *P. fluorescens* Pf-5 make up sub-clade 1. Sub-clade 2 is composed of *P. fluorescens* Q2-87, *P. fluorescens* Q8r1-96, and the distantly-related *P. fluorescens* Pf0-1. Four strains make up sub-clade 3: *P. fluorescens* strains A506, SS101, SBW25, and *Pseudomonas* sp. BG33R (previously called *P. synxantha* BG33R). These ten strains are environmental bacteria isolated from soil, the rhizosphere, or the phyllosphere. Each of the strains can function in biological control or suppress certain plant diseases (Table 4.1). Seven of the strains were recently sequenced, which, along with three previously-sequenced strains (37, 44, 45) facilitated investigation into genomic similarities. The ten strains share a core of 2831 predicted proteins representing 46-53% of each strains predicted proteome (Loper et al., unpublished). With only half of the proteins shared among the ten strains a high level of genomic diversity is indicated.

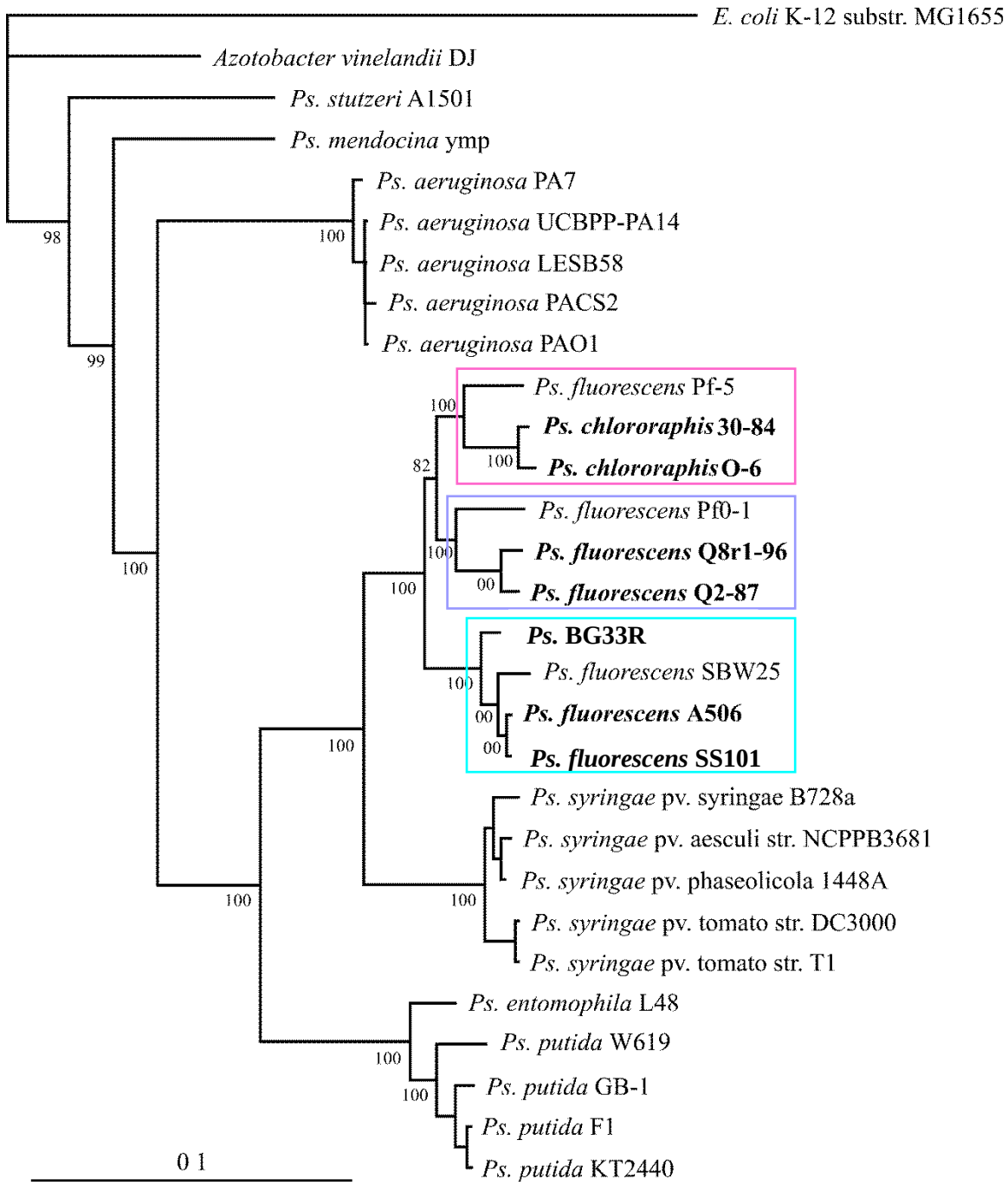


**Fig. 4.1. Bayesian analysis of select strains of *Pseudomonas* spp. having fully sequenced genomes based on multilocus sequence analysis (MLSA).** The three sub-clades of the species group are highlighted, sub-clade 1 in pink, sub-clade 2 in purple, and sub-clade 3 in blue. (Bayesian tree provided by Neal Wilson)

A goal of this study was to identify the TBDPs in the sequences of ten strains of the *P. fluorescens* group, and to identify the core TBDPs conserved in all ten genomes. Other members of the genus *Pseudomonas* have TBDPs numbering in the 20s and 30s. For example, *P. aeruginosa* PAO1 has 35, *P. putida* KT2440 has 30, *P. syringae* DC3000 has 25, and *P. entomophila* L48 has 31 (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). A recent survey of TonB-dependent outer-membrane proteins in the fluorescent pseudomonads focused on sequenced strains of *P. aeruginosa*, *P. syringae*, and *P. putida/P. entomophila* (7). The authors found high levels of diversity and identified a core set of TBDPs within the species of *Pseudomonas*. PhuR, which is involved in heme uptake, is the only TBDP present in all the *Pseudomonas* strains surveyed (7, 36). Within seven *P. aeruginosa* strains, a core of 26 TBDPs was identified. Three strains of *P. putida* have a core of 14; the inclusion of *P. entomophila* reduces the core to 11 TBDPs (7). The strains of *P. syringae* surveyed have a core of 13 TBDPs.

A second goal of this study was to assign putative functions to the TBDPs in the *P. fluorescens* group. Many of the TBDPs found in *P. aeruginosa* and other Gram-negative bacteria have been assigned functions, but the functions of many TBDPs remain unknown. Some of the substrates recognized by TBDPs are vitamin B12 (cobalamin) (43), copper (53), nickel (42), maltodextrins (29), sucrose (3) and ferric complexes of siderophores including pyochelin, enterobactin, aerobactin, ferrioxamine, ferrichrome, ferric-citrate, vibriobactin, and pyoverdines (6, 8, 15). The fluorescent pseudomonads produce pyoverdines that vary in the structure of the peptide chain and Fpv outer-membrane proteins, which are the TBDPs for ferric-pyoverdine uptake (4, 46). One Fpv is used for the uptake of the cognate pyoverdine, but many fluorescent pseudomonads have additional Fpv outer-membrane proteins for heterologous pyoverdine uptake (16, 26, 37). In Chapter 3, I proposed a strategy for assigning pyoverdine substrates to Fpv outer-membrane proteins based on the relationships observed between the Fpv sequences and structures of pyoverdine substrates in Pf-5. Continuing this strategy here, I assigned putative pyoverdine substrates to many Fpv outer-membrane proteins in ten strains of the *P. fluorescens* group.

**Table 4.1. Ten strains with biological control ability in the *P. fluorescens* group**

| Strain | Source | Target Disease | References |
|---|---|---|---|
| *P. chlororaphis* | | | |
| 30-84 | Wheat rhizosphere, Kansas, USA | Take-all | (38) |
| O-6 | Wheat rhizosphere, Utah, USA | Soft-rot, cucumber mosaic virus | (50) |
| *P. fluorescens* | | | |
| Pf-5 | Soil, Texas, USA | Pythium damping off | (22, 37) |
| Pf0-1 | Soil, Massachusetts, USA | Soil borne diseases | (44) |
| SBW25 | Sugar beet phyllosphere, Oxfordshire, UK | Soil borne diseases | (44) |
| Q8r1-96 | Wheat rhizosphere, Washington, USA | Take-all | (39) |
| Q2-87 | Wheat rhizosphere, Washington, USA | Take-all | (51) |
| A506 | Pear phyllosphere, California, USA | Fire blight | (28) |
| SS101 | Wheat rhizosphere, The Netherlands | Oomycete plant pathogens | (10) |
| *Pseudomonas* sp. | | | |
| BG33R | Peach rhizosphere, South Carolina, USA | Root knot nematode | (25) |

## Methods and materials

### Sequence compilation

The predicted proteomes of the ten strains in the *P. fluorescens* group were surveyed for TonB-dependent outer-membrane proteins based on the presence of conserved domains. Candidate TBDPs were compiled from each strain and submitted to Pfam to check for the presence of conserved receptor (Pfam: PF00593), plug (Pfam: PF07715) and N-terminal signaling domains (Pfam: PF07660) characteristic of TBDPs and the location of the domains within each protein. The TBDPs within each strain were categorized as receptors or transducers as described by Hartney et al. (20).

### Reciprocal BLASTP analysis

The TBDPs from each strain were compared to each of the other strains by reciprocal BLASTP analysis. TBDPs were considered orthologs if they shared greater than 60% amino acid identity over the entire protein.

### Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 4.0.2 (48). The clustalW based alignment option ws used to align the amino acid sequences with a gap open penalty of 3 and a gap extension penalty of 1.8. The aligned sequences were masked to remove gaps and subjected to Neighbor Joining and Maximum Parsimony analysis, the resulting consensus tree was used. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The Maximum Parsimony tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10

replicates). All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion Option).

**Bacterial strains and growth conditions**

*Pseudomonas* strains (Table 4.1) were grown on King's medium B (KMB) at 27°C for 24 h (24). Mutants used in crossfeeding and heterologous pyoverdine utilizations assays are *P. fluorescens* Pf-5 Δ*pvdI-pchA* (20), *P. fluorescens* Pf0-1 Δ*pvdI* (Mark Silby), *P. chlororaphis* 30-84 Δ*pvdL* (Leland Pierson III), *P. fluorescens* A506 $Pvd^-$ (Steve Lindow), and *P. fluorescens* SS101 Δ*pvdI* (Jos Raaijmakers).

**Bioinformatic prediction of the amino acid composition of the pyoverdine peptide chain**

The predicted amino acid sequences of the genes encoding the non-ribosomal peptide synthetases were submitted to the NRPS/PKS predictor (1) and the NRPS predictor (http://www-ab.informatik.uni-tuebingen.de/software/NRPSpredictor) (40, 47).

**Crossfeeding assays**

Crossfeeding of strains Pf-5 Δ*pvdI-pchA*, 30-84 Δ*pvdL*, and Pf0-1 *pvdI* was tested on KMB amended with 400 µM 2,2 dipyridyl, as described in Hartney et al. (20). Crossfeeding assays with the pyoverdine-deficient mutants of A506 and SS101 were performed on KMB amended with 800µM 2,2 dipyridyl as this was the concentration at which these two mutants were limited in growth. Crossfeeding assays with strains A506 $Pvd^-$ and SS101 Δ*pvdI* were done by placing agar plugs of donor strains on KMB amended with 800µM 2,2 dipyridyl. 5 µl of a 100-fold dilution of a 0.1 $OD_{600}$ suspension of the feeding strain were placed 10mm from the agar plug. Plates were incubated at 27°C. Readings were taken at 24 and 48 h. Assays were done on duplicate plates in multiple experiments.

**Purified pyoverdine recognition assays**

Pyoverdine mutants of 30-84, Pf0-1, A506, and SS101 were tested for their ability to recognize purified pyoverdines. Cells from the pyoverdine mutants were collected from KMB agar plates, suspended in sterile water to 0.1 $OD_{600}$, and diluted 100 fold in sterile water before spreading on KMB amended with 400 μM 2,2'-dipyridyl for 30-84 and Pf0-1 or 800 μM 2,2'-dipyridyl for A506 and SS101. 100 μL of the bacterial suspensions were spread on the plates to create a lawn. 5 μL of 8 mM pyoverdine solution was then placed on 5 mm diameter filter paper disks on the agar surface of these plates. Assays were done on duplicate plates in multiple experiments.

## Results and discussion

### Numbers of TonB-dependent outer-membrane proteins

The total number of TBDP genes within the strains of the *P. fluorescens* group varies between 45 (Pf-5) and 14 (Q2-87). The number of the two types of TBDPs, receptors and transducers, also varies among strains (Fig. 4.2). The range of receptors is from 27 in Pf-5 to ten in Q8r1-96 and Q2-87; the range of transducers is from 18 in Pf-5 to four in Q2-87. All strains have approximately twice as many receptors as transducers. Similarities in the number of TBDPs are found between some of the more related strains. For example, BG33R, SBW25, and SS101 all have 39 TBDPs (Fig. 4.2).

Orthologous TBDPs were defined as having >60% amino acid identity over the entire protein, being the reciprocal best hit, and clustering in a common clade in phylogenetic analysis. The clade with BG33R, SBW25, A506, and SS101 share the most TBDPs at 23 (Fig. 4.3a). The clade with Pf0-1, Q2-87 and Q8r1-96 share the fewest TBDPs at seven. This is due to the large difference in the total number of TBDPs between Pf0-1 (27) and Q2-87 (14) and Q8r1-96 (16). Higher levels of similarity, > 80% amino acid identity, are found between 30-84 and O-6, which share 25 TBDPs, A506 and SS101 sharing 33 TBDPs, and Q2-87 and Q8r1-96 sharing ten TBDPs. Five TBDPs are conserved in all ten strains (Fig. 4.3a).

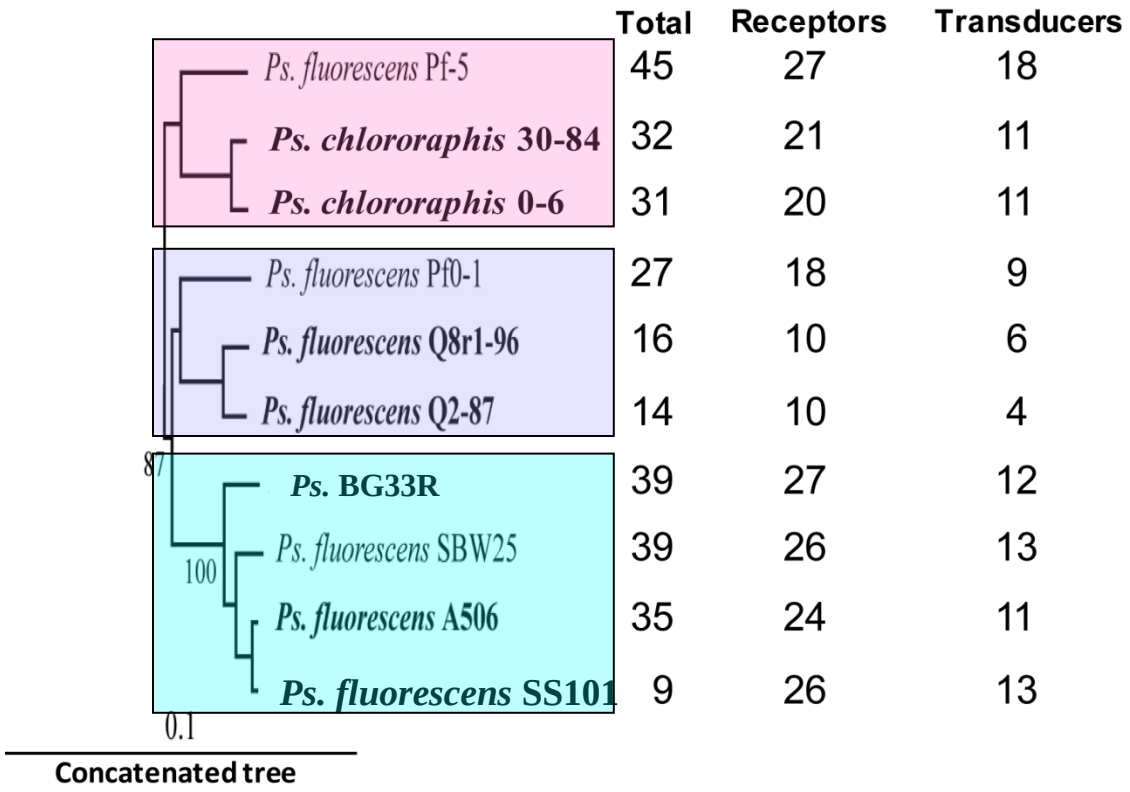


**Fig. 4.2. Number of TonB-dependent outer-membrane proteins in the strains of the *P. fluorescens* group.** Clade representing the *P. fluorescens* group (from Fig. 4.1), illustrating the phylogenetic relationships between the ten strains, with sub-clades highlighted corresponding to assignment in Figure 4.1. The total number of TBDPs found in each strain is listed along with the two types of TBDPs, receptors and transducers. Transducers have the addition of an N-terminal signaling domain that is not present in the receptors.

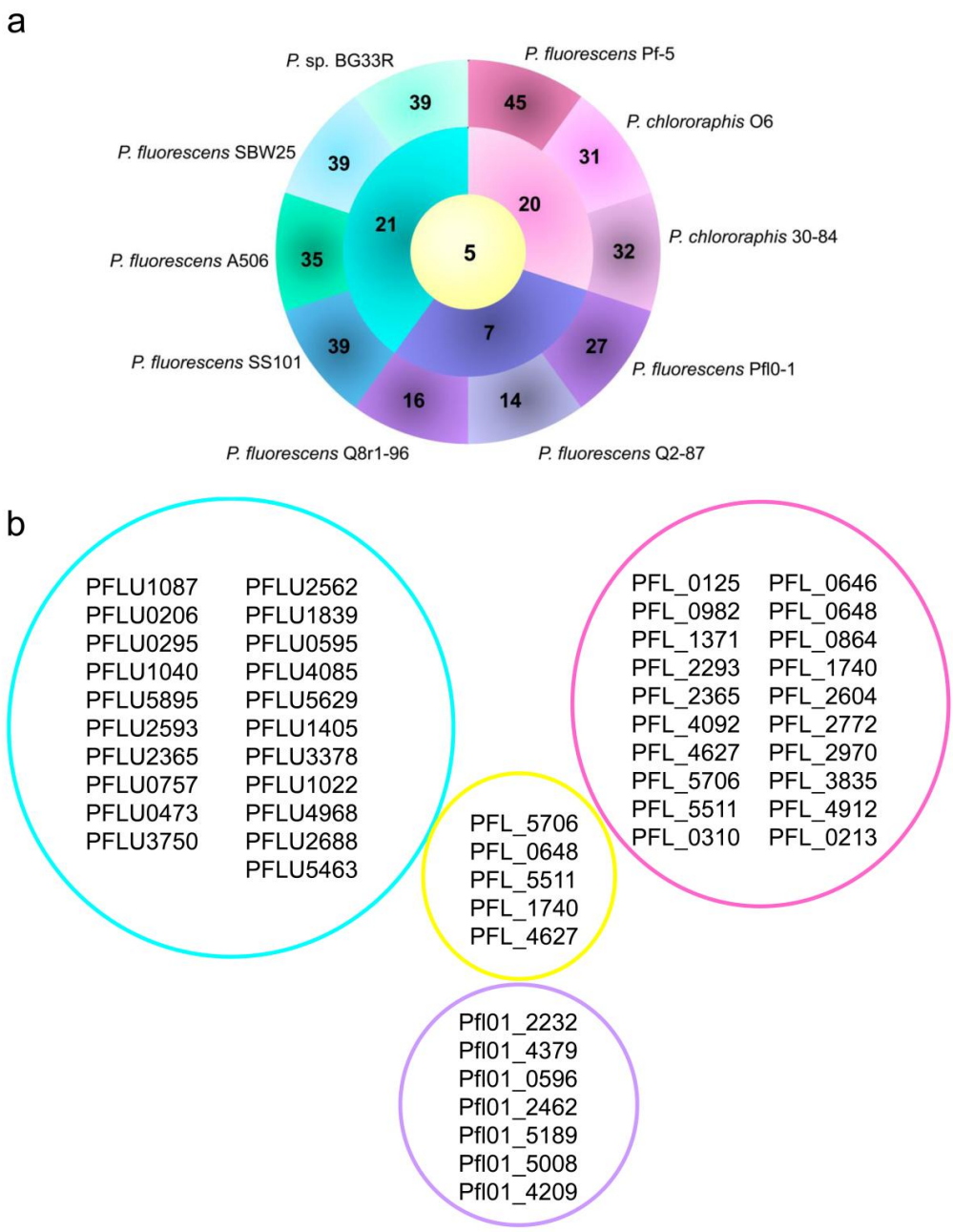


**Fig. 4.3. Diagram showing the number of conserved TBDPs in ten strains within the *P. fluorescens* group. a.** The number of TBDPs in each proteome (outside circle), the number of orthologous TBDPs shared by all strains in a given sub-clade (second circle from perimeter), and the number of orthologous TBDPs shared by all 10 strains (center). **b.** Lists of the orthologous TBDPs for SBW25 (blue circle, sub-clade 3), Pf-5 (pink circle, sub-clade 1), Pf0-1 (purple circle, sub-clade 2) corresponding to the three sub-clades and orthologs in Pf-5 to the five core TBDPs (yellow circle).

The distribution of the TBDPs within the three clades and within the *P. fluorescens* group is further illustrated in Figure 4.4a, a Venn diagram showing the TBDPs unique to an individual strain and specific combinations of strains. Pf-5 and SBW25 have the most TBDPs, 13 and seven, respectively, not found in other strains of the *P. fluorescens* group. Orthologs of these TBDPs are present in the proteomes of bacteria other than *P. fluorescens* group strains (Fig. 4.4a), suggesting horizontal gene transfer as discussed in Chapter 2. The most similarity is between A506, BG33R, and SS101, which have three TBDPs not found in the other strains of the species group. At the level of >60% amino acid identity, 73 TBDP types were identified. Seventeen of the 73 types were assigned putative functions (Fig. 4.4b). This number does not include the TBDPs with putative roles in pyoverdine uptake as they are too divergent from each other to conform to the >60% amino acid cutoff.

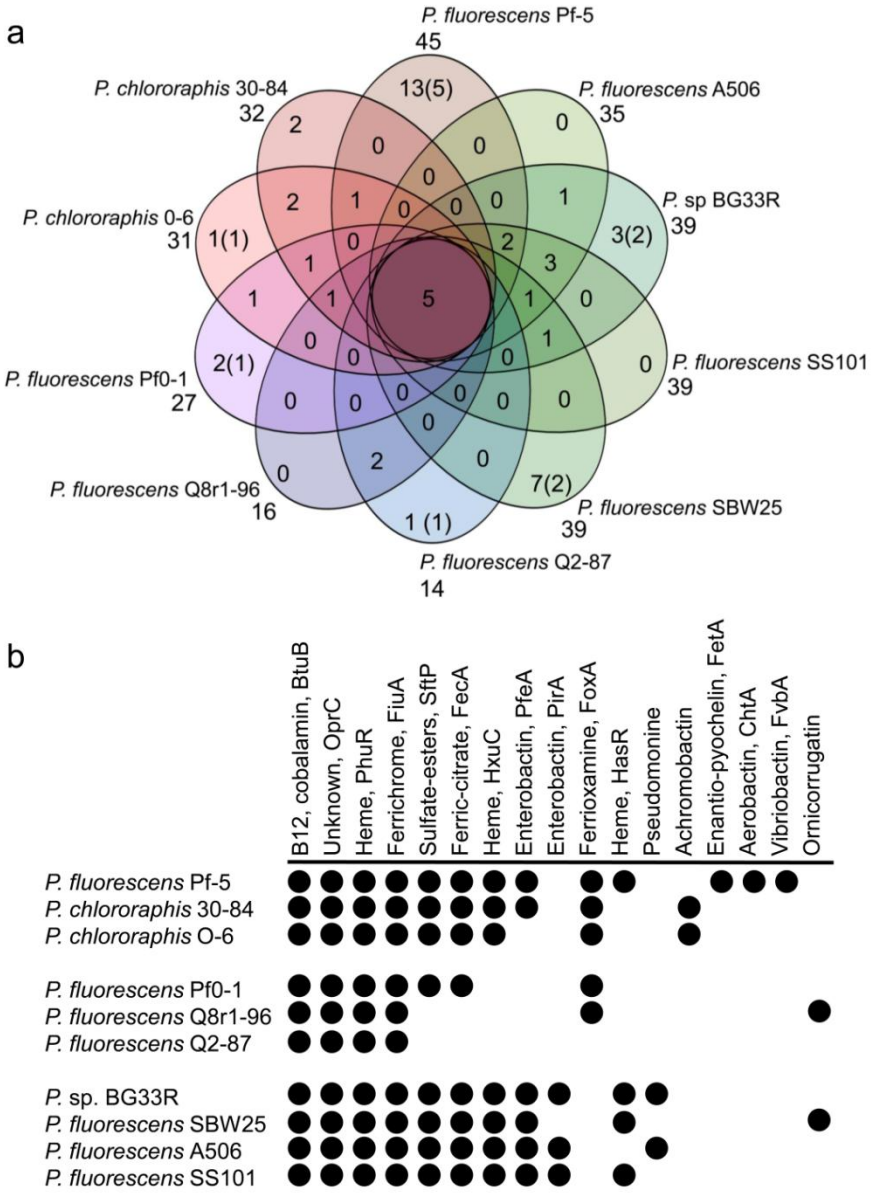


**Fig. 4.4. Conservation and diversity of TBDP types. a.** Venn diagram showing the number of TBDPs in strains of the *P. fluorescens* group. The total number of TBDPs is shown next to the strain name. TBDPs unique to each strain are shown in the outermost section with the number of TBDPs having orthologs in bacteria outside of the *P. fluorescens* group in parenthesis. **b.** TBDPs for which putative functions could be assigned among the ten strains.

**Core TonB-dependent outer-membrane proteins**

The core TBDPs includes orthologs of OprC, BtuB (vitamin B12/cobalamin), PhuR (heme), and FiuA (ferrichrome) and an unknown TBDP. The putative functions were assigned to each core TBDP by comparison to well-characterized TBDPs from other *Pseudomonas* spp. by amino acid percent identity and gene cluster organization (Fig. 4.5). The gene clusters in which the core TBDPs are located illustrate conservation of the TBDP and the surrounding genes (Fig. 4.5). The clades of the orthologous TBDPs making up the core are also shown illustrating evolutionary relationships of these proteins. The orthologs of OprC are in gene clusters having the most diversity in the number and arrangement of genes compared to the other gene clusters containing core TBDPs (Fig. 4.5a). OprC may have a role in copper uptake as it binds and is regulated by copper in *P. aeruginosa* (53). NosA, an ortholog of OprC from *P. stutzeri*, transports copper that is needed for nitrate reductase activity (27). However, *oprC* is not regulated by copper in *P. putida* KT2440 (33) and is up-regulated in the presence of zinc in Pf-5 (Lim et al. unpublished). Therefore, the substrate for OprC is not clearly defined and may differ among strains of *Pseudomonas* spp. The gene cluster containing the TBDP for vitamin B12 uptake is conserved among the strains (Fig. 4.5b) as are the gene clusters containing the ferrichrome TBDP (Fig. 4.5c) and the TBDP of unknown function (Fig. 4.5e). The putative heme TBDPs orthologous to PhuR are adjacent to ECF sigma and anti-sigma factors (Fig. 4.5d). PhuR orthologs have also been identified in strains of *P. aeruginosa*, *P. syringae*, *P. putida*, and *P. entomophila* (7). The recently sequenced *P. fluorescens* WH6 also has a PhuR ortholog as well as orthologs for BtuB, FiuA, OprC and the TBDP of unknown function. The number of conserved TBDPs within the ten strains increases to nine if the strains Q2-87 and Q8r1-96 are removed from the comparison, as they have the fewest TBDPs in their genomes. Two of the four additional TBDPs have putative functions for sulfate-ester (SftP) (23) and ferric citrate (FecA) (31) uptake, but the other two are unknown in function.

**Fig. 4.5. Gene clusters and clades from Neighbor Joining analysis of the core TBDPs.** Gene clusters and clades containing core TBDPs are shown for strains Pf-5 (PFL_), Pf0-1 (Pfl01_), SBW25 (PFLU), SS101 (PflSS101_), BG33R (PseBG33_), A506 (PflA506_), 30-84 (Pchl3084_), O-6 (PchlO6), Q2-87 (PflQ2_), and Q8r1-96 (PflQ8_). The five core TBDPs are **a.** OprC, **b.** Cobalamin (B12), **c.** Ferrichrome, **d.** Heme, and **e.** Unknown. Predicted gene functions are denoted by color: red, TBDP; brown, ABC transport; gold, membrane protein (other than ABC transport); green, biosynthesis; purple, ECF sigma factor and anti-sigma factor; yellow, regulatory (other than ECF sigma factor); blue, hypothetical. Genes whose functions appear unrelated to that of the TBDP are shown in white. Orthologs not readily identifiable by their position in the gene cluster are indicated by identical patterns.

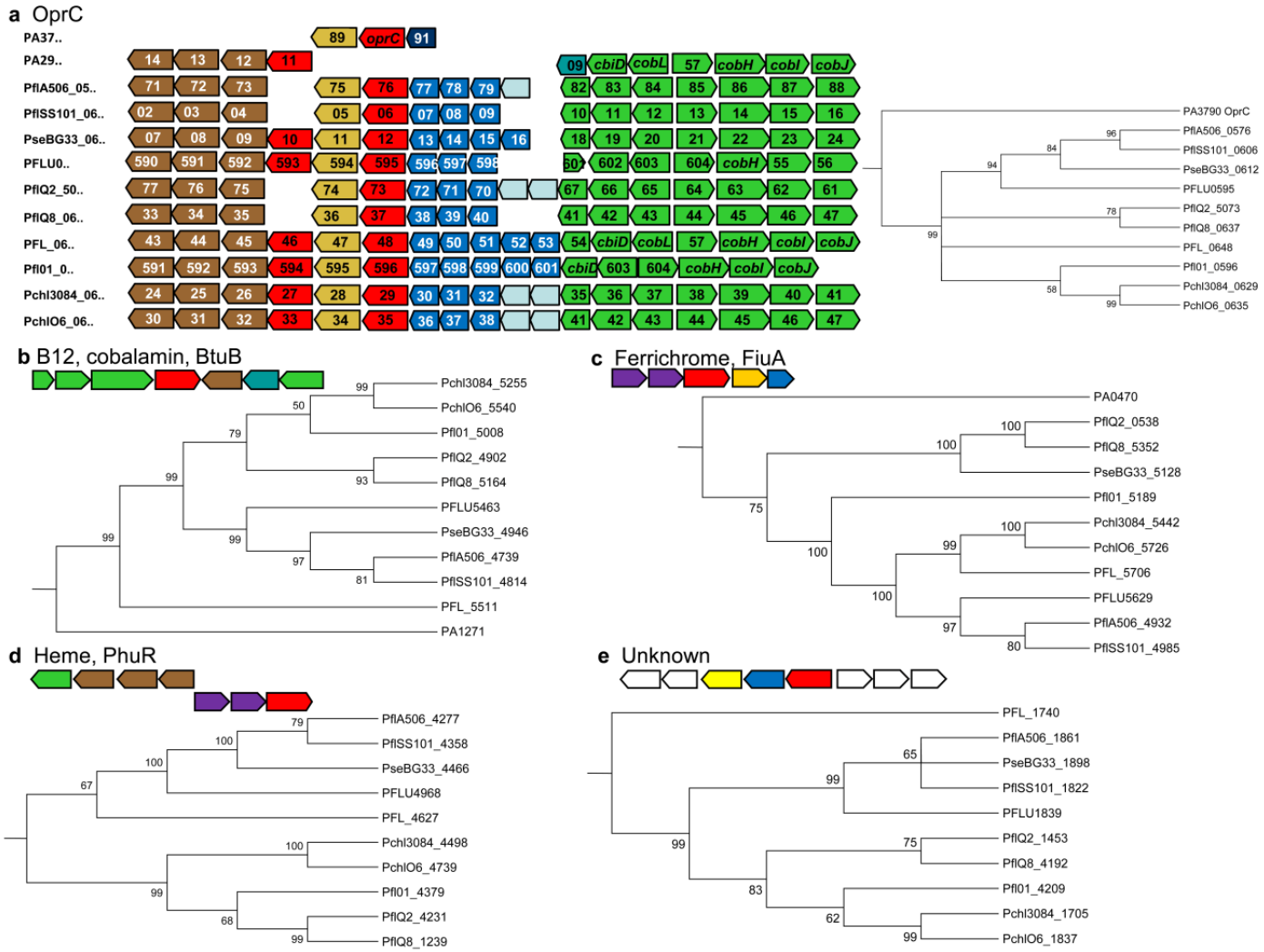
a OprC
PA37..
PA29..
PflA506_05..
PflSS101_06..
PseBG33_06..
PFLU0..
PflQ2_50..
PflQ8_06..
PFL_06..
Pfl01_0..
PchI3084_06..
PchIO6_06..
oprC
cbiD
cobL
cobH
cobI
cobJ
PA3790 OprC
PflA506_0576
PflSS101_0606
PseBG33_0612
PFLU0595
PflQ2_5073
PflQ8_0637
PFL_0648
Pfl01_0596
PchI3084_0629
PchIO6_0635
b B12, cobalamin, BtuB
PchI3084_5255
PchIO6_5540
Pfl01_5008
PflQ2_4902
PflQ8_5164
PFLU5463
PseBG33_4946
PflA506_4739
PflSS101_4814
PFL_5511
PA1271
c Ferrichrome, FiuA
PA0470
PflQ2_0538
PflQ8_5352
PseBG33_5128
Pfl01_5189
PchI3084_5442
PchIO6_5726
PFL_5706
PFLU5629
PflA506_4932
PflSS101_4985
d Heme, PhuR
PflA506_4277
PflSS101_4358
PseBG33_4466
PFLU4968
PFL_4627
PchI3084_4498
PchIO6_4739
Pfl01_4379
PflQ2_4231
PflQ8_1239
e Unknown
PFL_1740
PflA506_1861
PseBG33_1898
PflSS101_1822
PFLU1839
PflQ2_1453
PflQ8_4192
Pfl01_4209
PchI3084_1705
PchIO6_1837

**Fig. 4.5.**

**Putative functions of non-core TBDPs**

Some of TBDPs which are not part of the core are orthologs of well-characterized TBDPs (Fig. 4.4b). For example, Pf-5, SBW25 and BG33R have orthologs to HasR and the hemophore HasS (Fig. 4.6a) (7). An additional putative heme TBDP orthologous to HxuC from *P. aeruginosa* is found in A506, 30-84, O-6, BG33R, SBW25 and Pf-5 (6), bringing the number of heme TBDPs in Pf-5, SBW25, and BG33R to three. Eight of the strains surveyed have orthologs to SftP for sulfate-ester uptake (Fig. 4.6b). TBDPs with putative functions in enterobactin uptake were identified. Pf-5, SBW25 and 30-84 have orthologs to PfeA and the adjacent two component regulatory genes (Fig. 4.6c), whereas A506, SS101 and BG33R have orthologs to PirA but lack the contiguous two component regulatory genes (12-13). Orthologs to FoxA for the uptake of ferrioxamine were identified in Pf0-1, O-6, 30-84, and Pf-5 (Fig. 4.6d) (19). All the strains except Q2-87 and Q8r1-96 have orthologs to FecA for ferric citrate uptake (Fig. 4.6e) (8).

Some strains of fluorescent pseudomonads make a secondary siderophore (Fig. 4.4b). Pf-5 makes enantio-pyochelin with the TBDP FetA located in the biosynthetic gene cluster for uptake (20-21). SBW25 makes the secondary siderophore ornicorrugatin with a corresponding TBDP and there is an ortholog found in Q8r1-96 (5). TBDPs were found in gene clusters for a pseudomonine-like siderophore in A506 and BG33R. Gene clusters for the production of an achromobactin-like siderophore were identified in 30-84 and O-6 with an adjacent TBDP.

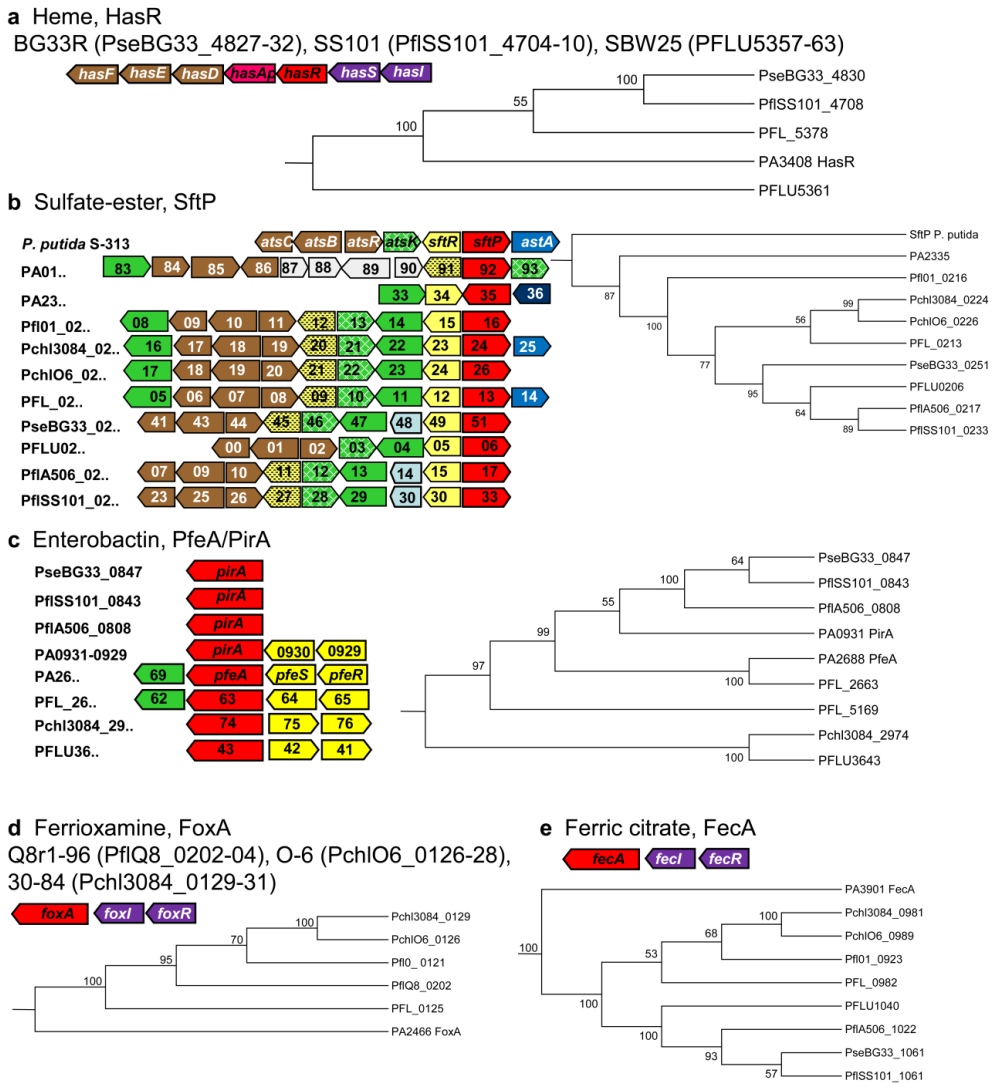


**Fig. 4.6. Gene clusters and clades from Neighbor Joining analysis of TBDPs with putative functions.** Gene clusters and clades containing TBDPs are shown for strains Pf-5 (PFL_), Pf0-1 (Pfl01_), SBW25 (PFLU), SS101 (PflSS101_), BG33R (PseBG33_), A506 (PflA506_), 30-84 (PchI3084_), O-6 (PflO6_), Q2-87 (PflQ2_), and Q8r1-96 (PflQ8_) with putative functions in the uptake of **a.** Heme, **b.** Sulfate-esters, **c.** Enterobactin, **d.** Ferrioxamine, **e.** Ferric citrate. Predicted gene functions are denoted by color: red, TBDP; brown, ABC transport; gold, membrane protein (other than ABC transport); green, biosynthesis; purple, ECF sigma factor and anti-sigma factor; yellow, regulatory (other than ECF sigma factor); pink, hemophore; blue, hypothetical. Genes whose functions appear unrelated to that of the TBDP are shown in white. Orthologs not readily identifiable by their position in the gene cluster are indicated by identical patterns.

**TBDP clusters and horizontal gene transfer**

In the process of comparing the TBDPs of the ten strains, I noticed that some TBDPs appear to be duplicated. Two contiguous TBDPs in BG33R, PseBG33_2355 and PseBG33_2354 are 55% identical at the amino acid level to each other and have 55% amino acid identity to Veis_2440 in *Verminephrobacter eiseniae* EF01_2, with the next most similar TBDPs found in *Nitrococcus mobilis* and *Paracoccus denitrificans*. The GC content of PseBG33_2354 (62.5%) differs significantly from BG33R's genomic average of 59.6%. Interestingly, these two TBDPs are located next to a biosynthetic gene cluster for a mycobactin-like siderophore in BG33R (11). Two TBDPs in SBW25, PFLU2509 and PFLU2941 are orthologs of PSPTO_1855 from *P. syringae* pv. tomato DC3000, a possible indication of a duplication event within SBW25, as they are 87% identical to each other.

Clustered TBDP genes, within two genes of each other, were found in Pf-5, 30-84, O-6, BG33R, Pf0-1, SBW25, and Q2-87. Pf-5 has three clusters (PFL_3176-3177; PFL_0646, PFL_0648; PFL_0992, PFL_0995). PFL_3176 and PFL_3177 are not related to each other but they are orthologous to PSEEN3436 and PSEEN3437 from *P. entomophila* (7). Clustered orthologs to PFL_0646 and PFL_0648 are found in 30-84, O-6, BG33R, Pf0-1, and SBW25. The clustered TBDPs PchlO6_0633 and PchlO6_0635 in the O-6 genome are 93% and 97% identical to Pchl3084_0505 and Pchl3084_0507, respectively, from 30-84. Q2-87 (PflQ2_2928 and PflQ2_2925) and O-6 (PchlO6_3255 and PchlO6_3258) share another pair of clustered orthologous genes. The conservation of these clustered TBDPs within some of the strains suggests horizontal transfer or the loss of TBDPs from some strains.

Candidates for horizontal gene transfer (HGT) were identified as some of the strains have TBDPs without orthologs within the pseudomonads (Fig. 4.4a). In O-6, PchlO6_3555 is 75% identical to Acav_1899 from *Acidovorax avenae* subsp. *avenae* and is phylogenetically related to Fpv outer-membrane proteins (Fig. 4.7a). In Q2-87

PflQ2_3242 is 45% identical to a TBDP in *Nitrococcus mobilis* Nb-231 and is similar to TBDPs found in *Ralstonia eutropha* and *Achromobacter xylosoxidans*. This TBDP is adjacent to a cluster of genes with characteristics of siderophore production and is in the Fpv outer-membrane protein clade (Fig. 4.8a). Pf01_2342 is 56% identical to Smal_3152 from *Stentrophomonas maltophilia* R551-3 and TBDPs in other *Stentrophomonas* spp., and has a significantly different % GC of 64% compared to the genomic average of 60.6%. In Pf-5, the candidates for horizontal gene transfer are described in Hartney et al. (20).

## Pyoverdine iron-acquisition systems

### Characterization of pyoverdines: bioinformatic structure predictions and crossfeeding of Pf-5

For the seven recently sequenced strains, the length and amino acid composition of the pyoverdine peptide chains were predicted bioinformatically from the amino acid sequences of the non-ribosomal peptide synthetases in the pyoverdine biosynthetic gene clusters. The predicted pyoverdine peptide chain structures of 30-84 and O-6 and the Pf-5 pyoverdine are identical in the first four amino acid residues (Asp-FOHOrn-Lys-Thr) with minor differences in the N-terminal portion of the peptide chain (Table 4.2). The predicted pyoverdine peptide chains of BG33R, SS101, and A506 are very similar; the only difference in the first four residues is due to an unknown in the sequence of the BG33R pyoverdine, and the last three residues are conserved. The predicted peptide chains of Q2-87 and Q8r1-96 are both eight amino acids long and are conserved in five of the residues at identical positions in the peptide chains (Table 4.2). Therefore, the strains within each sub-clade produce identical or very similar pyoverdines. The seven strains were tested for crossfeeding of Pf-5 Δ*pvdI-pchC* and derivatives with deletions in the six *fpv* genes (Table 4.2). Pf-5 was crossfed by BG33R, A506, 30-84, O-6, and SS101, but not by Q2-87 and Q8r1-96. Strains 30-84 and O-6 did not crossfeed the *fpvZ* mutant. BG33R did not crossfeed the *fpvU* mutant (Table 4.2). The predicted amino acid composition of the BG33R pyoverdine is similar to the pyoverdines produced by other

pseudomonads that were recognized by FpvU (Chapter 3). They may differ however, because not all of the amino acid residues in BG33R pyoverdine could be predicted bioinformatically. SS101 did not crossfeed the *fpvY* mutant of Pf-5. The peptide chain of the pyoverdine produced by strain SS101 is identical to that of the *P. fluorescens* 18.1 pyoverdine, which is taken up with high affinity by FpvAI, an ortholog of FpvY (18). It appears that the first four amino acids of the SS101 peptide chain (Lys-Gly-FOHOrn) conform exactly to the sequence of pyoverdines transported by FpvAI (18) and FpvU (Chapter 3). The observation that strain SS101 crossfed Pf-5 via FpvY rather than FpvU indicates that other residues in the peptide chain also confer specificity for pyoverdine binding. A506 was able to crossfeed all of the single *fpv* mutants, therefore A506 and a pyoverdine deficient mutant of A506 were tested for crossfeeding of double *fpv* mutants of Pf-5 Δ*pvdI-pchC* siderophore mutant. A506 did not crossfeed a mutant of Pf-5 deficient in both *fpv*U and *fpv*Y (Table 4.2). The pyoverdine-deficient mutant of A506 did not promote the growth of the Pf-5 siderophore mutant or any of the *fpv* mutants tested. This same result was observed in crossfeeding assays with *P. fluorescens* WCS374, which has the same peptide chain structure as A506 (Chapter 3). Therefore, the results of the crossfeeding assays were as expected based on the ferric-pyoverdines assigned to the Fpv outer-membrane proteins of Pf-5 in Chapter 3.

**Table 4.2. Crossfeeding of single *fpv* mutants of Pf-5 in the Δ*pvdI-pchC* background**

| Test Strains | *fpv* mutants[a] | | | | | | Bioinformatic prediction of peptide chain[b] |
|---|---|---|---|---|---|---|---|
| | ***fpvZ*** | ***fpvU*** | ***fpvX*** | ***fpvW*** | ***fpvY*** | ***fpvV*** | |
| *P. chlororaphis* 30-84 | – | + | + | + | + | + | Asp-FOHOrn-Lys-Thr-Gly/Ala-Gly/Ala-FOHOrn-?/Ala |
| *P. chlororaphis* O-6 | – | + | + | + | + | + | Asp-FOHOrn-Lys-Thr-Gly-Gly-FOHOrn-Lys |
| *P.* sp.BG33R | + | - | + | + | + | + | Ser-?-Gly-FOHOrn-Lys-FOHOrn-?/Ser |
| *P. fluorescens* SS101 | + | + | + | + | - | + | Ser-Lys-Gly-FOHOrn-Ser-Ser-Gly-Lys-FOHOrn-Ser |
| *P. fluorescens* A506 | + | +* | + | + | +* | + | Ser-Lys-Gly-FOHOrn-Lys-FOHOrn-Ser |
| *P. fluorescens* Q2-87 | - | - | - | - | - | - | Ser-AcOHOrn-Gly-Gly-Ser-Ser-Asp-Thr/Dhb |
| *P. fluorescens* Q8r1-96 | - | - | - | - | - | - | Ala/Gly-AcOHOrn-Ala-Gly-Ser-Ala/Gly-Asp-Thr/Dhb |

[a]+ indicates growth of the mutant in the presence of the test strain. – indicates no growth of the mutant in the presence of the test strain. * Negative crossfeeding obtained with a *fpvU fpvY* mutant. [b]Underline denotes D-amino acids. FOHOrn is δN-formyl- δN-hydroxy-ornithine. Dhb is diamino-butanoic acid. AcOHOrn is δN-acetyl- δN-hydroxy-ornithine.

**Bioinformatic and phylogenetic analysis of Fpv outer-membrane proteins**

The amino acid sequences of the six characterized Fpv outer-membrane proteins from Pf-5 were individually submitted to BLASTP analysis against the predicted proteomes of the nine other strains of the *P. fluorescens* group. Orthologs were found in all of the strains at a cutoff of >60% amino acid identity (% ID) (Table 4.3). A506 and BG33R have orthologs of FpvU. Six of the strains have Fpv outer-membrane proteins similar to FpvW (55-68%ID). SBW25 has an ortholog of FpvX, and *P. chlororaphis* 30-84 and O-6 have orthologs of FpvZ. To identify any Fpv outer-membrane proteins not found in the initial BLASTP analysis, the TBDPs from each strain were subjected to BLASTP against the protein sequences of the other strains. Additional Fpv outer-membrane proteins were identified in Q2-87, BG33R, SS101, and SBW25. It was observed that the % ID between the Fpv outer-membrane proteins of the ten strains covers a wide range, but there is a basal level of amino acid identity at approximately 32-35%. This level of identity exceeds the 20% ID between any Fpv and a TBDP of different function.

Fpv outer-membrane proteins involved in ferric-pyoverdine uptake are present in all ten strains. In each strain, one *fpv* is located in the pyoverdine-biosynthesis gene cluster and two to five additional *fpv* genes are located elsewhere in the genome. Phylogenetic analysis indicates distinct clades and sub-clades of Fpv outer-membrane proteins (Fig. 4.7a). Four sub-clades include Fpv outer-membrane proteins that are located in pyoverdine biosynthetic gene clusters (denoted by an asterisk in Fig. 4.7a). The TBDPs with putative roles in ferric-pyoverdine uptake are both receptors (black) and transducers (blue) (Fig. 4.7a). Pf-5 has Fpv transducers only but all other strains have both Fpv receptors and transducers. Fpv outer-membrane proteins in the receptor class cluster separately from Fpv outer-membrane proteins in the transducer class, although there is a level of relatedness as the Fpv receptors form clades with Fpv transducers at external and internal branch points (Fig. 4.7a). The Fpv outer-membrane proteins

identified in this study are not considered as part of the core due to their diversity within and between the ten strains, as no one Fpv is found in all ten strains.

**Table 4.3. Reciprocal BLASTP analysis of Fpv outer-membrane proteins of the *P. fluorescens* group**

| **Pf-5** | **Pf-5** | % ID | **30-84** | % ID | **O6** | % ID | **SBW25** | % ID | **A506** | % ID | **BG33R** | % ID | **SS101** | % ID | **Pf0-1** | % ID | **Q8r1-96** | % ID | **Q2-87** | % ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fpv | | Pchl3084_ | | PchlO6_ | | PFLU | | PflA506_ | | PseBG33_ | | PflSS101_ | | Pfl01_ | | PflQ8_ | | PflQ2_ | |
| FpvW | | | 3355 | 68 | 3657 | 68 | 5798 | 40 | 1983 | 55 | 2016 | 55 | 1938 | 55 | 1848 | 41 | 2057 | 68 | 1672 | 38 |
| FpvU | | | 2488 | 41 | 4229 | 38 | 2545 | 72 | 3090 | 70 | 2445 | 70 | 3096 | 57 | 2583 | 40 | 3550 | 36 | 0688 | 37 |
| FpvV | | | 2488 | 36 | 4229 | 33 | 3633 | 42 | 3090 | 36 | 2445 | 34 | 3154 | 34 | 2462 | 39 | 3550 | 35 | 0688 | 35 |
| FpvX | | | 2488 | 55 | 3015 | 41 | 3633 | 75 | 3090 | 41 | 2445 | 39 | 3154 | 40 | 2583 | 43 | 3550 | 37 | 0688 | 37 |
| FpvY | | | 2488 | 38 | 3555 | 39 | 2545 | 58 | 3090 | 52 | 2445 | 54 | 3154 | 55 | 2583 | 40 | 2057 | 34 | 0688 | 35 |
| FpvZ | | | 3950 | 83 | 4229 | 83 | 3378 | 49 | 2860 | 47 | 2654 | 42 | 2880 | 46 | 2462 | 48 | 3550 | 46 | 0688 | 47 |
| | | | | | | | | | | | | | | | | | | | | |
| **Q2-87** | | | | | | | | | | | | | | | | | | | | |
| PflQ2_3242 | FpvY | 35 | 3355 | 33 | 3657 | 32 | 2545 | 35 | 3090 | 34 | 2445 | 32 | 3096 | 35 | 1848 | 33 | 2057 | 32 | | |
| | | | | | | | | | | | | | | | | | | | | |
| **BG33R** | | | | | | | | | | | | | | | | | | | | |
| PseBG33_3069 | FpvX | 40 | 2783 | 72 | 3015 | 73 | 2688 | 89 | 3008 | 91 | | | 2361 | 91 | 2583 | 71 | 2057 | 35 | 0688 | 34 |
| PseBG33_2658 | FpvZ | 47 | 3950 | 45 | 4229 | 46 | 3378 | 85 | 2860 | 88 | | | 288 | 88 | 2462 | 81 | 3550 | 79 | 0688 | 80 |
| PseBG33_5305 | FpvW | 40 | 3355 | 41 | 3657 | 41 | 5798 | 94 | 1983 | 41 | | | 1938 | 42 | 1848 | 56 | 3969 | 51 | 1672 | 52 |
| | | | | | | | | | | | | | | | | | | | | |
| **SS101** | | | | | | | | | | | | | | | | | | | | |
| PflSS101_2361 | FpvX | 40 | 2783 | 72 | 3015 | 72 | 2688 | 88 | 3008 | 97 | 3069 | 91 | | | 2583 | 72 | 2057 | 35 | 0688 | 34 |
| | | | | | | | | | | | | | | | | | | | | |
| **SBW25** | | | | | | | | | | | | | | | | | | | | |
| PFLU2688 | FpvX | 41 | 2783 | 74 | 3015 | 74 | | | 3008 | 88 | 3069 | 89 | 2361 | 88 | 2583 | 72 | 3550 | 33 | 1672 | 32 |

**Fig. 4.7. Phylogenetic analysis of Fpv outer-membrane proteins and association with pyoverdine structures. a**. Maximum Parsimony analysis of Fpv outer-membrane proteins from the *P. fluorescens* group, close orthologs and characterized Fpv outer-membrane proteins. The characterized Fpv outer-membrane proteins from Pf-5 are in red. An asterisk indicates that the TBDP is in a pyoverdine biosynthetic gene cluster, and blue indicates that the Fpv is a transducer. Branches are collapsed for bootstraps <50. Abbreviations for species are as follows: *P. fluorescens* A506 (PflA506_), *P. fluorescens* SS101 (PflSS101_), *P. fluorescens* Q2-87 (PflQ2_), *P. fluorescens* Q8r1-96 (PflQ8_), *P. fluorescens* Pf-5 (PFL_), *P. fluorescens* SBW25 (PFLU), *P. fluorescens* Pf01 (Pfl01_), *P.* sp. BG33R (PseBG33_), *P. chlororaphis* 30-84 (Pchl3084_), *P. chlororaphis* O-6 (PchlO6_), *P. putida* GB-1 (PputGB1_), *Acidovorax avenae* subsp. *avenae* (Acav_), *P. putida* W619 (PputW619_), *P. fluorescens* WH6 (PFWH6_), *P. aeruginosa* LESB58 (Pa LESB58), *P. aeruginosa* PAO1 (PA), *P.* sp. M114 , *P. putida* (Pp), *P. brassicacearum* subsp. brassicacearum NFM421 (PSEBR_), *P. aeruginosa* ATCC27853 (Pa 27853), and *P. aeruginosa* 7NSK2 (Pa 7NSK2). **b**. Pyoverdine peptide chains associated with the Fpv functional groups. Underline denotes D-amino acids. Parentheses define cyclic residues. cOHOrn is cyclo-hydroxy-ornithine. FOHOrn is δN-formyl- δN-hydroxy-ornithine. εLys is Lys linked by its ε-NH2. OHAsp is threo-β-hydroxy-aspartic acid. Dab is diamino-butanoic acid. AcOHOrn is δN-acetyl- δN-hydroxy-ornithine. A slash between two amino acids means that the pyoverdine peptide chain recognized by the associated Fpv contains either of the amino acids.

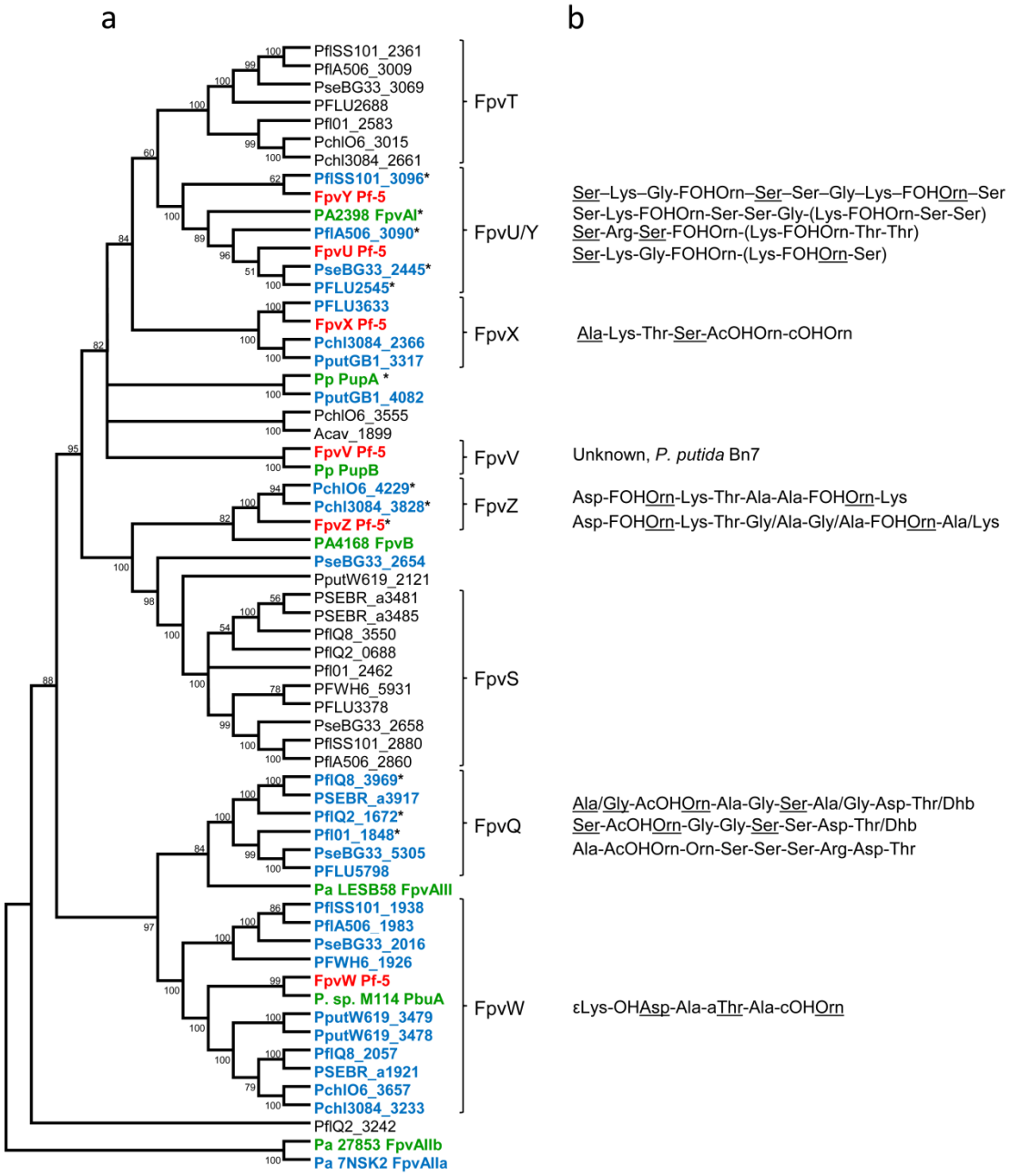
a
b
PflSS101_2361
PflA506_3009
PseBG33_3069
PFLU2688
Pfl01_2583
PchlO6_3015
Pchl3084_2661
FpvT
PflSS101_3096*
FpvY Pf-5
PA2398 FpvAI*
PflA506_3090*
FpvU Pf-5
PseBG33_2445*
PFLU2545*
FpvU/Y
Ser–Lys–Gly-FOHOrn–Ser–Ser–Gly–Lys–FOHOrn–Ser
Ser-Lys-FOHOrn-Ser-Ser-Gly-(Lys-FOHOrn-Ser-Ser)
Ser-Arg-Ser-FOHOrn-(Lys-FOHOrn-Thr-Thr)
Ser-Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser)
PFLU3633
FpvX Pf-5
Pchl3084_2366
PputGB1_3317
FpvX
Ala-Lys-Thr-Ser-AcOHOrn-cOHOrn
Pp PupA *
PputGB1_4082
PchlO6_3555
Acav_1899
FpvV Pf-5
Pp PupB
FpvV
Unknown, P. putida Bn7
PchlO6_4229*
Pchl3084_3828*
FpvZ Pf-5*
FpvZ
Asp-FOHOrn-Lys-Thr-Ala-Ala-FOHOrn-Lys
Asp-FOHOrn-Lys-Thr-Gly/Ala-Gly/Ala-FOHOrn-Ala/Lys
PA4168 FpvB
PseBG33_2654
PputW619_2121
PSEBR_a3481
PSEBR_a3485
PflQ8_3550
PflQ2_0688
Pfl01_2462
PFWH6_5931
PFLU3378
PseBG33_2658
PflSS101_2880
PflA506_2860
FpvS
PflQ8_3969*
PSEBR_a3917
PflQ2_1672*
Pfl01_1848*
PseBG33_5305
PFLU5798
FpvQ
Ala/Gly-AcOHOrn-Ala-Gly-Ser-Ala/Gly-Asp-Thr/Dhb
Ser-AcOHOrn-Gly-Gly-Ser-Ser-Asp-Thr/Dhb
Ala-AcOHOrn-Orn-Ser-Ser-Ser-Arg-Asp-Thr
Pa LESB58 FpvAIII
PflSS101_1938
PflA506_1983
PseBG33_2016
PFWH6_1926
FpvW Pf-5
P. sp. M114 PbuA
PputW619_3479
PputW619_3478
PflQ8_2057
PSEBR_a1921
PchlO6_3657
Pchl3084_3233
FpvW
εLys-OHAsp-Ala-aThr-Ala-cOHOrn
PflQ2_3242
Pa 27853 FpvAIIb
Pa 7NSK2 FpvAIIa

**Fig. 4.7.**

**Functional characterization of Fpv outer-membrane proteins**

Mutants deficient in pyoverdine biosynthesis in strains 30-84, Pf0-1, A506 and SS101 were tested for growth on an iron-limited medium in the presence of the members of the *P. fluorescens* group. These strains were also tested in crossfeeding assays with *Pseudomonas* strains that produce pyoverdines previously linked to specific Pf-5 Fpv outer-membrane proteins (20) (Chapter 3) (Fig. 4.8). 30-84 was crossfed by *P. chlororaphis* O-6, *P. fluorescens* Pf-5 and B10, and *P. putida* CS111. Pf0-1 was crossfed by *P. fluorescens* Pf-5, Q2-87, and Q8r1-96, *P. chlororaphis* 30-84 and O-6. A506 was crossfed by *P. aeruginosa* PAO1, *P. fluorescens* SS101, SBW25, Pf-5, B10, *P.*sp. BG33R, *P. chlororaphis* 30-84, O-6, and *P. reactans* NCPPB387. SS101 was crossfed by *P. aeruginosa* PAO1, *P. fluorescens* SS101, SBW25, Pf-5, B10, *P.* sp. BG33R, *P. chlororaphis* 30-84, O-6, *P. rhodesiae* 92-104 and *P. reactans* NCPPB387.

There is some correlation between the ability to crossfeed the pyoverdine mutants and which clade the feeding strains are in, which is related to the similar pyoverdine peptide chains produced by the more related strains (Table 4.2). The pyoverdines produced by the strains of the Pf-5 clade crossfed all four strains (A506, SS101, 30-84 and Pf0-1). The pyoverdines produced by the strains in the SBW25 clade crossfed Pf-5, A506, and SS101; in addition, SS101 crossfed Pf0-1. The pyoverdines produced by Q2-87 and Q8r1-96 crossfed Pf0-1 only, and Pf0-1 crossfed Pf-5 only (20).

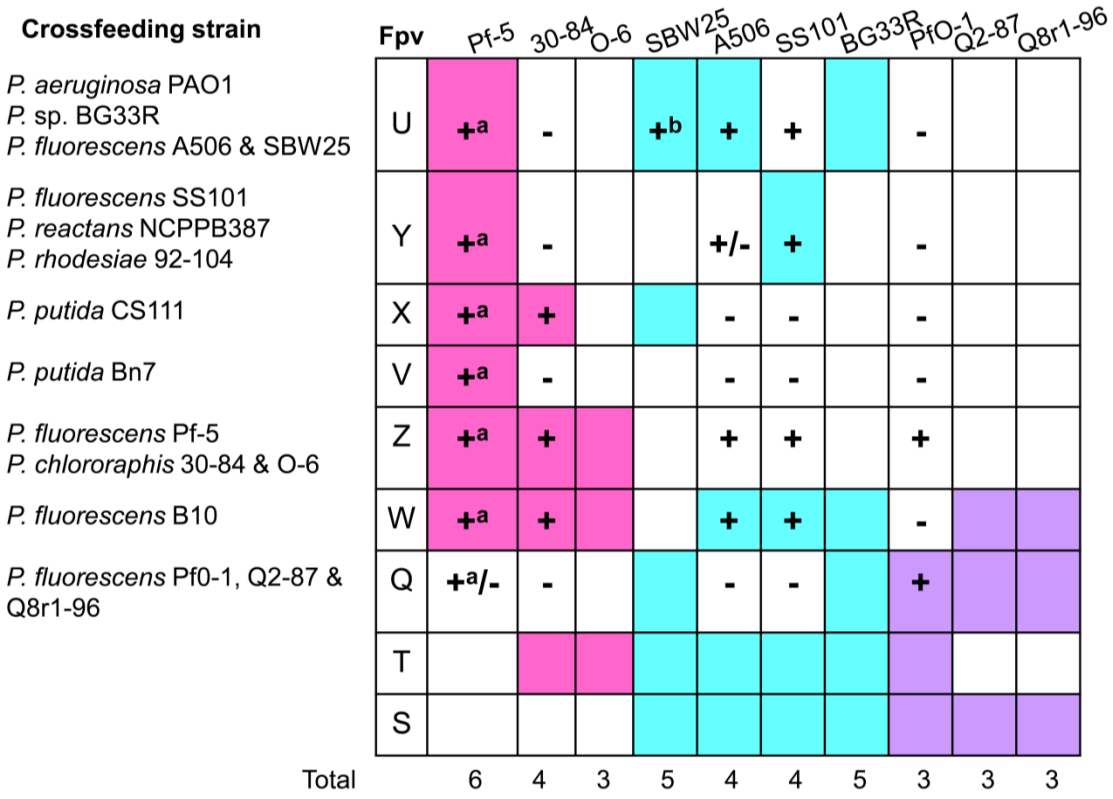


**Fig. 4.8. Crossfeeding of strains in the *P. fluorescens* group by *Pseudomonas* strains producing pyoverdines recognized by the characterized Fpv outer-membrane proteins of Pf-5[a] (Chapter 3).** Colored boxes represent the presence of the Fpv in the strain. Pink corresponds to the Pf-5, 30-84, and O-6 clade. Blue corresponds to the SBW25, A506, SS101, and BG33R clade. Purple corresponds to the Pf0-1, Q2-87, and Q8r1-96 clade. + means growth of the mutant and - means no growth of the mutant in the presence of the pyoverdine producing strain (i.e., crossfeeding strain). Open boxes lacking a plus or minus indicate that the crossfeeding test was not done. [b]Published in Moon et al. (34).

To identify the recognition capabilities for diverse pyoverdine peptide chain structures, mutants deficient in pyoverdine biosynthesis in strains 30-84, Pf0-1, A506, and SS101 were tested for crossfeeding by heterologous ferric-pyoverdines produced by *Pseudomonas* strains, which are unique in structure and known to be recognized by the Fpv outer-membrane proteins of Pf-5 (20) (Chapter 3) (Table 4.4).

The *P. chlororaphis* 30-84 pyoverdine mutant was able to grow in the presence of the purified pyoverdines produced by *P. fluorescens* B10, Pf-5, and *P. putida* SB8.3 (Table 4.4). The pyoverdine deficient mutant of SS101 was able to grow in the presence of the purified pyoverdines from B10, *P. aeruginosa* PAO1, *P. rhodesiae* CFML92-104 and Pf-5 (Table 4.4). The pyoverdine mutant of Pf0-1 was able to grow in the presence of the purified pyoverdines from *P. rhodesiae* CFML 92-104 and *P. fluorescens* Pf-5. The pyoverdine mutant of A506 was able to grow in the presence of the ferric-pyoverdines from *P. fluorescens* B10 and Pf-5, and *P. aeruginosa* PAO1 (Table 4.4).

The three ferric-pyoverdine types produced by *P. aeruginosa* strains (52) were tested, resulting in differing utilization patterns between the pyoverdine mutants of 30-84, Pf0-1, A506, and SS101 (Table 4.4). A506 and SS101 were able to recognize the type I and type II ferric-pyoverdines. Pf0-1 was able to recognize the type II and type III ferric-pyoverdines, and 30-84 was able to recognize the type III ferric-pyoverdine.

**Table 4.4. Purified ferric-pyoverdine stimulated growth assay**

| Strain | *fpv** | Pyoverdine peptide chain structure[a] | Pvd- Mutants[b] | | | |
|---|---|---|---|---|---|---|
| | | | **30-84** | **Pf0-1** | **A506** | **SS101** |
| *P. rhodesiae* CFML92-104 | Y | Ser–Lys–FOHOrn–Ser–Ser–Gly–(Lys–FOHOrn–Ser–Ser) (4) | - | + | - | + |
| *P. aeruginosa* PA01 | U | Ser-Arg-Ser-FOHOrn-(Lys-FOHOrn-Thr-Thr) (Type I pyoverdine) (14) | - | - | + | + |
| *P. putida* SB8.3 | X | Ala-Lys-Thr-Ser-AOHOrn-cOHOrn (32) | + | - | - | - |
| *P. putida* Bn7 | V | Unknown | - | - | - | - |
| *P. fluorescens* Pf-5 | Z | Asp-FOHOrn-Lys-Thr-Ala-Ala-FOHOrn-Lys (20) | + | + | + | + |
| *P. fluorescens* B10 | W | εLys-OHAsp-Ala-aThr-Ala-cOHOrn (49) | + | - | + | + |
| *P. aeruginosa* 7NSK2 | | Ser-FOHOrn-Orn-Gly-aThr-Ser-cOHOrn (Type II pyoverdine) (9) | - | + | + | + |
| *P. aeruginosa* Pa6 | | Ser-cDab-FOHOrn-Gln-Gln-FOHOrn-Gly (Type III pyoverdine) (17) | + | + | - | - |

[a]Underline denotes D-amino acids. Parentheses define cyclic residues. cOHOrn is cyclo-hydroxy-ornithine. FOHOrn is δN-formyl- δN-hydroxy-ornithine. OHAsp is threo-β-hydroxy-aspartic acid. Dab is diamino-butanoic acid. AOHOrn is δN-acetyl- δN-hydroxy-ornithine. [b]+ sign indicates growth of the pyoverdine deficient mutant in the presence of the pyoverdine producing strain, – sign indicates no growth of the pyoverdine deficient mutant in the presence of the pyoverdine producing strain. *See Chapter 3.

**Fpv functional groups**

Based on % amino acid identity, crossfeeding/ferric-pyoverdine growth promotion assays, and phylogenetic analysis, eight Fpv functional groups were identified (Fig. 4.7b; Fig. 4.8). Fpv outer-membrane proteins orthologous to the characterized Fpv outer-membrane proteins of Pf-5 were identified in each of the nine strains and correlated with specific ferric-pyoverdines through crossfeeding and purified ferric-pyoverdine utilization assays.

Orthologs of FpvU and FpvY form the FpvU/Y functional group. Strains Pf-5, SS101 (PflSS101_3069), A506 (PflA506_3090), BG33R (PseBG33_2445), and SBW25 (PFLU2545) have Fpv outer-membrane proteins in this group, including FpvAI from *P. aeruginosa* PAO1. All of the ferric-pyoverdines recognized by this group have a Ser in position one and similar residues making up the rest of the peptide chain. The Fpv outer-membrane proteins in this group, except for Pf-5, are located in pyoverdine biosynthetic regions (Fig. 4.7a). The overlapping nature of FpvY and FpvU is supported by SS101 and A506 having orthologs of Y and U, respectively. *P. aeruginosa* PAO1 was able to crossfeed SS101 even though it does not have an ortholog of FpvU, so it is feasible that SS101 is using PflSS101_3096 to take up PAO1's pyoverdine. A506 has PflA506_3090 as an ortholog to FpvU and is able to grow in the presence of ferric-pyoverdines recognized by FpvU. A506 is also able to recognize the pyoverdines from SS101 and *P. reactans* NCPPB387, which are linked to FpvY, but not the *P. rhodesiae* CFML92-104 pyoverdine, which appears to be specific to FpvY.

The FpvX functional group contains orthologous Fpv outer-membrane proteins from Pf-5, SBW25 (PFLU3633), and 30-84 (Pchl3084_2366) (Fig. 4.7b). The ferric-pyoverdines recognized by these Fpv outer-membrane proteins are produced by *P. putida* strains CS111 and SB8.3 (Fig. 4.8).

Orthologs of FpvZ, which are found in Pf-5, 30-84 (Pchl3084_3828), and O-6 (PchlO6_4229), make up the FpvZ functional group. These three TBDP genes are

located in the pyoverdine biosynthetic gene clusters of these strains and are involved in the uptake of the cognate ferric-pyoverdines (Fig. 4.7).

FpvV, which is found in Pf-5 and is orthologous to *P. putida* PupB, makes up the FpvV functional group. Pf-5 is the only strain with FpvV, and only Pf-5 could grow in the presence of the *P. putida* Bn7 pyoverdine (Fig. 4.8).

Orthologs of FpvW, found in all the *P. fluorescens* group strains except Pf0-1, SBW25 and Q2-87, make up the FpvW functional group. The pyoverdine mutants with Fpv outer-membrane proteins in this group were able to grow in the presence of the pyoverdine produced by *P. fluorescens* B10 (Fig. 4.8).

Orthologs found in strains Q2-87, Q8r1-96, Pf0-1, BG33R, and SBW25 make up the FpvQ functional group. The Fpv outer-membrane proteins from Q2-87 (PflQ2_1672), Q8r1-96 (PflQ8_3969), and Pf0-1 (Pfl01_1848) are located in pyoverdine biosynthetic gene clusters of these strains (Fig. 4.7a). Pf0-1 showed positive crossfeeding with the strains Q2-87 and Q8r1-96, whereas all of the other strains tested had negative crossfeeding with these strains (Fig. 4.8). Pfl01_1848 is phylogenetically related to Fpv outer-membrane proteins from these two strains and is the likely candidate for the recognition of the pyoverdines produced by Q2-87 and Q8r1-96 (Fig. 4.7a).

The FpvS and FpvT functional groups are made up of receptors found in all the strains except Pf-5 and also include Fpv outer-membrane proteins from *P. fluorescens* WH6 and *P. brassicacearum* subsp. brassicacearum NFM421. To our knowledge this is the first report of TBDPs of the receptor type being putative Fpv outer-membrane proteins. There is some indication that these Fpv receptors are involved in pyoverdine recognition as some strains were able to grow in the presence of pyoverdines despite a lack of the associated Fpv transducer. Pf0-1, A506, and SS101 do not have an ortholog of FpvZ but growth was promoted with the Pf-5 ferric-pyoverdine and in crossfeeding assays with Pf-5, 30-84 and O-6 (Fig. 4.8). Pfl01_2462, PflSS101_2880, and

PflA506_2860 are in the FpvS functional group (Fig. 4.7a), which may be how these strains are recognizing the pyoverdines produce by Pf-5, 30-84, and O-6.

**Conclusion**

Two levels of core TBDPs are evident in the *P. fluorescens* group, one at the species group level and the other within the more related strains. Within the *P. fluorescens* group is a core of five TBDPs. The five conserved TBDPs are used for the uptake of vitamin B12 (cobalamin) and substrates with bound metals such as iron and copper. These metals function as co-factors for proteins in essential metabolic pathways. Putative functions of TBDPs outside the core are largely related to iron uptake primarily from siderophores, but the majority of the TBDP functions are unknown. The heme binding TBDP PhuR was identified in all ten strains surveyed here and was also identified in the eleven strains surveyed by Cornelis and Bodilis (7). PhuR appears to be core to the genus *Pseudomonas*. The TBDP core for the *P. fluorescens* group is small compared to the TBDP cores identified in other *Pseudomonas* species (7), which is reasonable due to the diversity of the strains within this group. Within this species group, 84 TBDP types were identified, made up of 17 TBDP types with putative functions, eight Fpv functional groups, three additional Fpv outer-membrane proteins, and 56 unknown TBDP types.

TBDPs with putative functions in ferric-pyoverdine uptake are found within each strain. The abundance and diversity of the Fpv outer-membrane proteins in this species group indicates the importance of acquiring iron through heterologous pyoverdine siderophores. The characterized Fpv outer-membrane proteins of Pf-5, *P. aeruginosa* PAO1 and *P. putida* facilitated the identification of 28 Fpv outer-membrane proteins in the seven recently-sequenced strains and provides a method for Fpv identification in other pyoverdine-producing bacteria. The strategy proposed in Chapter 3 for identifying and predicting ferric-pyoverdine recognition of Fpv outer-membrane proteins was successful for the majority of the Fpv outer-membrane proteins present in the strains of

this study. Some of the Fpv outer-membrane proteins are divergent from the currently characterized Fpv outer-membrane proteins, preventing association with ferric-pyoverdines. Mutagenesis of these Fpv outer-membrane proteins followed by assays with ferric-pyoverdines of known structures not recognized by the currently characterized Fpv outer-membrane proteins may identify substrates for these Fpv outer-membrane proteins.

The ten strains evaluated in this study are associated with soil, root and leaf surfaces (Table 4.1). These are diverse environments, which provide challenges to the associated bacteria in nutrient acquisition (2, 30, 41). TonB-dependent outer-membrane proteins have been found to aid in the uptake of essential minerals, particularly iron, as well as carbohydrates (3, 5, 27, 42). The diversity in the numbers and types of TBDPs found in the strains of the *P. fluorescens* group reflects the diverse nature of these strains as well as their commonalities.

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**Chapter 5: Concluding remarks**

TonB-dependent outer-membrane proteins (TBDPs) are a large family of paralogous proteins found in Gram-negative bacteria. They are structurally very similar to each other, but very diverse in their functions. There is ~20-25% amino acid similarity over all TBDPs, which is enough to maintain the amino acid residues required for the formation of the secondary and tertiary structures required for functionality. The types of TBDPs within the bacterial species surveyed here appear to have differing origins, with some acquired through horizontal gene transfer and duplication events, while others are ancestral to these strains. Horizontal gene transfer appears to be a common mechanism for moving TBDPs among Gram-negative bacteria, but it is not easy to tell the direction of TBDP transfer.

In Chapter 2, a combination of bioinformatic and phylogenetic analyses and bio-assays were employed to characterize the 45 TBDPs of *P. fluorescens* Pf-5. Motifs defining constituent domains were identified and the presence or absence of the N-terminal signaling domain was used to distinguish the 27 TonB-dependent receptors (TBDRs) from the 18 TonB-dependent transducers (TBDTs) in the Pf-5 proteome. Phylogenetic analyses of the TBDPs from Pf-5 and characterized orthologs from other *Pseudomonas* spp. allowed the assignment of putative functions to certain TBDRs and TBDTs of Pf-5.

With the initial investigations of the TBDPs in the Pf-5 proteome, it became apparent that a good portion of the transducers are involved in iron acquisition, whereas the receptors may have broader functions that largely remain unknown. The prevalence of TBDPs for the binding and uptake of ferric-substrates directed further investigations into the utilization capabilities of Pf-5 for various iron-containing compounds. Pf-5 exhibited a remarkable capacity to utilize ferric-citrate, heme, and the siderophores ferrichrome, ferrioxamine B, enterobactin, and aerobactin, as well as pyoverdines with diverse structures produced by different *Pseudomonas* spp.. The ability of Pf-5 to be

crossfed by such a variety of pyoverdine-producing *Pseudomonas* spp. was an exciting prospect as six Fpv outer-membrane proteins of Pf-5 were predicted to be involved in heterologous pyoverdine uptake.

In Chapter 3, I discuss the characterization of the six Fpv outer-membrane proteins in Pf-5. The association between the Fpv outer-membrane proteins of Pf-5 and specific pyoverdines with known peptide chain structures is the culmination of making and testing many mutants in crossfeeding assays and with purified pyoverdines. Previous investigations into the association between the Fpv outer-membrane proteins within a bacterial strain and heterologous pyoverdines were limited by either too few Fpv outer-membrane proteins or too few heterologous pyoverdines tested. The presence of six functional Fpv outer-membrane proteins within Pf-5 and the 37 *Pseudomonas* strains tested make this research novel and a good model for further characterization of Fpv outer-membrane proteins and associated pyoverdines. There are currently ~70 structurally characterized pyoverdines, many of them being unique (3). The individual Fpv outer-membrane proteins used for heterologous pyoverdine uptake show diversity in the portions of the protein responsible for pyoverdine binding as the individual Fpv outer-membrane proteins are used for the uptake of a specific set of structurally related pyoverdines. The specificity of the Fpv for certain structures of pyoverdines indicates parallel evolution of the Fpv and the pyoverdine (3, 5). As pyoverdines with new peptide chains arose, Fpv outer-membrane proteins used for uptake developed to recognize the new pyoverdine structure.

The recent genome sequencing of seven plant-associated strains within the *P. fluorescens* group gave me an opportunity to extend the analysis of TBDPs to these strains, as discussed in Chapter 4. I manually annotated the TBDPs and associated genes, such as those encoding ECF sigma factors and TonB proteins, in each of the genomes. With the completion of the annotation of the TBDPs, I set out to see if a core set of TBDPs is present in the ten strains of the *P. fluorescens* group. The TBDPs of the *P. fluorescens* group form two levels of core TBDPs, one at the species group level and the

other within sub-clades of related strains. The related strains *P. fluorescens* WH6 and *P. brassicacearum* sp. brassicacearum the same core of five distinct TBDPs (4, 6). My analysis showed (i) the number of TBDPs varies between strains; (ii) higher levels of conservation of TBDPs within the more related strains; (iii) a core of five TBDPs conserved among all strains; (iv) putative functions for 28 TBDP types; (v) and high levels of diversity among the Fpv functional groups.

Phylogenetic analysis of the TBDPs in the *P. fluorescens* group indicates a high level of redundancy for the uptake of certain compounds, notably ferrioxamine/ferrichrome, ferric-citrate, heme, and pyoverdines. The number of TBDPs in certain phylogenetic clades, such as those with putative functions in heme and pyoverdine acquisition, exceeds the number found in other bacteria such as *P. aeruginosa* PAO1 (1-2). The diversity and complexity of the TBDPs with roles in iron uptake clearly indicate the importance of iron in the biology of Pf-5 and related *Pseudomonas* spp..

A multi-faceted approach of BLASTP analysis, phylogenetics and pyoverdine growth promotion assays developed in the characterization of the Fpv outer-membrane proteins from Pf-5 was employed to identify Fpv outer-membrane proteins in the *P. fluorescens* group and associate them with pyoverdine peptide chain structures. Fpv outer-membrane proteins were found in multiple copies within each strain. I identified eight Fpv functional groups and three additional Fpv outer-membrane proteins. The abundance and diversity of the Fpv outer-membrane proteins in this species group indicates the importance of acquiring iron through heterologous pyoverdine siderophores. The characterized Fpv outer-membrane proteins of Pf-5, *P.* sp. M114, *P. aeruginosa* PAO1 and *P. putida* WCS358 facilitated the identification of 28 Fpv outer-membrane proteins in the seven recently-sequenced *Pseudomonas* species group strains and provides a method for Fpv identification in other pyoverdine-producing bacteria. Siderotyping is a method of classifying pyoverdine producing pseudomonads based on structural similarities of the cognate pyoverdine to aid in taxonomy (4). An important counterpart to this is the Fpv outer-membrane protein composition of a strain. Each strain has an Fpv

for its own pyoverdine that also may be able to recognize structurally-similar pyoverdines. The Fpv for the cognate pyoverdine adds another criterion, along with siderotype, by which bacterial strains can be characterized.

In summary, the ten strains of the *P. fluorescens* group have 317 TBDPs that fall into 84 types. Seventy three TBDP types are composed of TBDPs sharing >60 % amino acid identity (Fig. 5.1). An additional 11 TBDP types are made up of the Fpv outer-membrane proteins found in these strains. Of the 73 types, 17 of them have been assigned putative functions. This is a small proportion of the total, leaving ample room for further investigation. Experiments to look at changes in the expression of the TBDPs in the presence of diverse substrates may provide candidates for more targeted analysis to further research of the unknown TBDPs.

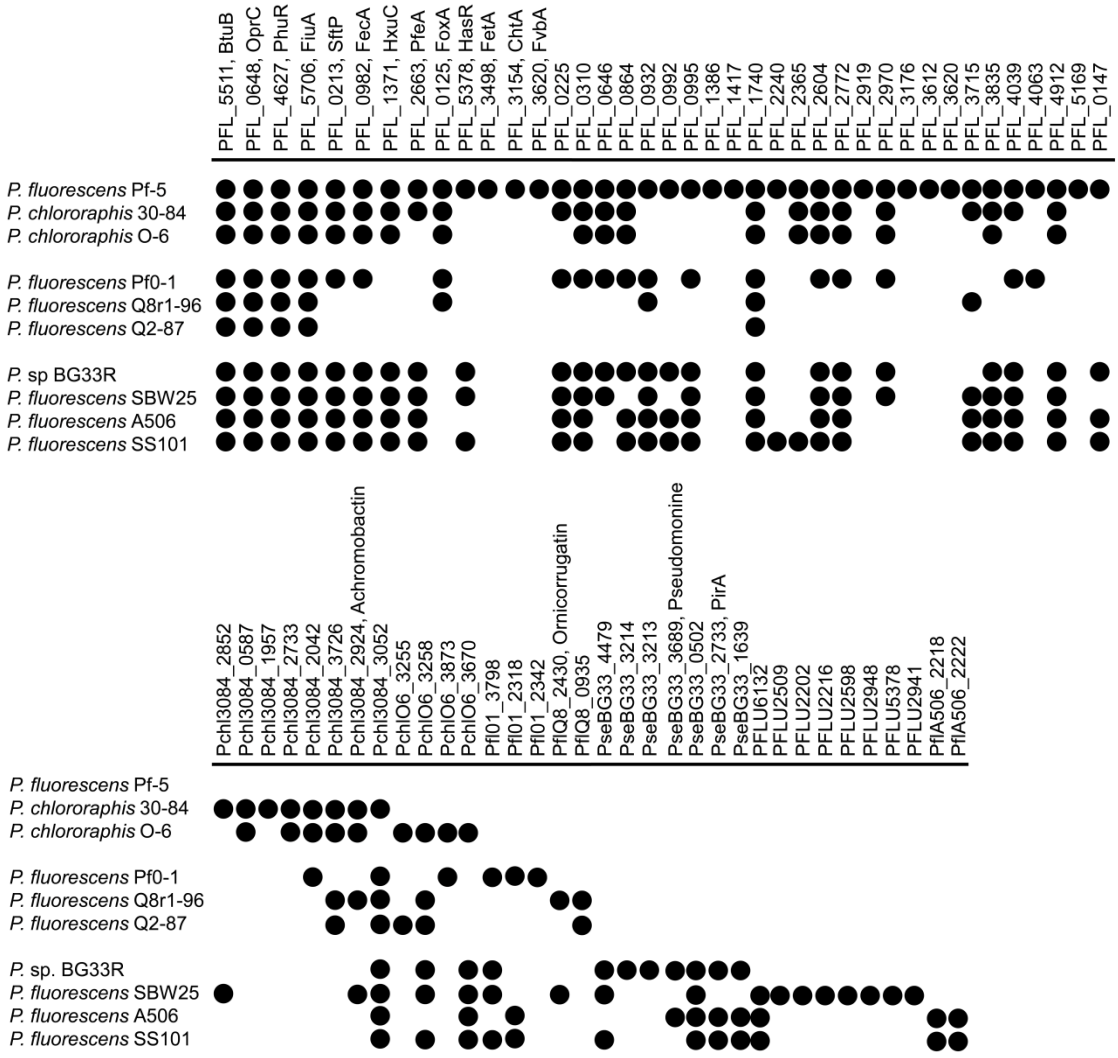


**Fig. 5.1. TBDP types within the *P. fluorescens* group.** A black circle indicates a TBDP orthologous to the TBDP with the listed locus tag at > 60 % amino acid identity, Fpv outer-membrane proteins are not included. Strain locus tags are as follows, Pf-5 (PFL_), 30-84 (Pchl3084_), O-6 (PchlO6_), Pf0-1 (Pfl01_), BG33R (PseBG33_), SBW25 (PFLU), and A506 (PflA506_).

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# APPENDICES

**Appendix 1. Contiguous gene regions surrondoing TBDP genes in *P. fluorescens* Pf-5.** TBDPs are highlighted in green. Transcription regulators are in orange. TonB protein complex genes are in blue. ABC transport genes are in yellow.

| PFL | name | Function | PAO1 homolog | Other homologs | Fur box motif |
|---|---|---|---|---|---|
| 117 | gcdH | glutaryl-CoA dehydrogenase | PA0447 | | |
| 118 | | CoA-transferase, family III | PA0446 | | |
| 122 | rrlA | 23S ribosomal RNA | | | |
| 6292 | PFL_r01 | 5S ribosomal RNA | | | |
| 123 | rrfA | 5S ribosomal RNA | | | |
| 124 | | PepSY-associated TM helix domain protein | PA2465 | | |
| 125 | | TBDP, ferrichrome-iron receptor, Transducer | PA2466 optS | | |
| 126 | | sigma factor regulatory protein, FecR/PupR family | PA2467 | | |
| 127 | | RNA polymerase sigma-70 factor, ECF subfamily | PA2468 | | TAATGATAAT |
| 128 | pctA | chemotactic transducer PctA | | PP2249, Pfl01_0128 | |
| 144 | | efflux transporter, outer membrane factor lipoprotein, NodT family | PA2837 | PP1273 | |
| 145 | | RNA polymerase sigma-70 factor, ECF subfamily | | PP3086 | |
| 146 | | sigma factor regulatory protein, FecR/PupR family | | PP3085 | TAATGATAAT |
| 147 | | TBDP, ferrichrome-iron receptor, Transducer | | PP3084 | |
| 148 | | CHP | | | |
| 149 | | CHP | PA5441 | | |
| 150 | | membrane protein, putative | | | |
| 207 | atsB | sulfate ester ABC transporter, permease protein AtsB | PA0185 | | |
| 208 | atsR | sulfate ester ABC transporter, periplasmic sulfate ester-binding protein AtsR | PA0186 | | |
| 209 | | transcriptional regulator SftR homolog | PA0191 | | |
| 210 | atsK | alkylsulfatase AtsK | PA0193 | | |
| 211 | | sulfatase domain protein | PA2333 | | |
| 212 | sftR | transcriptional regulator SftR | PA2334 | | |
| 213 | sftP | TBDP SftP, sulfate-ester, Receptor | PA2335 optO | | |
| 214 | | HP | PA2336 | | |
| 215 | | HP | | | |
| 216 | | HP | | | |
| 217 | | lipoprotein, putative | PA5403 | | |
| 218 | | CHP | PA5402 | | |
| 249 | dcyD | D-cysteine desulfhydrase | | PSPTO_5179 | |
| 250 | | serine acetyltransferase, putative | | PSEEN0208 | |
| 251 | betT | high-affinity choline transporter, BCCT family, BetT | PA3933 | | |
| 252 | | oxidoreductase, short chain dehydrogenase/reductase family | | PSEEN2587 | |
| 253 | | transcriptional regulator, LysR family | | | |
| 254 | | transcriptional regulator, LacI family | | Pfl01_0249, PSPPH_4858 | |
| 255 | | TBDP, Receptor | PA2335 | Pfl01_0250 | |
| 256 | | nitrilotriacetate monooxygenase component A, NtaA | | Pfl01_0251 | |
| 257 | | L-glyceraldehyde 3-phosphate reductase YqhZ | | Pfl01_0252 | |
| 258 | | auxiliary transport protein, membrane fusion protein (MFP) family | | | |
| 259 | | drug resistance transporter, EmrB/QacA subfamily | | PSEEN2707 | |
| 260 | | transcriptional regulator, AraC family | | | GATAAT |
| 261 | | AMP-binding protein | | | |
| 304 | rfbA | glucose-1-phosphate thymidylyltransferase | PA5163 | | |
| 305 | rfbB | dTDP-glucose 4,6-dehydratase | PA5161 | | |
| 306 | | phosphotransferase family/aminotransferase, class III | | PP4154 | |
| 307 | aguB | N-carbamoylputrescine amidase | PA0293 | | |
| 308 | aguA | agmatine deiminase | PA0292 | | |
| 309 | | HP | | | |
| 310 | | TBDP, Receptor | | PP0267, PSEEN0263 | GATAAT |
| 311 | | CHP | PA0058 | | |
| 312 | | HP | | | |
| 313 | oprQ | outer membrane porin OprE3 | PA2760 | | |
| 314 | | HP | | | |
| 315 | | HP | | | |
| 316 | | TPR domain protein | | | |
| 642 | | CHP | | | |
| 643 | | iron-compound ABC transporter, permease protein | PA2914 | | |
| 644 | | iron compound ABC transporter, iron compound-binding protein | PA2913 | | |
| 645 | | iron compound ABC transporter, ATP-binding protein | PA2912 | | |
| 646 | | TBDP, Receptor | | | |
| 647 | | PepSY-associated membrane protein | PA3789 | | |
| 648 | oprC | TBDP, copper, OprC, Receptor | PA3790 | | |
| 649 | | HP | PA3791 | | |
| 650 | | Protein of unknown function (DUF461) family | PA3785 | | |
| 651 | | HP | PA3786 | | |
| 652 | | HP | | PSEEN3939 | |
| 653 | | HP | | PSEEN3938 | |
| 654 | | Precorrin-6x reductase CbiJ/CobK | PA2909 | | |

# Appendix 1. Continued

| PFL | name | Function | PAO1 homolog | Other homologs | Fur box motif |
|---|---|---|---|---|---|
| 858 | fruR | fructose transport system repressor FruR | PA3563 | | GATAAT |
| 859 | | phosphoenolpyruvate-protein phosphotransferase, EIIA/HPr/EI components | PA3562 | | |
| 860 | fruK | 1-phosphofructokinase | PA3561 | | |
| 861 | fruA | PTS system, fructose-specific IIBC component | PA3560 | | |
| 862 | | PhoD family protein | PA3910 | | |
| 863 | | flavodoxin/oxidoreductase NAD binding domain | PA4513 | | |
| 864 | | TBDP, Receptor | PA4514 piuA | | |
| 865 | | oxidoreductase, 2OG-Fe(II) oxygenase family | PA4515 | | |
| 866 | | Sel1 domain protein | PA4516 | | |
| 867 | | ornithine decarboxylase | PA4519 | | |
| 868 | opuCA | glycine betaine/carnitine/choline ABC transporter, ATP-binding protein | PA3891 | | |
| 869 | opuCB | glycine betaine/carnitine/choline ABC transporter, permease protein | PA3890 | | |
| 870 | opuCC | glycine betaine/L-proline ABC transporter, periplasmic substrate-binding protei | PA3889 | | |
| | | | | | |
| 926 | ttq2F | toluene tolerance protein Ttq2F | PA4451 | | |
| 927 | murA | UDP-N-acetylglucosamine 1-carboxyvinyltransferase | PA4450 | | |
| 928 | his G | ATP phosphoribosyltransferase | | | |
| 929 | hisD | histidinol dehydrogenase | PA4448 | | |
| 930 | hisC | histidinol-phosphate aminotransferase | PA4447 | | |
| 931 | | HP | | PP3326 | |
| 932 | | TBDP, Receptor | PA4837 | | |
| 933 | algW | serine protease AlgW | PA4446 | | |
| 934 | | CHP | PA4445 | | |
| 935 | cysD | sulfate adenylyltransferase, small subunit | PA4443 | | |
| 936 | cysN | sulfate adenylate transferase, large subunit/adenylylsulfate kinase, putative | PA4442 | | |
| 937 | | acyltransferase | PA0834 | | |
| 938 | pta | phosphate acetyltransferase | PA0835 | | |
| | | | | | |
| 979 | sdaA | L-serine ammonia-lyase 2 | | | |
| 980 | | transcriptional regulator, LysR family | | | |
| 981 | | CHP | | | |
| 982 | | TBDP, ferric-citrate, FecA, Transducer | PA3901 fecA | | |
| 983 | | anti-sigma factor, FecR | PA3900 | | |
| 984 | | RNA polymerase sigma factor FacI | PA3899 | | |
| 985 | | HP | PA0800 | | |
| 986 | | PepSY-associated TM helix domain protein | PA0801 | | |
| 987 | | HP | PA0802 | | |
| 988 | | RNA polymerase sigma-70 factor, ECF subfamily | PA0149 | | |
| 989 | | sigma factor regulatory protein, FecR/PupR family | PA0150 | | |
| 990 | | transport energizing protein, ExbD family | | PSPPH 0912 | GATAAT |
| 991 | tonB | TonB domain protein | | PSPPH 0913 | |
| 992 | | TBDP, Receptor | | PSPPH 0914 | |
| 993 | | transporter, MotA/TolQ/ExbB proton channel family | | PSPPH 0915 | |
| 994 | | Ser/Thr protein phosphatase/5'-nucleotidase domain protein | | PP1414 | |
| 995 | | TBDP, Transducer | PA0151 | | |
| 996 | | HP | | | |
| 997 | | acid phosphatase, putative | | | |
| 998 | | outer membrane porin, OprD family | | | |
| 999 | | transcriptional regulator, AraC family | PA3898 | | GATAAT |
| 1000 | | integral membrane protein, DUF6 family | PA3897 | | |
| 1001 | ghrB2 | glyoxylate/hydroxypyruvate reductase B | PA3896 | | |
| | | | | | |
| 1367 | | CobW/P47K family protein | | PSPPH 3929 | |
| 1368 | | HP | | PSPPH 3928 | |
| 1369 | | HP | | | |
| 1370 | | HP | | | |
| 1371 | hxuC | TBDP, heme, Transducer | PA1302 hxuC | | |
| 1372 | | sigma factor regulatory protein, FecR/PupR family | | PSEEN4332 | |
| 1373 | | RNA polymerase sigma-70 factor, ECF subfamily | PA1300 | | |
| 1374 | | rhodanese-like domain protein | PA2603 | | |
| | | | | | |
| 1383 | | renal dipeptidase family protein | | | |
| 1384 | | amine oxidase, flavin-containing | | | |
| 1385 | | endoribonuclease L-PSP family protein | | | |
| 1386 | | TBDP, Receptor | PA2089 | | |
| 1387 | | alkanesulfonate ABC transporter, periplasmic substrate-binding protein | | | |
| 1388 | | HP | | | |
| 1389 | | HP | | | |
| | | | | | |
| 1411 | | FAD dependent oxidoreductase | PA1267 | | |
| 1412 | | proline racemase family protein | PA1268 | | |
| 1413 | | dihydrodipicolinate synthetase family protein | PA1254 | | |
| 1414 | | aspartate:proton symporter YveA | | PP1259 | |
| 1415 | aldH | NADP-dependent fatty aldehyde dehydrogenase | | | |
| 1416 | | malate/L-lactate dehydrogenase family protein | PA1252 | | |
| 1417 | | TBDP, Receptor | | | |
| 1418 | | acetyltransferase, GNAT family | | | |
| 1419 | | HP | | | |
| 1420 | | HP | | Pfl01 3187 | |
| 1421 | | HP | | | |
| 1422 | | HP | | | |
| 1423 | | HP | | | |

# Appendix 1. Continued

| PFL | name | Function | PAO1 homolog | Other homologs | Fur box motif |
|---|---|---|---|---|---|
| 1737 | fabA | 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase | | | |
| 1738 | | sensor histidine kinase/response regulator | | | |
| 1739 | | HP | | | |
| 1740 | | TBDP, Receptor | PA2070 | | |
| 1741 | gpsA | glycerol-3-phosphate dehydrogenase | | | |
| 1742 | | HP | | | |
| 1743 | sixA | phosphohistidine phosphatase SixA | | | |
| | | | | | |
| 2237 | | MASE2 domain/diguanylate cyclase domain protein | | | |
| 2238 | | iron compound ABC transporter, iron compound-binding protein, putative | | | |
| 2239 | | HP | | | |
| 2240 | | TBDP, Receptor | PA2289 | | |
| 2241 | astA | arginine N-succinyltransferase, beta subunit | | | |
| 2242 | astA | arginine N-succinyltransferase, alpha subunit | | | |
| 2243 | | amino acid transporter, AAT family | | | |
| | | | | | |
| 2288 | | phenazine biosynthesis protein, PhzF family | PA1367 | | |
| 2289 | | HP | | | |
| 2290 | hemB | delta-aminolevulinic acid dehydratase | | Pfl01 3751 | |
| 2291 | | RNA polymerase sigma factor FecI | PA1912 | | TAAGTAATAG |
| 2292 | | sigma factor regulatory protein FecR | | | |
| 2293 | | TBDP, Transducer | PA4221 fptA | | |
| 2294 | | transcriptional regulator, LysR family | | | |
| 2295 | | oxidoreductase, short-chain dehydrogenase/reductase family | | | |
| 2296 | | FAD dependent oxidoreductase | | | |
| | | | | | |
| 2361 | | phosphotransferase family/peptidase, M23 family/aminotransferase, class III | | PP3361 | |
| 2362 | | transcriptional regulator, AsnC family | | PP3362 | |
| 2363 | | RNA polymerase sigma-70 factor, ECF subfamily | | Pfl01 3796 | TAATAGTAAT |
| 2364 | | sigma factor regulatory protein, FecR/PupR family | | PSPPH 2748 | |
| 2365 | | TBDP, Transducer | PA4897 optI | | Fur box |
| 2366 | | lipoprotein, putative | PA2581 | | |
| 2367 | | HP | PA1906 | | |
| 2368 | | HP | | | |
| | | | | | |
| 2388 | | membrane protein, putative | | | |
| 2389 | | HP | | | |
| 2390 | | hydrolase, NUDIX family | | PSPPH 2763 | |
| 2391 | fpvU | TBDP, Transducer | PA2398 fpvA | | |
| 2392 | | sigma factor regulatory protein, FecR/PupR family | | | |
| 2393 | | RNA polymerase sigma-70 family protein | | | |
| 2394 | | HP | | | |
| | | | | | |
| 2524 | | radical SAM domain protein | | Pfl01 3469 | |
| 2525 | | HP | | Pfl01 3468 | |
| 2526 | | HP | | | |
| 2527 | fpvV | TBDP Transducer | | PSEEN2529 | |
| 2528 | pupR | sigma factor regulatory protein PupR | | | |
| 2529 | pupI | RNA polymerase sigma-70 factor, ECF subfamily | | | |
| 2530 | ilvE | branched-chain amino acid aminotransferase | PA5013 | | |
| 2531 | bkdC | 2-oxoisovalerate dehydrogenase E3 component, dihydrolipoyl dehydrogenase | PA2250 | PA14 35490 | |
| | | | | | TAATAG |
| 2601 | | HP | | | |
| 2602 | | HP | PA1968 | | |
| 2603 | | ABC transporter, quaternary amine uptake transporter (QAT) family, substrate- | PA5103 | | |
| 2604 | | TBDP, Receptor | | Pfl01 3318 | |
| 2605 | | HP | | | |
| 2606 | phaG | probable K(+)/H(+) antiporter subunit G | PA1059 | | |
| 2607 | phaF | probable K(+)/H(+) antiporter subunit F | PA1058 | | |
| | | | | | |
| 2660 | | TRAP transporter, DctM subunit | PA0886 | | |
| 2661 | mag1 | DNA-3-methyladenine glycosylase | | | |
| 2662 | | Putative esterase superfamily | PA2689 | | |
| 2663 | | TBDP PfeA, Receptor | PA2688, 0931 pirA | | AGTAATAGTAAG |
| 2664 | pfeS | sensor protein PfeS | PA2687 | | |
| 2665 | pfeR | transcriptional activator PfeR | PA2686 | | |
| 2666 | | YheO-like PAS domain protein | | | |
| | | | | | |
| 2766 | | HP | | | |
| 2767 | | FAD linked oxidase domain protein | PA3026 | | |
| 2768 | | FAD dependent oxidoreductase | PA3025 | | |
| 2769 | | carbohydrate kinase, FGGY family | PA3024 | | |
| 2770 | | HP | | | |
| 2771 | | HP | | | |
| 2772 | | TBDP, Receptor | PA2289 | PP4137 | |
| 2773 | | HP | | | |
| 2774 | | HP | | | |
| 2775 | | beta-lactamase | | PSPTO 2834 | |
| 2776 | | HP | | | |
| 2777 | | Ypar31 protein | | | |
| 2778 | | amidase family protein | PA4163 | | |

# Appendix 1. Continued

| PFL | name | Function | PAO1 homolog | Other homologs | Fur box motif |
|---|---|---|---|---|---|
| 2912 | | betaine aldehyde dehydrogenase, putative | | PP0708 | |
| 2913 | | alcohol dehydrogenase, iron-containing | PA1146 | | |
| 2914 | | amino acid permease, APC family | PA1147 | | |
| 2915 | | transcriptional regulator, LysR family | | PSPPH 3618 | |
| 2916 | | transporter, major facilitator family | | | |
| 2917 | | oxidoreductase membrane protein, FAD-binding | PA0545 | | |
| 2918 | | monooxygenase, SsuD family | PA2598 | | |
| 2919 | | TBDP, Receptor | | | |
| 2920 | | HP | | | |
| 2921 | | bacterial extracellular solute-binding proteins, family 5 | | | |
| 2922 | | monooxygenase, SsuD family | | | |
| 2923 | | HP | | PSEEN3419 | |
| | | | | | |
| 2967 | | HP | | | |
| 2968 | | HP | | | |
| 2969 | | HP | | | |
| 2970 | | TBDP, Receptor | PA2911 | | |
| 2971 | | NAD-dependent epimerase/dehydratase family protein | | | TAATAGAAC |
| 2972 | | HP | PA3064 PelA | PA14 24480 | |
| 2973 | | HP | PA3063 PelB | PA14 24490 | |
| | | | | | |
| 3151 | | ATPase, AFG1 family | PA2353 | Pfl01 2579 | |
| 3152 | | HP | | Pfl01 2578 | |
| 3153 | | DNA-binding protein | | Pfl01 2577 | |
| 3154 | | TBDP, Transducer | PA4675 iutA/chtA | | |
| 3155 | | sigma factor regulatory protein, FecR/PupR family | | PP2192 | |
| 3156 | | RNA polymerase sigma-70 factor, ECF subfamily | | | |
| 3157 | yqfO | high-affinity xanthine transporter, NCS2 family, YqfO | | Pfl01 2573 | |
| | | | | | |
| 3170 | | ABC transporter, ATP-binding protein | | PSEEN3426 | |
| 3171 | | ABC transporter, ATP-binding protein | | PSEEN3427 | |
| 3172 | | monooxygenase, SsuD family | | PSEEN3428 | |
| 3173 | | monooxygenase, putative | | PSEEN3429 | |
| 3174 | | monooxygenase, putative | | PSEEN3430 | |
| 3175 | | HP | | | |
| 3176 | | TBDP, Receptor | PA2089 | PSEEN3436 | |
| 3177 | | TBDP OptO, Receptor | PA2335 optO | PSEEN3437 | |
| 3178 | | ABC transporter, permease protein | | PSEEN3438 | |
| 3179 | | ABC transporter, permease protein | | PSEEN3439 | |
| 3180 | | ABC transporter, ATP-binding protein | | PSEEN3440 | |
| 3181 | | putative nitrate/nitrite/cyanate ABC transporter, NitT family, permease protein | PA2327 | | |
| 3182 | | putative nitrate/nitrite/cyanate ABC transporter, NitT family, periplasmic nitrate | PA2328 | | |
| | | | | | |
| 3312 | | phosphotransferase system, EIIC domain/cyclic diguanylate phosphodiesterase (EAL) domain protein | | | |
| 3313 | | RNA polymerase sigma-70 factor, ECF subfamily | | | |
| 3314 | | sigma factor regulatory protein, FecR/PupR family | | | |
| 3315 | fpvX | TBDP, Transducer | | | |
| 3316 | | 3-demethylubiquinone-9 3-methyltransferase domain protein | PA2721 | | |
| 3317 | | transcriptional regulator, AraC family | | | |
| 3318 | | translocator protein, LysE family | | | |
| | | | | | |
| 3482 | | transcriptional regulator, MarR family/acetyl transferase, GNAT family | | | GATAAT |
| 3483 | | RNA polymerase sigma-70 factor, ECF subfamily | | | |
| 3484 | | sigma factor regulatory protein, putative | | | |
| 3485 | | TBDP, Transducer | | | |
| 3486 | | HP | | Pfl01 1951 | |
| 3487 | | HP | | | |
| 3488 | pchA | isochorismate synthase | PA4231 | | |
| | | | | | |
| 3495 | pchH | ABC transporter, ATP-binding/permease protein | PA4223 | | |
| 3496 | pchD | salicyl-AMP ligase | PA4228 | | |
| 3497 | | regulatory protein Pchr | PA4227 | | TAAAGGATAAT |
| 3498 | fetA | TBDP FetA, Receptor | | PSEEN3225 | |
| 3499 | | PepSY-associated membrane protein | | | |
| 3500 | | iron-chelate uptake ABC transporter, FeCT family, periplasmic iron-chelate-binding protein, puta | | Pmen 0797 | |
| 3501 | | iron-chelate uptake ABC transporter, FeCT family, permease protein | | Pmen 0798 | |
| | | | | | |
| 3609 | | flavin reductase like domain protein | | PSEEN3104 | |
| 3610 | | RNA polymerase sigma-70 family protein | | | TGATAATCA |
| 3611 | | sigma factor regulatory protein, FecR/PupR family | | | |
| 3612 | | TBDP, Transducer | | | |
| 3613 | | Di-haem cytochrome c peroxidase family protein | | | |
| 3614 | | phosphoenolpyruvate-dependent sugar phosphotransferase system, EIIA 2 | | | |
| 3615 | | efflux transporter, outer membrane factor lipoprotein, NodT family | | | |
| 3616 | | efflux transporter, RND family, MFP subunit | | | |
| 3617 | | RND transporter, hydrophobe/amphiphile efflux-1 (HAE1) family | | | |
| 3618 | | molybdenum-pterin-binding protein | | | |
| 3619 | | monooxygenase, NtaA/SnaA/SoxA family | PA4155 | | |
| 3620 | | TBDP ferric vibriobactin, Receptor | PA4156 | | |
| 3621 | | transcriptional regulator, IclR family | PA4157 | | |
| 3622 | fepC | ferric enterobactin ABC transporter, ATP-binding protein FepC | PA4158 | | |
| 3623 | fepB | ferric enterobactin ABC transporter, periplasmic ferric enterobactin-binding pro | PA4159 | | GTAATAGTATAC |
| | | | | | |
| 3709 | | oxidoreductase, FAD-linked | PA5327 | | |
| 3710 | | HP | PA5328 | | |
| 3711 | tonB | tonB protein, putative | PA5531 | PSPPH 2987 | Fur box |
| 3712 | tolR | tolR protein | | | |
| 3713 | tolQ | tolQ protein | | | |
| 3714 | | 3-phytase family protein | | | |
| 3715 | | TBDP, Receptor | | | |
| 3716 | | diguanylate cyclase (GGDEF) domain protein | | | |
| 3717 | | transcriptional regulator, AraC family | | | |
| 3718 | | aldehyde dehydrogenase (NAD) family protein | PA2323 | | |
| 3719 | | membrane protein, putative | | PSPTO 0520 | |
| 3720 | moeA | molybdopterin biosynthesis MoeA protein | | | |
| 3721 | moaB | molybdenum cofactor biosynthesis protein B | | PSEEN4045 | TAATAG |

## Appendix 1. Continued

| PFL | name | Function | PAO1 homolog | Other homologs | Fur box motif |
|---|---|---|---|---|---|
| 3832 | | HP | | | |
| 3833 | | HP | | | |
| 3834 | | HP | | | |
| 3835 | | TBDP UfrA, Receptor | PA1910 ufrA | | |
| 3836 | | oxidoreductase, FAD-binding | | | |
| 3837 | | transcriptional regulator, LysR family | | | |
| 3838 | | transporter, major facilitator family | | | |
| 4036 | | transcriptional regulator, LysR family | | | GAGATAATCA |
| 4037 | | transcriptional regulator, AsnC family | | | |
| 4038 | | transporter, major facilitator family | PA3532 | | |
| 4039 | | TBDP FecA-like, Transducer | PA3268 | | |
| 4040 | | sigma factor regulatory protein, FecR/PupR family | | | |
| 4041 | | RNA polymerase sigma-70 factor, ECF subfamily | | | |
| 4042 | | HP | | | |
| 4060 | | putative membrane protein | | | |
| 4061 | | HP | | | GTAATAGT |
| 4062 | | HP | PA0435 | | |
| 4063 | | TBDP, Receptor | PA0434 | | |
| 4064 | | HP | PA0433 | | |
| 4065 | ndvB | NdvB protein | | | |
| 4066 | | transcriptional regulator-related protein | | | |
| 4089 | pvdO | protein of unknown function DUF323 | PA2395 | | |
| 4090 | pvdF | N(5)-hydroxyornithine transformylase PvdF | PA2396 | | |
| 4091 | pvdE | pyoverdine ABC export system, permease/ATP-binding protein | PA2397 | | |
| 4092 | | TBDP, Transducer | PA4168 fpvB | | |
| 4093 | | NRPS | PA2402 | | |
| 4094 | | NRPS | PA2399 | | |
| 4095 | | NRPS | | | |
| 4624 | mgsA | methylglyoxal synthase | | Pmen 1310 | |
| 4625 | | RNA polymerase sigma-70 factor, ECF subfamily | | Pfl01 4377 | GATAAT |
| 4626 | | sigma factor regulatory protein, FecR/PupR family | | Pfl01 4378 | |
| 4627 | phuR | TBDP, PhuR, Transducer | PA4710 phuR | Pfl01 4379 | |
| 4628 | hemO | heme oxygenase | PA0672 | Pfl01 4380 | |
| 4629 | | HP | | Pfl01 4381 | |
| 4630 | | methyl-accepting chemotaxis protein | | Pfl01 4382 | |
| 4906 | | putative proline-specific permease, ProY family | PA0789 | PSEEN1179 | |
| 4907 | | HP | | | |
| 4908 | | transcriptional regulator, PadR family | | PSEEN1177 | |
| 4909 | | siderophore-interacting protein family protein | | PSEEN1176 | |
| 4910 | | membrane protein, putative | | PSEEN1175 | |
| 4911 | | HP | | PSEEN1174 | |
| 4912 | | TBDP, Receptor | PA0781 | | |
| 4913 | | transcriptional regulator, GntR family | | | ATATAATAGATA |
| 4914 | | benzoate transport protein | | PSEEN3859 | |
| 4915 | oprB2 | carbohydrate-selective porin OprB | | PSEEN1171 | |
| 4916 | gcd | quinoprotein glucose dehydrogenase | PA2290 | | |
| 4917 | | HP | | | |
| 4918 | | lipoprotein, putative | | | |
| 5166 | | ribosomal protein L25, Ctc-form | PA4671 | PSPTO 1103 | |
| 5167 | pth | peptidyl-tRNA hydrolase | PA4672 | PSPTO 1102 | |
| 5168 | ychF | GTP-binding protein YchF | PA4673 | PSPTO 1101 | |
| 5169 | | TBDP, Receptor | | PSPTO 0784 | |
| 5170 | pobA | 4-hydroxybenzoate 3-monooxygenase | PA0247 | PSPTO 1907 | |
| 5171 | pobR | transcriptional regulator PobR | PA0248 | PSPTO 1908 | |
| 5172 | fsr | fosmidomycin resistance protein | | PSPTO 3799 | |
| 5375 | hasE | type 1 secretion system membrane fusion protein HasE | PA3405 | | |
| 5376 | hasD | type 1 secretion system ATPase HasD | PA3406 | | |
| 5377 | hasAp | heme acquisition protein HasAp | PA3407 | | |
| 5378 | hasR | TBDP HasR, Transducer | PA3408 hasR | | |
| 5379 | hasS | sigma factor regulatory protein HasS | PA3409 | | |
| 5380 | hasI | RNA polymerase sigma-70 factor, ECF subfamily | PA3410 | | |
| 5381 | aroQ | 3-dehydroquinate dehydratase, type II | PA0245 | | |
| 5508 | xseB | exodeoxyribonuclease VII, small subunit | | | |
| 5509 | ispA | geranyltranstransferase | | | |
| 5510 | dxs | 1-deoxy-D-xylulose-5-phosphate synthase | | | |
| 5511 | btuB | TBDP, B12, BtuB, Receptor | PA1271 | | |
| 5512 | | periplasmic cobalamin-binding protein | | | |
| 5513 | | HP | | | |
| 5514 | ribA | GTP cyclohydrolase II | | | TAATAG |
| 5703 | dnaQ | exonuclease | PA3232 | | |
| 5704 | | RNA polymerase sigma-70 family protein | PA0472 | | |
| 5705 | | sigma factor regulatory protein, FecR/PupR family | PA0471 | | |
| 5706 | | TBDR FiuA, Transducer | PA0470 fiuA | | |
| 5707 | | PepSY-associated membrane protein | PA1909 | | |
| 5708 | | HP | | Pfl01 5191 | |
| 5709 | | HP | | | |

**Appendix 2: Reciprocal BLASTP analysis of the *P. fluorescens* group.** Color scale applied to the % amino acid ID values goes from red for the most similar to green for the least similar.

### A. *P. fluorescens* Pf-5

| Pf-5 | A506 | | | 30-84 | | | O-6 | | |
|---|---|---|---|---|---|---|---|---|---|
| Locus tag | | M.W. | % ID | | M.W. | % ID | | M.W. | % ID |
| PFL_0125 ferrioxamine | PflA506_0139 | 88.8 | 36 | Pchl3084_0129 | 90.6 | 82 | PchlO6_0126 | 91.2 | 81 |
| PFL_0147 ferrichrome | PflA506_0139 | | 73 | Pchl3084_0129 | | 38 | PchlO6_0126 | | 38 |
| PFL_0213 Sulphate | PflA506_0217 | 82.9 | 88 | Pchl3084_0224 | 85.2 | 90 | PchlO6_0225 | | 90 |
| PFL_0255 | PflA506_3446 | 76 | 88 | Pchl3084_0273 | 81.9 | 87 | PchlO6_0225 | 85.2 | 32 |
| PFL_0310 | PflA506_0292 | 77 | 79 | Pchl3084_0320 | 77.4 | 80 | PchlO6_0324 | 77.5 | 80 |
| PFL_0646 | PflA506_3017 | 78.1 | 30 | Pchl3084_0627 | 79 | 77 | PchlO6_0633 | 78 | 77 |
| PFL_0648 copper | PflA506_0576 | 74 | 88 | Pchl3084_0629 | 76.9 | 87 | PchlO6_0635 | 77 | 87 |
| PFL_0864 | PflA506_0798 | | 82 | Pchl3084_0855 | 82 | 84 | PchlO6_0861 | 82.1 | 84 |
| PFL_0932 | PflA506_0745 | 76.6 | 81 | Pchl3084_3174 | 77.8 | 43 | PchlO6_0324 | | 30 |
| PFL_0982 citrate | PflA506_1022 | 84.9 | 87 | Pchl3084_0981 | 85.7 | 86 | PchlO6_0989 | 85.5 | 86 |
| PFL_0992 | PflA506_1030 | 82.5 | 76 | Pchl3084_3848 | 78.1 | 23 | PchlO6_4138 | 78.2 | 23 |
| PFL_0995 | PflA506_1001 | 87.1 | 77 | Pchl3084_3848 | 78.1 | 42 | PchlO6_4138 | 78.2 | 42 |
| PFL_1371 heme HxuC | PflA506_1363 | 91.6 | 64 | Pchl3084_1354 | 95.5 | 85 | PchlO6_1431 | 95.5 | 85 |
| PFL_1386 | PflA506_5184 | 86 | 38 | Pchl3084_2164 | 97.7 | 25 | PchlO6_3258 | 89.6 | 47 |
| PFL_1417 | PflA506_0798 | | 42 | Pchl3084_0855 | | 42 | PchlO6_0861 | | 42 |
| PFL_1740 | PflA506_1861 | 77.3 | 79 | Pchl3084_1705 | 77.6 | 84 | PchlO6_1837 | 77.6 | 84 |
| PFL_2240 | PflA506_4759 | | 25 | Pchl3084_3848 | | 24 | PchlO6_4138 | | 24 |
| PFL_2293 pyoverdine | PflA506_1983 | 90.4 | 55 | Pchl3084_3355 | 89.3 | 68 | PchlO6_3657 | 89.2 | 68 |
| PFL_2365 heme | PflA506_4277 | 90.3 | 32 | Pchl3084_2211 | 103.9 | 82 | PchlO6_2435 | 104 | 82 |
| PFL_2391 pyoverdine | PflA506_3089 | 87.6 | 70 | Pchl3084_2488 | 89.2 | 41 | PchlO6_4229 | 91.1 | 38 |
| PFL_2527 pyoverdine | PflA506_3089 | | 36 | Pchl3084_2488 | | 36 | PchlO6_4229 | | 33 |
| PFL_2604 | PflA506_3017 | | 81 | Pchl3084_2407 | 78.7 | 82 | PchlO6_2626 | 78.7 | 81 |
| PFL_2663 enterobactin | PflA506_0808 | 81.3 | 50 | Pchl3084_2974 | 72.3 | 28 | PchlO6_5540 | | 22 |
| PFL_2772 | PflA506_3139 | 77.3 | 61 | Pchl3084_2519 | 77.2 | 82 | PchlO6_2744 | 77.5 | 83 |
| PFL_2919 | PflA506_0798 | 81.3 | 23 | Pchl3084_0709 | 94.3 | 22 | PchlO6_0716 | 94.5 | 22 |
| PFL_2970 | PflA506_3017 | | 31 | Pchl3084_0627 | | 61 | PchlO6_0633 | | 62 |
| PFL_3154 aerobactin | PflA506_3811 | 77.9 | 29 | Pchl3084_3950 | 91.2 | 25 | PchlO6_4229 | | 22 |
| PFL_3176 | PflA506_3446 | | 30 | Pchl3084_0273 | | 30 | PchlO6_3255 | 81 | 29 |
| PFL_3177 | PflA506_5184 | | 38 | Pchl3084_2164 | | 26 | PchlO6_3670 | 86.2 | 37 |
| PFL_3315 pyoverdine | PflA506_3089 | | 41 | Pchl3084_2488 | | 55 | PchlO6_3015 | 82.6 | 41 |
| PFL_3485 pyoverdine | PflA506_3089 | | 52 | Pchl3084_2488 | | 38 | PchlO6_3555 | 74.6 | 39 |
| PFL_3498 enantio-pch | PflA506_0139 | 88.8 | 41 | Pchl3084_5442 | | 39 | PchlO6_5726 | | 39 |
| PFL_3612 | PflA506_1995 | 86.6 | 30 | Pchl3084_3828 | 88.4 | 30 | PchlO6_4739 | 94.3 | 22 |
| PFL_3620 | PflA506_3446 | | 20 | Pchl3084_0273 | | 21 | PchlO6_3255 | | 25 |
| PFL_3715 | PflA506_2346 | 92.5 | 85 | Pchl3084_3605 | 93.6 | 86 | PchlO6_0861 | | 25 |
| PFL_3835 | PflA506_2311 | 78.2 | 74 | Pchl3084_3659 | 79.7 | 80 | PchlO6_3952 | 80 | 81 |
| PFL_4039 citrate | PflA506_1995 | | 77 | Pchl3084_3828 | | 86 | PchlO6_0989 | | 45 |
| PFL_4063 | PflA506_3017 | | 55 | Pchl3084_2407 | | 57 | PchlO6_2626 | | 58 |
| PFL_4092 pyoverdine | PflA506_2859 | 78.8 | 47 | Pchl3084_3950 | | 83 | PchlO6_4229 | | 83 |
| PFL_4627 heme PhuR | PflA506_4277 | | 76 | Pchl3084_4498 | 94.3 | 74 | PchlO6_4739 | | 74 |
| PFL_4912 | PflA506_1059 | 71.9 | 82 | Pchl3084_4710 | 72.8 | 86 | PchlO6_4957 | 72.9 | 85 |
| PFL_5169 | PflA506_0808 | | 31 | Pchl3084_2974 | | 31 | PchlO6_5540 | | 25 |
| PFL_5378 heme HasR | PflA506_4277 | | 28 | Pchl3084_1354 | | 25 | PchlO6_4739 | | 28 |
| PFL_5511 B12 | PflA506_4759 | 69.5 | 68 | Pchl3084_5255 | 70.3 | 71 | PchlO6_5540 | 70.3 | 69 |
| PFL_5706 ferrichrome | PflA506_4932 | 88.2 | 87 | Pchl3084_5442 | 88.1 | 94 | PchlO6_5726 | 88 | 94 |
| | | 86.56 | | | 90.39 | | | 88.56 | |

## A. *P. fluorescens* Pf-5, Continued

| Pf-5 | Q8r1-96 | | | Q2-87 | | | BG33R | | |
|---|---|---|---|---|---|---|---|---|---|
| Locus tag | | M.W. | % ID | | M.W. | % ID | | M.W. | % ID |
| PFL_0125 ferrioxamine | PflQ8_0202 | 91.6 | 81 | PflQ2_0538 | 88.7 | 36 | PseBG33_5128 | 88.4 | 36 |
| PFL_0147 ferrichrome | PflQ8_5352 | 88.8 | 38 | PflQ2_0538 | | 38 | PseBG33_0163 | 88.9 | 72 |
| PFL_0213 Sulphate | PflQ8_0935 | | 35 | PflQ2_4530 | | 36 | PseBG33_0251 | 83 | 88 |
| PFL_0255 | PflQ8_0935 | 86.9 | 30 | PflQ2_4530 | 86.6 | 31 | PseBG33_3630 | 79.7 | 88 |
| PFL_0310 | PflQ8_4827 | 77.5 | 32 | PflQ2_3112 | 78.1 | 27 | PseBG33_0325 | 76.8 | 79 |
| PFL_0646 | PflQ8_2798 | | 26 | PflQ2_2980 | | 26 | PseBG33_0610 | | 72 |
| PFL_0648 copper | PflQ8_0637 | 77 | 85 | PflQ2_5073 | 76.8 | 84 | PseBG33_0612 | | 83 |
| PFL_0864 | PflQ8_3242 | 78.3 | 27 | PflQ2_3112 | | 26 | PseBG33_0836 | 80.6 | 82 |
| PFL_0932 | PflQ8_4827 | | 84 | PflQ2_3112 | | 46 | PseBG33_0782 | 76.5 | 81 |
| PFL_0982 citrate | PflQ8_5352 | | 21 | PflQ2_1695 | 88.5 | 43 | PseBG33_1061 | 84.9 | 87 |
| PFL_0992 | PflQ8_2798 | | 22 | PflQ2_2980 | | 22 | PseBG33_1069 | 82.6 | 77 |
| PFL_0995 | PflQ8_2798 | 77.6 | 42 | PflQ2_2980 | 74.7 | 42 | PseBG33_1043 | 87.2 | 75 |
| PFL_1371 heme HxuC | PflQ8_1239 | 94.1 | 23 | PflQ2_4231 | 94.3 | 23 | PseBG33_1530 | 93.9 | 64 |
| PFL_1386 | PflQ8_5164 | | 28 | PflQ2_2928 | 89.9 | 46 | PseBG33_3617 | 85.9 | 44 |
| PFL_1417 | PflQ8_3242 | | 27 | PflQ2_3112 | | 26 | PseBG33_0836 | | 42 |
| PFL_1740 | PflQ8_4192 | 78.9 | 81 | PflQ2_1453 | 78.9 | 82 | PseBG33_1898 | 77.6 | 79 |
| PFL_2240 | PflQ8_5164 | | 24 | PflQ2_4902 | | 25 | PseBG33_4946 | | 25 |
| PFL_2293 pyoverdine | PflQ8_2057 | 89.7 | 68 | PflQ2_1672 | 89.8 | 38 | PseBG33_2016 | 90.2 | 55 |
| PFL_2365 heme | PflQ8_1239 | | 33 | PflQ2_4231 | | 31 | PseBG33_4831 | 95.9 | 26 |
| PFL_2391 pyoverdine | PflQ8_3550 | 79.7 | 36 | PflQ2_0688 | 80 | 37 | PseBG33_2445 | 88.4 | 70 |
| PFL_2527 pyoverdine | PflQ8_3550 | | 35 | PflQ2_0688 | | 35 | PseBG33_2445 | | 34 |
| PFL_2604 | PflQ8_4827 | | 25 | PflQ2_3112 | | 24 | PseBG33_2513 | 78.2 | 81 |
| PFL_2663 enterobactin | PflQ8_0637 | | 21 | PflQ2_4902 | | 22 | PseBG33_0847 | 81.3 | 51 |
| PFL_2772 | PflQ8_2057 | | 20 | PflQ2_1695 | | 20 | PseBG33_3265 | 77.6 | 62 |
| PFL_2919 | PflQ8_2798 | 77.6 | 24 | PflQ2_2980 | | 25 | PseBG33_0610 | 77.4 | 25 |
| PFL_2970 | PflQ8_0202 | | 24 | PflQ2_2980 | | 27 | PseBG33_0610 | | 60 |
| PFL_3154 aerobactin | PflQ8_1239 | | 22 | PflQ2_4231 | | 22 | PseBG33_0163 | | 23 |
| PFL_3176 | PflQ8_0935 | | 29 | PflQ2_4530 | | 30 | PseBG33_3630 | | 30 |
| PFL_3177 | PflQ8_4192 | | 22 | PflQ2_2928 | | 35 | PseBG33_5403 | 85.9 | 38 |
| PFL_3315 pyoverdine | PflQ8_3550 | | 37 | PflQ2_0688 | | 37 | PseBG33_2445 | | 39 |
| PFL_3485 pyoverdine | PflQ8_2057 | | 34 | PflQ2_0688 | | 35 | PseBG33_2445 | | 54 |
| PFL_3498 enantio-pch | PflQ8_5352 | | 42 | PflQ2_0538 | | 42 | PseBG33_0163 | | 41 |
| PFL_3612 | PflQ8_1239 | | 20 | PflQ2_1695 | | 29 | PseBG33_2445 | 88.4 | 29 |
| PFL_3620 | PflQ8_2430 | 75.9 | 28 | PflQ2_2925 | 78.8 | 23 | PseBG33_2355 | 74.3 | 22 |
| PFL_3715 | PflQ8_3000 | 93.1 | 85 | PflQ2_4231 | | 22 | PseBG33_0836 | | 25 |
| PFL_3835 | PflQ8_2987 | 87.2 | 27 | PflQ2_2980 | | 26 | PseBG33_3219 | 79.1 | 74 |
| PFL_4039 citrate | PflQ8_3969 | 87.9 | 21 | PflQ2_1695 | | 83 | PseBG33_2027 | 86.8 | 77 |
| PFL_4063 | PflQ8_3242 | | 22 | PflQ2_3112 | | 22 | PseBG33_2513 | | 55 |
| PFL_4092 pyoverdine | PflQ8_3550 | | 46 | PflQ2_0688 | | 47 | PseBG33_2654 | 87.3 | 42 |
| PFL_4627 heme PhuR | PflQ8_1239 | | 74 | PflQ2_4231 | | 73 | PseBG33_4466 | 91.6 | 76 |
| PFL_4912 | PflQ8_1239 | | 26 | PflQ2_1695 | | 26 | PseBG33_1099 | 71.8 | 82 |
| PFL_5169 | PflQ8_5164 | | 24 | PflQ2_4902 | | 23 | PseBG33_0847 | | 31 |
| PFL_5378 heme HasR | PflQ8_1239 | | 27 | PflQ2_4231 | | 27 | PseBG33_4831 | 96 | 76 |
| PFL_5511 B12 | PflQ8_5164 | 70.2 | 67 | PflQ2_4902 | 70.3 | 66 | PseBG33_4946 | 69.7 | 68 |
| PFL_5706 ferrichrome | PflQ8_5352 | | 66 | PflQ2_0538 | | 66 | PseBG33_5128 | | 68 |
| | | 87.05 | | | 86 | | | 89.8 | |

## A. *P. fluorescens* Pf-5, Continued

| Pf-5 | ss101 | | | Pf0-1 | | P. putida GB-1 | | SBW25 | | WH6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus tag | | M.W. | % ID | Gene | % ID | Gene | % ID | Gene | %ID | Gene | %ID |
| PFL_0125 ferrioxamine | PflSS101_4985 | 88.2 | 36 | Pfl01_0121 | 86 | PputGB1_0178 | 71 | PFLU2598 | 39 | PFWH6_5394 | 35 |
| PFL_0147 ferrichrome | PflSS101_0139 | 88.9 | 72 | Pfl01_5189 | 38 | PputGB1_3322 | 46 | PFLU2598 | 42 | PFWH6_5394 | 37 |
| PFL_0213 Sulphate | PflSS101_0233 | 83 | 88 | Pfl01_0216 | 90 | PputGB1_0235 | 74 | PFLU0206 | 93 | PFWH6_2193 | 21 |
| PFL_0255 | PflSS101_3463 | 75.9 | 88 | Pfl01_0250 | 86 | PputGB1_0235 | 32 | PFLU4085 | 90 | PFWH6_2193 | 24 |
| PFL_0310 | PflSS101_0311 | 76.9 | 79 | Pfl01_0293 | 81 | PputGB1_0293 | 75 | PFLU0295 | 80 | PFWH6_0295 | 79 |
| PFL_0646 | PflSS101_2915 | 77.9 | 29 | Pfl01_0594 | 81 | PputGB1_2060 | 62 | PFLU0593 | 77 | PFWH6_0295 | 25 |
| PFL_0648 copper | PflSS101_0606 | 73.9 | 89 | Pfl01_0596 | 87 | PputGB1_4894 | 81 | PFLU0595 | 88 | PFWH6_0621 | 82 |
| PFL_0864 | PflSS101_0832 | | 83 | Pfl01_0798 | 66 | PputGB1_0904 | 65 | PFLU0295 | 29 | PFWH6_5214 | 28 |
| PFL_0932 | PflSS101_0775 | 76.6 | 81 | Pfl01_0874 | 87 | PputGB1_0293 | 33 | PFLU0757 | 83 | PFWH6_0820 | 25 |
| PFL_0982 citrate | PflSS101_1061 | 84.9 | 87 | Pfl01_0923 | 88 | PputGB1_4596 | 78 | PFLU1040 | 89 | PFWH6_1047 | 87 |
| PFL_0992 | PflSS101_1069 | 82.4 | 76 | Pfl01_0923 | 23 | PputGB1_4761 | 27 | PFLU1022 | 32 | PFWH6_1796 | 57 |
| PFL_0995 | PflSS101_1043 | 87.2 | 75 | Pfl01_0931 | 77 | PputGB1_4588 | 69 | PFLU1022 | 78 | PFWH6_1030 | 75 |
| PFL_1371 heme HxuC | PflSS101_1406 | 93.7 | 64 | Pfl01_4379 | 24 | PputGB1_1005 | 23 | PFLU1405 | 68 | PFWH6_1417 | 65 |
| PFL_1386 | PflSS101_3450 | 85.9 | 44 | Pfl01_2342 | 38 | PputGB1_2657 | 48 | PFLU4069 | 47 | PFWH6_3831 | 43 |
| PFL_1417 | PflSS101_0832 | | 42 | Pfl01_0798 | 42 | PputGB1_0904 | 43 | PFLU0295 | 30 | PFWH6_0820 | 41 |
| PFL_1740 | PflSS101_1822 | 77.4 | 80 | Pfl01_4209 | 82 | PputGB1_3742 | 72 | PFLU1839 | 81 | PFWH6_1770 | 79 |
| PFL_2240 | PflSS101_2030 | 71.2 | 74 | Pfl01_5008 | 25 | PputGB1_0571 | 27 | PFLU5463 | 26 | PFWH6_5214 | 26 |
| PFL_2293 pyoverdine | PflSS101_1938 | 90.4 | 55 | Pfl01_1848 | 41 | PputGB1_1585 | 56 | PFLU5798 | 40 | PFWH6_0820 | 23 |
| PFL_2365 heme | PflSS101_3593 | 104 | 78 | Pfl01_4379 | 31 | PputGB1_1523 | 32 | PFLU5361 | 27 | PFWH6_4738 | 31 |
| PFL_2391 pyoverdine | PflSS101_3096 | 88.2 | 57 | Pfl01_2583 | 40 | PputGB1_0693 | 68 | PFLU2545 | 72 | PFWH6_1926 | 35 |
| PFL_2527 pyoverdine | PflSS101_3096 | | 34 | Pfl01_2462 | 39 | PputGB1_0692 | 67 | PFLU3633 | 42 | PFWH6_3086 | 34 |
| PFL_2604 | PflSS101_2915 | | 81 | Pfl01_3318 | 81 | PputGB1_2047 | 56 | PFLU2593 | 82 | PFWH6_0766 | 82 |
| PFL_2663 enterobactin | PflSS101_0843 | 81.3 | 49 | Pfl01_2342 | 24 | PputGB1_1870 | 51 | PFLU3643 | 29 | PFWH6_5214 | 21 |
| PFL_2772 | PflSS101_3147 | 77.5 | 63 | Pfl01_2318 | 86 | PputGB1_3519 | 31 | PFLU3750 | 64 | PFWH6_3525 | 63 |
| PFL_2919 | PflSS101_0832 | 82.3 | 21 | Pfl01_0594 | 30 | PputGB1_3333 | 83 | PFLU0593 | 26 | PFWH6_0820 | 24 |
| PFL_2970 | PflSS101_2915 | | 31 | Pfl01_0594 | 62 | PputGB1_2060 | 72 | PFLU0593 | 59 | PFWH6_0295 | 25 |
| PFL_3154 aerobactin | PflSS101_3832 | 77.9 | 29 | Pfl01_4379 | 22 | PputGB1_3544 | 77 | PFLU2545 | 28 | PFWH6_5931 | 24 |
| PFL_3176 | PflSS101_3463 | | 30 | Pfl01_0250 | 31 | PputGB1_3348 | 84 | PFLU4085 | 30 | PFWH6_2193 | 26 |
| PFL_3177 | PflSS101_5234 | 86 | 38 | Pfl01_2342 | 35 | PputGB1_3349 | 85 | PFLU5895 | 39 | PFWH6_3831 | 37 |
| PFL_3315 pyoverdine | PflSS101_3096 | | 40 | Pfl01_2583 | 43 | PputGB1_3317 | 55 | PFLU3633 | 75 | PFWH6_3086 | 37 |
| PFL_3485 pyoverdine | PflSS101_3096 | | 55 | Pfl01_2583 | 40 | PputGB1_0693 | 59 | PFLU2545 | 57 | PFWH6_1926 | 35 |
| PFL_3498 enantio-pch | PflSS101_0139 | | 41 | Pfl01_5189 | 42 | PputGB1_0747 | 45 | PFLU2598 | 44 | PFWH6_5994 | 39 |
| PFL_3612 | PflSS101_1951 | 86.6 | 31 | Pfl01_3784 | 29 | PputGB1_0911 | 29 | PFLU5361 | 32 | PFWH6_1417 | 30 |
| PFL_3620 | PflSS101_3463 | | 20 | Pfl01_0250 | 23 | PputGB1_1801 | 72 | PFLU3218 | 30 | PFWH6_2193 | 28 |
| PFL_3715 | PflSS101_3074 | 92.5 | 85 | Pfl01_4379 | 24 | PputGB1_1005 | 22 | PFLU3698 | 88 | PFWH6_0820 | 22 |
| PFL_3835 | PflSS101_2208 | 78.9 | 74 | Pfl01_3798 | 28 | PputGB1_1424 | 66 | PFLU2562 | 74 | PFWH6_2397 | 74 |
| PFL_4039 citrate | PflSS101_1951 | | 77 | Pfl01_3784 | 88 | PputGB1_0911 | 76 | PFLU1040 | 46 | PFWH6_1047 | 45 |
| PFL_4063 | PflSS101_2915 | | 55 | Pfl01_3318 | 61 | PputGB1_2047 | 59 | PFLU2593 | 58 | PFWH6_1926 | 54 |
| PFL_4092 pyoverdine | PflSS101_2880 | 78.9 | 46 | Pfl01_2462 | 47 | PputGB1_4082 | 38 | PFLU3378 | 49 | PFWH6_3086 | 47 |
| PFL_4627 heme PhuR | PflSS101_4358 | 93.2 | 76 | Pfl01_4379 | 77 | PputGB1_1005 | 69 | PFLU4968 | 78 | PFWH6_4738 | 75 |
| PFL_4912 | PflSS101_1097 | 71.9 | 82 | Pfl01_3784 | 22 | PputGB1_4359 | 78 | PFLU1087 | 88 | PFWH6_0820 | 83 |
| PFL_5169 | PflSS101_0843 | | 31 | Pfl01_5008 | 26 | PputGB1_1870 | 30 | PFLU3643 | 33 | PFWH6_5214 | 24 |
| PFL_5378 heme HasR | PflSS101_4708 | 96.1 | 77 | Pfl01_4379 | 27 | PputGB1_1005 | 26 | PFLU5361 | 52 | PFWH6_4738 | 27 |
| PFL_5511 B12 | PflSS101_4814 | 69.5 | 67 | Pfl01_5008 | 74 | PputGB1_0571 | 65 | PFLU5463 | 69 | PFWH6_5214 | 67 |
| PFL_5706 ferrichrome | PflSS101_4985 | | 88 | Pfl01_5189 | 81 | PputGB1_0378 | 79 | PFLU5629 | 89 | PFWH6_5394 | 86 |
| | | 89.09 | | | | | | | | | |

## A. *P. chlororaphis* 30-84

| 30-84 | | O-6 | | Q8r1-96 | | Q2-87 | | BG33R | | SS101 | | Pf-5 | | A506 | | SBW25 | | Pf01 | | WH6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ORF | Locus tag | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | %ID | | %ID |
| ORF00002 | Pchl3084_0007 | PchlO6_0126 | 93 | PflQ8_0202 | 85 | PflQ2_0538 | 36 | PseBG33_5128 | 36 | PflSS101_0139 | 36 | PFL_0125 | 85 | PflA506_0139 | 37 | PFLU2598 | 41 | Pfl01_0121 | 89 | PFWH6_5394 | 35 |
| ORF00104 | Pchl3084_0102 | PchlO6_0225 | 90 | PflQ8_0935 | 35 | PflQ2_4530 | 37 | PseBG33_0251 | 89 | PflSS101_0233 | 89 | PFL_0213 | 92 | PflA506_0217 | 89 | PFLU0206 | 94 | Pfl01_0216 | 90 | PFWH6_2193 | 21 |
| ORF00156 | Pchl3084_0151 | PchlO6_0225 | 33 | PflQ8_0935 | 29 | PflQ2_4530 | 31 | PseBG33_3630 | 87 | PflSS101_3463 | 88 | PFL_0255 | 93 | PflA506_3446 | 88 | PFLU4085 | 90 | Pfl01_0205 | 91 | PFWH6_2193 | 24 |
| ORF00206 | Pchl3084_0198 | PchlO6_0861 | 96 | PflQ8_4827 | 33 | PflQ2_3112 | 27 | PseBG33_0836 | 77 | PflSS101_0832 | 77 | PFL_0310 | 81 | PflA506_1983 | 57 | PFLU0295 | 80 | Pfl01_0293 | 78 | PFWH6_0295 | 79 |
| ORF00524 | Pchl3084_0505 | PchlO6_0633 | 94 | PflQ8_2798 | 27 | PflQ2_2980 | 27 | PseBG33_0610 | 73 | PflSS101_2915 | 29 | PFL_0646 | 83 | PflA506_3017 | 29 | PFLU0593 | 77 | Pfl01_0594 | 84 | PFWH6_0295 | 26 |
| ORF00526 | Pchl3084_0507 | PchlO6_4739 | 95 | PflQ8_0637 | 86 | PflQ2_5073 | 85 | PseBG33_4466 | 85 | PflSS101_4358 | 85 | PFL_0648 | 89 | PflA506_0576 | 85 | PFLU0595 | 84 | Pfl01_0596 | 88 | PFWH6_0621 | 83 |
| ORF00609 | Pchl3084_0587 | PchlO6_0716 | 97 | PflQ8_3969 | 25 | PflQ2_1672 | 24 | PseBG33_2654 | 27 | PflSS101_5488 | 22 | PFL_2919 | 27 | PflA506_5433 | 22 | PFLU2598 | 22 | Pfl01_0293 | 34 | PFWH6_5931 | 22 |
| ORF00750 | Pchl3084_0733 | PchlO6_0861 | 96 | PflQ8_3242 | 28 | PflQ2_3112 | 27 | PseBG33_0836 | 85 | PflSS101_0832 | 85 | PFL_0864 | 86 | PflA506_0798 | 86 | PFLU0295 | 30 | Pfl01_0798 | 68 | PFWH6_0820 | 86 |
| ORF00878 | Pchl3084_0859 | PchlO6_0989 | 92 | PflQ8_5352 | 21 | PflQ2_1695 | 42 | PseBG33_1061 | 83 | PflSS101_1061 | 83 | PFL_0982 | 90 | PflA506_1022 | 84 | PFLU1040 | 89 | Pfl01_0923 | 89 | PFWH6_1047 | 84 |
| ORF01255 | Pchl3084_1232 | PchlO6_1431 | 94 | PflQ8_1239 | 24 | PflQ2_4231 | 24 | PseBG33_1530 | 65 | PflSS101_1406 | 65 | PFL_1371 | 89 | PflA506_1363 | 65 | PFLU1405 | 69 | Pfl01_4379 | 24 | PFWH6_1417 | 66 |
| ORF01622 | Pchl3084_1583 | PchlO6_1837 | 97 | PflQ8_4192 | 85 | PflQ2_1453 | 86 | PseBG33_1898 | 81 | PflSS101_1822 | 81 | PFL_1740 | 84 | PflA506_1861 | 81 | PFLU1839 | 83 | Pfl01_4209 | 85 | PFWH6_1770 | 83 |
| ORF01992 | Pchl3084_1957 | PchlO6_0324 | 32 | PflQ8_3242 | 28 | PflQ2_3112 | 28 | PseBG33_0325 | 32 | PflSS101_0311 | 32 | PFL_0310 | 34 | PflA506_0292 | 32 | PFLU0295 | 34 | Pfl01_0293 | 34 | PFWH6_0295 | 33 |
| ORF02079 | Pchl3084_2042 | PchlO6_2381 | 92 | PflQ8_5164 | 34 | PflQ2_2928 | 24 | PseBG33_5403 | 25 | PflSS101_5234 | 25 | PFL_3177 | 27 | PflA506_5184 | 25 | PFLU2202 | 27 | Pfl01_3740 | 90 | PFWH6_3831 | 28 |
| ORF02129 | Pchl3084_2089 | PchlO6_2435 | 94 | PflQ8_1239 | 30 | PflQ2_4231 | 29 | PseBG33_4831 | 27 | PflSS101_3593 | 81 | PFL_2365 | 85 | PflA506_1363 | 22 | PFLU5361 | 25 | Pfl01_4379 | 32 | PFWH6_4738 | 26 |
| ORF02337 | Pchl3084_2285 | PchlO6_0633 | 95 | PflQ8_4827 | 25 | PflQ2_3112 | 24 | PseBG33_0610 | 81 | PflSS101_0832 | 82 | PFL_2604 | 83 | PflA506_3017 | 82 | PFLU2593 | 81 | Pfl01_3318 | 87 | PFWH6_0820 | 25 |
| ORF02421 | Pchl3084_2366 | PchlO6_3555 | 43 | PflQ8_3550 | 39 | PflQ2_0688 | 38 | PseBG33_2445 | 41 | PflSS101_3096 | 42 | PFL_3315 | 56 | PflA506_3089 | 44 | PFLU3633 | 55 | Pfl01_2583 | 41 | PFWH6_3086 | 39 |
| ORF02454 | Pchl3084_2397 | PchlO6_2744 | 96 | PflQ8_1239 | 22 | PflQ2_4231 | 22 | PseBG33_3265 | 64 | PflSS101_3147 | 65 | PFL_2772 | 86 | PflA506_3139 | 63 | PFLU3750 | 65 | Pfl01_2318 | 91 | PFWH6_3525 | 64 |
| ORF02719 | Pchl3084_2661 | PchlO6_3015 | 95 | PflQ8_3550 | 33 | PflQ2_0688 | 34 | PseBG33_3069 | 72 | PflSS101_2361 | 72 | PFL_2719 | 43 | PflA506_3008 | 72 | PFLU2688 | 74 | Pfl01_2583 | 78 | PFWH6_3086 | 35 |
| ORF02792 | Pchl3084_2733 | PchlO6_3108 | 93 | PflQ8_3242 | 29 | PflQ2_3112 | 29 | PseBG33_0836 | 36 | PflSS101_0832 | 37 | PFL_1417 | 40 | PflA506_0798 | 37 | PFLU2365 | 29 | Pfl01_0798 | 35 | PFWH6_0820 | 36 |
| ORF02912 | Pchl3084_2852 | PchlO6_5540 | 25 | PflQ8_1239 | 24 | PflQ2_4231 | 23 | PseBG33_0847 | 28 | PflSS101_0843 | 28 | PFL_5169 | 33 | PflA506_0808 | 28 | PFLU3643 | 88 | Pfl01_4379 | 24 | PFWH6_5214 | 25 |
| ORF02986 | Pchl3084_2924 | PchlO6_3307 | 94 | PflQ8_2987 | 82 | PflQ2_0538 | 23 | PseBG33_2000 | 39 | PflSS101_2109 | 39 | PFL_3835 | 29 | PflA506_2218 | 39 | PFLU4093 | 85 | Pfl01_3798 | 42 | PFWH6_3849 | 82 |
| ORF03122 | Pchl3084_3052 | PchlO6_4138 | 30 | PflQ8_3242 | 71 | PflQ2_3112 | 71 | PseBG33_2364 | 72 | PflSS101_2136 | 72 | PFL_0932 | 44 | PflA506_2242 | 73 | PFLU2365 | 75 | Pfl01_2232 | 74 | PFWH6_2249 | 71 |
| ORF03307 | Pchl3084_3233 | PchlO6_3657 | 94 | PflQ8_2057 | 83 | PflQ2_1672 | 40 | PseBG33_2016 | 58 | PflSS101_1938 | 56 | PFL_2293 | 72 | PflA506_1983 | 57 | PFLU5798 | 42 | Pfl01_1848 | 41 | PFWH6_1926 | 57 |
| ORF03565 | Pchl3084_3483 | PchlO6_4739 | 24 | PflQ8_3000 | 86 | PflQ2_4231 | 24 | PseBG33_4466 | 23 | PflSS101_0832 | 86 | PFL_3715 | 87 | PflA506_2346 | 86 | PFLU3698 | 89 | Pfl01_4379 | 25 | PFWH6_0820 | 23 |
| ORF03620 | Pchl3084_3537 | PchlO6_3952 | 92 | PflQ8_2987 | 27 | PflQ2_2980 | 24 | PseBG33_3219 | 74 | PflSS101_2207 | 73 | PFL_3835 | 81 | PflA506_2311 | 73 | PFLU2562 | 75 | Pfl01_3798 | 28 | PFWH6_2397 | 73 |
| ORF03792 | Pchl3084_3706 | PchlO6_0989 | 46 | PflQ8_3969 | 22 | PflQ2_1695 | 86 | PseBG33_2027 | 79 | PflSS101_1951 | 82 | PFL_4039 | 90 | PflA506_1995 | 79 | PFLU1040 | 46 | Pfl01_3784 | 88 | PFWH6_1047 | 45 |
| ORF03815 | Pchl3084_3726 | PchlO6_4138 | 95 | PflQ8_2798 | 81 | PflQ2_2980 | 83 | PseBG33_1043 | 42 | PflSS101_1043 | 42 | PFL_0995 | 43 | PflA506_1001 | 42 | PFLU1022 | 42 | Pfl01_0931 | 42 | PFWH6_1030 | 41 |
| ORF03922 | Pchl3084_3828 | PchlO6_4229 | 89 | PflQ8_3550 | 50 | PflQ2_0688 | 48 | PseBG33_2654 | 44 | PflSS101_2880 | 47 | PFL_4092 | 85 | PflA506_2859 | 49 | PFLU3378 | 49 | Pfl01_2462 | 48 | PFWH6_3086 | 49 |
| ORF04457 | Pchl3084_4376 | PchlO6_4739 | 95 | PflQ8_1239 | 81 | PflQ2_4231 | 79 | PseBG33_4753 | 73 | PflSS101_4358 | 73 | PFL_4627 | 79 | PflA506_4277 | 72 | PFLU4968 | 75 | Pfl01_4379 | 84 | PFWH6_4738 | 73 |
| ORF04674 | Pchl3084_4588 | PchlO6_4957 | 94 | PflQ8_5164 | 22 | PflQ2_1695 | 23 | PseBG33_1099 | 84 | PflSS101_1097 | 84 | PFL_4912 | 91 | PflA506_1059 | 84 | PFLU1087 | 88 | Pfl01_0594 | 43 | PFWH6_5214 | 22 |
| ORF05222 | Pchl3084_5133 | PchlO6_5540 | 94 | PflQ8_5164 | 83 | PflQ2_4902 | 82 | PseBG33_4946 | 73 | PflSS101_4814 | 73 | PFL_5511 | 73 | PflA506_4759 | 74 | PFLU5463 | 77 | Pfl01_5008 | 83 | PFWH6_5214 | 74 |
| ORF05401 | Pchl3084_5228 | PchlO6_5726 | 99 | PflQ8_5352 | 67 | PflQ2_0538 | 67 | PseBG33_5128 | 68 | PflSS101_4985 | 90 | PFL_5706 | 95 | PflA506_4932 | 88 | PFLU5629 | 90 | Pfl01_5189 | 83 | PFWH6_5394 | 86 |

## B. *P. chlororaphis* O-6

| O-6 | | Q8r1-96 | % ID | Q2-87 | % ID | BG33R | % ID | SS101 | % ID | A506 | % ID | Pf-5 | % ID | 30-84 | % ID | SBW25 | %ID | Pf01 | %ID | WH6 | %ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| ORF00005 | PchlO6_0126 | PflQ8_0202 | 85 | PflQ2_0538 | 36 | PseBG33_5128 | 36 | PflSS101_4985 | 35 | PflA506_0139 | 36 | PFL_0125 | 86 | Pchl3084_0129 | 92 | PFLU2598 | 41 | Pfl01_0121 | 90 | PFWH6_5394 | 35 |
| ORF00108 | PchlO6_0225 | PflQ8_0935 | 35 | PflQ2_4530 | 37 | PseBG33_0251 | 89 | PflSS101_0233 | 89 | PflA506_0217 | 88 | PFL_0213 | 95 | Pchl3084_0224 | 89 | PFLU0206 | 94 | Pfl01_0216 | 90 | PFWH6_2193 | 21 |
| ORF00209 | PchlO6_0324 | PflQ8_4827 | 33 | PflQ2_3112 | 28 | PseBG33_0325 | 77 | PflSS101_0311 | 77 | PflA506_0292 | 77 | PFL_0310 | 81 | Pchl3084_0320 | 96 | PFLU0295 | 79 | Pfl01_0293 | 78 | PFWH6_0295 | 78 |
| ORF00530 | PchlO6_0633 | PflQ8_2798 | 26 | PflQ2_2980 | 26 | PseBG33_0610 | 73 | PflSS101_2915 | 30 | PflA506_3017 | 30 | PFL_0646 | 82 | Pchl3084_0627 | 93 | PFLU0593 | 78 | Pfl01_0594 | 85 | PFWH6_0295 | 26 |
| ORF00532 | PchlO6_0635 | PflQ8_0637 | 88 | PflQ2_5073 | 87 | PseBG33_0612 | 85 | PflSS101_0606 | 85 | PflA506_0576 | 85 | PFL_0648 | 89 | Pchl3084_0629 | 97 | PFLU0595 | 84 | Pfl01_0596 | 89 | PFWH6_0621 | 85 |
| ORF00618 | PchlO6_0716 | PflQ8_3969 | 25 | PflQ2_1672 | 24 | PseBG33_2654 | 27 | PflSS101_5488 | 21 | PflA506_5433 | 21 | PFL_2919 | 27 | Pchl3084_0709 | 97 | PFLU2598 | 22 | Pfl01_0293 | 34 | PFWH6_5931 | 21 |
| ORF00767 | PchlO6_0861 | PflQ8_3242 | 28 | PflQ2_3112 | 27 | PseBG33_0836 | 85 | PflSS101_0832 | 85 | PflA506_0798 | 85 | PFL_0864 | 86 | Pchl3084_0855 | 96 | PFLU0295 | 30 | Pfl01_0798 | 68 | PFWH6_0767 | 85 |
| ORF00900 | PchlO6_0989 | PflQ8_2987 | 24 | PflQ2_1695 | 43 | PseBG33_1061 | 85 | PflSS101_1061 | 85 | PflA506_1022 | 85 | PFL_0982 | 90 | Pchl3084_0981 | 93 | PFLU1040 | 90 | Pfl01_0923 | 90 | PFWH6_1047 | 85 |
| ORF01351 | PchlO6_1431 | PflQ8_1239 | 24 | PflQ2_4231 | 23 | PseBG33_1530 | 64 | PflSS101_1406 | 63 | PflA506_1363 | 66 | PFL_1371 | 87 | Pchl3084_1354 | 94 | PFLU1405 | 68 | Pfl01_4379 | 24 | PFWH6_1417 | 65 |
| ORF01761 | PchlO6_1837 | PflQ8_4192 | 84 | PflQ2_1453 | 86 | PseBG33_1898 | 81 | PflSS101_1822 | 81 | PflA506_1861 | 81 | PFL_1740 | 85 | Pchl3084_1705 | 97 | PFLU1839 | 83 | Pfl01_4209 | 84 | PFWH6_1770 | 83 |
| ORF02295 | PchlO6_2381 | PflQ8_5164 | 34 | PflQ2_2928 | 24 | PseBG33_5403 | 25 | PflSS101_5234 | 25 | PflA506_5184 | 25 | PFL_3177 | 27 | Pchl3084_2164 | 92 | PFLU2202 | 27 | Pfl01_3740 | 90 | PFWH6_3831 | 28 |
| ORF02350 | PchlO6_2435 | PflQ8_1239 | 30 | PflQ2_4231 | 30 | PseBG33_4831 | 27 | PflSS101_3593 | 80 | PflA506_1363 | 23 | PFL_2365 | 84 | Pchl3084_2211 | 94 | PFLU5361 | 27 | Pfl01_4379 | 32 | PFWH6_4738 | 26 |
| ORF02550 | PchlO6_2626 | PflQ8_4827 | 25 | PflQ2_3112 | 24 | PseBG33_2513 | 81 | PflSS101_2915 | 82 | PflA506_3017 | 82 | PFL_2604 | 83 | Pchl3084_3771 | 95 | PFLU2593 | 81 | Pfl01_3318 | 87 | PFWH6_0621 | 85 |
| ORF02670 | PchlO6_2744 | PflQ8_1239 | 21 | PflQ2_4231 | 21 | PseBG33_3265 | 62 | PflSS101_3147 | 63 | PflA506_3139 | 61 | PFL_2772 | 86 | Pchl3084_2519 | 94 | PFLU3750 | 65 | Pfl01_2318 | 92 | PFWH6_3525 | 62 |
| ORF02949 | PchlO6_3015 | PflQ8_3550 | 33 | PflQ2_0688 | 34 | PseBG33_3069 | 73 | PflSS101_2361 | 72 | PflA506_3008 | 72 | PFL_3315 | 42 | Pchl3084_2783 | 95 | PFLU2688 | 74 | Pfl01_2583 | 78 | PFWH6_3086 | 34 |
| ORF03045 | PchlO6_3108 | PflQ8_3242 | 29 | PflQ2_3112 | 29 | PseBG33_0836 | 36 | PflSS101_0832 | 36 | PflA506_0798 | 37 | PFL_1417 | 41 | Pchl3084_2855 | 93 | PFLU2365 | 30 | Pfl01_0798 | 36 | PFWH6_0820 | 35 |
| ORF03204 | PchlO6_3255 | PflQ8_2430 | 27 | PflQ2_2925 | 90 | PseBG33_3630 | 30 | PflSS101_3463 | 30 | PflA506_3446 | 30 | PFL_3176 | 30 | Pchl3084_0273 | 30 | PFLU4085 | 31 | Pfl01_0250 | 31 | PFWH6_2193 | 27 |
| ORF03207 | PchlO6_3258 | PflQ8_5164 | 25 | PflQ2_2928 | 90 | PseBG33_3617 | 59 | PflSS101_3450 | 60 | PflA506_5184 | 37 | PFL_1386 | 49 | Pchl3084_2164 | 34 | PFLU4069 | 60 | Pfl01_2342 | 37 | PFWH6_3831 | 61 |
| ORF03257 | PchlO6_3307 | PflQ8_2987 | 83 | PflQ2_0538 | 23 | PseBG33_2000 | 39 | PflSS101_2109 | 39 | PflA506_2218 | 39 | PFL_3835 | 29 | Pchl3084_3046 | 95 | PFLU4093 | 86 | Pfl01_3798 | 42 | PFWH6_3849 | 82 |
| ORF03519 | PchlO6_3555 | PflQ8_3550 | 38 | PflQ2_0688 | 38 | PseBG33_2445 | 36 | PflSS101_3096 | 38 | PflA506_3089 | 40 | PFL_3315 | 41 | Pchl3084_2488 | 43 | PFLU3633 | 42 | Pfl01_2583 | 37 | PFWH6_3086 | 38 |
| ORF03614 | PchlO6_3657 | PflQ8_2057 | 81 | PflQ2_1672 | 39 | PseBG33_2016 | 58 | PflSS101_1938 | 57 | PflA506_1983 | 58 | PFL_2293 | 72 | Pchl3084_3355 | 92 | PFLU5798 | 42 | Pfl01_1848 | 41 | PFWH6_1926 | 57 |
| ORF03627 | PchlO6_3670 | PflQ8_4192 | 22 | PflQ2_2928 | 36 | PseBG33_5403 | 81 | PflSS101_5234 | 81 | PflA506_5184 | 81 | PFL_3177 | 38 | Pchl3084_2164 | 27 | PFLU5895 | 83 | Pfl01_2342 | 41 | PFWH6_3831 | 38 |
| ORF03833 | PchlO6_3872 | PflQ8_5164 | 26 | PflQ2_2928 | 32 | PseBG33_2166 | 37 | PflSS101_1702 | 37 | PflA506_5184 | 32 | PFL_3177 | 37 | Pchl3084_2164 | 65 | PFLU2216 | 26 | Pfl01_3121 | 89 | PFWH6_3831 | 32 |
| ORF03835 | PchlO6_3873 | PflQ8_1239 | 25 | PflQ2_2928 | 36 | PseBG33_2166 | 35 | PflSS101_5234 | 24 | PflA506_5184 | 24 | PFL_3177 | 34 | Pchl3084_2164 | 26 | PFLU2202 | 40 | Pfl01_3124 | 84 | PFWH6_3831 | 37 |
| ORF03918 | PchlO6_3952 | PflQ8_2987 | 26 | PflQ2_2980 | 25 | PseBG33_3219 | 74 | PflSS101_2208 | 74 | PflA506_2311 | 73 | PFL_3835 | 81 | Pchl3084_3659 | 96 | PFLU2562 | 77 | Pfl01_3798 | 28 | PFWH6_2397 | 73 |
| ORF04103 | PchlO6_4138 | PflQ8_2798 | 82 | PflQ2_2980 | 84 | PseBG33_1043 | 42 | PflSS101_1043 | 42 | PflA506_1001 | 42 | PFL_0995 | 43 | Pchl3084_3848 | 95 | PFLU1022 | 41 | Pfl01_0931 | 41 | PFWH6_1030 | 40 |
| ORF04196 | PchlO6_4229 | PflQ8_3550 | 50 | PflQ2_0688 | 49 | PseBG33_2654 | 44 | PflSS101_2880 | 47 | PflA506_2859 | 49 | PFL_4092 | 85 | Pchl3084_3950 | 89 | PFLU3378 | 49 | Pfl01_2462 | 48 | PFWH6_3086 | 49 |
| ORF04706 | PchlO6_4739 | PflQ8_1239 | 81 | PflQ2_4231 | 79 | PseBG33_4466 | 74 | PflSS101_4358 | 74 | PflA506_4277 | 73 | PFL_4627 | 79 | Pchl3084_4498 | 95 | PFLU4968 | 75 | Pfl01_4379 | 84 | PFWH6_4738 | 73 |
| ORF04935 | PchlO6_4957 | PflQ8_1239 | 27 | PflQ2_1695 | 22 | PseBG33_1099 | 86 | PflSS101_1097 | 86 | PflA506_1059 | 86 | PFL_4912 | 91 | Pchl3084_4710 | 96 | PFLU1087 | 89 | Pfl01_5008 | 30 | PFWH6_5214 | 22 |
| ORF05521 | PchlO6_5540 | PflQ8_5164 | 80 | PflQ2_4902 | 80 | PseBG33_4946 | 71 | PflSS101_4814 | 71 | PflA506_4759 | 72 | PFL_5511 | 72 | Pchl3084_5255 | 92 | PFLU5463 | 76 | Pfl01_5008 | 83 | PFWH6_5214 | 71 |
| ORF05708 | PchlO6_5726 | PflQ8_5352 | 66 | PflQ2_0538 | 66 | PseBG33_5128 | 67 | PflSS101_4985 | 88 | PflA506_4932 | 87 | PFL_5706 | 94 | Pchl3084_5442 | 97 | PFLU5629 | 89 | Pfl01_5189 | 83 | PFWH6_5394 | 84 |

## C. *P. fluorescens* Pf0-1

| Pf0-1 | Pf-5 | SBW25 | | Q2-87 | | Q8r1-96 | | 30-84 | | O-6 | | A506 | | BG33R | | SS101 | |
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| Pfl01_0216 | PFL_0213 | 90 | PFLU0206 | 89 | PflQ2_4530 | 36 | PflQ8_0935 | 34 | PchI3084_0224 | 80 | PchIO6_0225 | 80 | PflA506_0217 | 82 | PseBG33_0251 | 82 | PflSS101_0233 | 82 |
| Pfl01_0250 | PFL_0255 | 86 | PFLU4085 | 92 | PflQ2_4530 | 30 | PflQ8_0935 | 29 | PchI3084_0273 | 83 | PchIO6_0225 | 31 | PflA506_3446 | 84 | PseBG33_3630 | 84 | PflSS101_3463 | 85 |
| Pfl01_0293 | PFL_0310 | 81 | PFLU0295 | 81 | PflQ2_3112 | 28 | PflQ8_4827 | 30 | PchI3084_0320 | 77 | PchIO6_0324 | 77 | PflA506_0292 | 77 | PseBG33_0325 | 77 | PflSS101_0311 | 77 |
| Pfl01_0594 | PFL_0646 | 81 | PFLU0593 | 78 | PflQ2_2980 | 26 | PflQ8_2798 | 27 | PchI3084_0627 | 80 | PchIO6_0633 | 81 | PflA506_3017 | 31 | PseBG33_0610 | 73 | PflSS101_2915 | 31 |
| Pfl01_0596 | PFL_0648 | 87 | PFLU0595 | 88 | PflQ2_5073 | 87 | PflQ8_0637 | 87 | PchI3084_0629 | 88 | PchIO6_0635 | 89 | PflA506_0576 | 87 | PseBG33_0612 | 86 | PflSS101_0606 | 87 |
| Pfl01_0798 | PFL_0864 | 66 | PFLU0295 | 29 | PflQ2_3112 | 26 | PflQ8_3242 | 26 | PchI3084_0855 | 66 | PchIO6_0861 | 66 | PflA506_0798 | 64 | PseBG33_0836 | 64 | PflSS101_0832 | 64 |
| Pfl01_0874 | PFL_0932 | 85 | PFLU0757 | 83 | PflQ2_3112 | 46 | PflQ8_4827 | 83 | PchI3084_3174 | 42 | PchIO6_0324 | 31 | PflA506_0745 | 82 | PseBG33_0782 | 81 | PflSS101_0775 | 82 |
| Pfl01_0923 | PFL_0982 | 88 | PFLU1040 | 89 | PflQ2_1695 | 41 | PflQ8_5352 | 21 | PchI3084_0981 | 85 | PchIO6_0989 | 85 | PflA506_1022 | 83 | PseBG33_1061 | 84 | PflSS101_1061 | 84 |
| Pfl01_0931 | PFL_0995 | 77 | PFLU1022 | 79 | PflQ2_2980 | 43 | PflQ8_2798 | 43 | PchI3084_3848 | 41 | PchIO6_4138 | 40 | PflA506_1001 | 75 | PseBG33_1043 | 76 | PflSS101_1043 | 76 |
| Pfl01_1848 | PFL_2293 | 42 | PFLU5798 | 57 | PflQ2_1672 | 53 | PflQ8_3969 | 52 | PchI3084_3355 | 39 | PchIO6_3657 | 39 | PflA506_1983 | 41 | PseBG33_5305 | 55 | PflSS101_1938 | 41 |
| Pfl01_2232 | PFL_0932 | 45 | PFLU2365 | 86 | PflQ2_3112 | 79 | PflQ8_3242 | 80 | PchI3084_3174 | 71 | PchIO6_5726 | 29 | PflA506_2242 | 82 | PseBG33_2364 | 82 | PflSS101_2136 | 81 |
| Pfl01_2318 | PFL_2772 | 86 | PFLU3750 | 66 | PflQ2_4231 | 22 | PflQ8_1239 | 22 | PchI3084_2519 | 89 | PchIO6_2744 | 90 | PflA506_3139 | 62 | PseBG33_3265 | 63 | PflSS101_3147 | 64 |
| Pfl01_2342 | PFL_3177 | 34 | PFLU5895 | 41 | PflQ2_2928 | 36 | PflQ8_5164 | 29 | PchI3084_2974 | 21 | PchIO6_3670 | 39 | PflA506_5184 | 40 | PseBG33_5403 | 41 | PflSS101_5234 | 41 |
| Pfl01_2462 | PFL_4092 | 46 | PFLU3378 | 82 | PflQ2_1672 | 35 | PflQ8_3550 | 80 | PchI3084_3950 | 45 | PchIO6_4229 | 45 | PflA506_2859 | 77 | PseBG33_2658 | 77 | PflSS101_2880 | 77 |
| Pfl01_2583 | PFL_3315 | 42 | PFLU2688 | 72 | PflQ2_1327 | 30 | PflQ8_3550 | 31 | PchI3084_2783 | 74 | PchIO6_3015 | 74 | PflA506_3008 | 68 | PseBG33_3069 | 68 | PflSS101_2361 | 69 |
| Pfl01_2952 truncated | PFL_4063 | 51 | PFLU2593 | 36 | PflQ2_2980 | 27 | PflQ8_2798 | 27 | PchI3084_2407 | 50 | PchIO6_2626 | 51 | PflA506_3017 | 48 | PseBG33_2513 | 48 | PflSS101_2915 | 48 |
| Pfl01_3121 | PFL_2202 | 25 | PFLU3177 | 37 | PflQ2_2928 | 24 | PflQ8_5164 | 26 | PchI3084_2164 | 65 | PchIO6_3872 | 86 | PflA506_5184 | 29 | PseBG33_3617 | 24 | PflSS101_3450 | 24 |
| Pfl01_3124 | PFL_2202 | 36 | PFLU3177 | 33 | PflQ2_2928 | 23 | PflQ8_5352 | 26 | PchI3084_2164 | 26 | PchIO6_3873 | 81 | PflA506_5184 | 24 | PseBG33_3617 | 23 | PflSS101_3450 | 23 |
| Pfl01_3318 | PFL_2604 | 81 | PFLU2593 | 82 | PflQ2_3112 | 24 | PflQ8_4827 | 25 | PchI3084_2407 | 86 | PchIO6_2626 | 86 | PflA506_3017 | 85 | PseBG33_2513 | 83 | PflSS101_2915 | 85 |
| Pfl01_3740 | PFL_2202 | 28 | PFLU3177 | 27 | PflQ2_1564 | 25 | PflQ8_5164 | 36 | PchI3084_2164 | 84 | PchIO6_2381 | 84 | PflA506_5184 | 25 | PseBG33_5318 | 24 | PflSS101_5234 | 25 |
| Pfl01_3784 | PFL_4039 | 88 | PFLU1040 | 45 | PflQ2_1695 | 84 | PflQ8_0202 | 20 | PchI3084_3828 | 86 | PchIO6_0989 | 44 | PflA506_1995 | 80 | PseBG33_2027 | 78 | PflSS101_1951 | 81 |
| Pfl01_3798 | PFL_3835 | 28 | PFLU3566 | 68 | PflQ2_1672 | 21 | PflQ8_2987 | 40 | PchI3084_3046 | 42 | PchIO6_3307 | 42 | PflA506_2218 | 37 | PseBG33_2000 | 60 | PflSS101_1922 | 59 |
| Pfl01_4209 | PFL_1740 | 82 | PFLU1839 | 80 | PflQ2_1453 | 87 | PflQ8_4192 | 85 | PchI3084_1705 | 84 | PchIO6_1837 | 84 | PflA506_1861 | 78 | PseBG33_1898 | 78 | PflSS101_1822 | 79 |
| Pfl01_4379 | PFL_4627 | 75 | PFLU4968 | 71 | PflQ2_4231 | 80 | PflQ8_1239 | 81 | PchI3084_4498 | 81 | PchIO6_4739 | 81 | PflA506_4277 | 71 | PseBG33_4466 | 72 | PflSS101_4358 | 71 |
| Pfl01_5008 | PFL_5511 | 74 | PFLU5463 | 75 | PflQ2_4902 | 80 | PflQ8_5164 | 80 | PchI3084_5255 | 83 | PchIO6_5540 | 82 | PflA506_4759 | 77 | PseBG33_4946 | 76 | PflSS101_4814 | 76 |
| Pfl01_5189 | PFL_5706 | 81 | PFLU5629 | 81 | PflQ2_0538 | 68 | PflQ8_5352 | 67 | PchI3084_5442 | 81 | PchIO6_5726 | 80 | PflA506_4932 | 77 | PseBG33_5128 | 68 | PflSS101_4985 | 78 |

## D. *P. fluorescens* Q8r1-96

| Q8r1-96 | | Q2-87 | | Pf-5 | | A506 | | 30-84 | | O-6 | | SS101 | | BG33R | | Pf0-1 | | SBW25 | | WH6 | |
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| ORF00502 | PflQ8_0637 | PflQ2_5073 | 95 | PFL_0648 | 86 | PflA506_0576 | 85 | Pchl3084_0629 | 88 | PchlO6_0635 | 88 | PflSS101_0606 | 84 | PseBG33_0612 | 85 | Pfl01_0596 | 86 | PFLU0595 | 85 | PFWH6_0502 | 84 |
| ORF00804 | PflQ8_4827 | PflQ2_3112 | 45 | PFL_0932 | 84 | PflA506_0745 | 83 | Pchl3084_3174 | 44 | PchlO6_0324 | 32 | PflSS101_0775 | 83 | PseBG33_0782 | 82 | Pfl01_0874 | 85 | PFLU0757 | 84 | PFWH6_0766 | 83 |
| ORF01477 | PflQ8_4192 | PflQ2_1453 | 92 | PFL_1740 | 82 | PflA506_1861 | 79 | Pchl3084_1722 | 85 | PchlO6_1837 | 84 | PflSS101_1822 | 79 | PseBG33_1898 | 78 | Pfl01_4209 | 85 | PFLU1839 | 80 | PFWH6_1770 | 81 |
| ORF01699 | PflQ8_3969 | PflQ2_1672 | 86 | PFL_2293 | 41 | PflA506_1983 | 40 | Pchl3084_3355 | 39 | PchlO6_3657 | 39 | PflSS101_1938 | 40 | PseBG33_5305 | 49 | Pfl01_1848 | 53 | PFLU5798 | 50 | PFWH6_1926 | 39 |
| ORF02118 | PflQ8_3550 | PflQ2_0688 | 82 | PFL_4092 | 47 | PflA506_2859 | 79 | Pchl3084_3950 | 47 | PchlO6_4229 | 47 | PflSS101_2880 | 80 | PseBG33_2658 | 80 | Pfl01_2462 | 86 | PFLU3378 | 84 | PFWH6_3086 | 79 |
| ORF02446 | PflQ8_3242 | PflQ2_3112 | 89 | PFL_0932 | 46 | PflA506_2242 | 77 | Pchl3084_3174 | 68 | PchlO6_4138 | 31 | PflSS101_2136 | 76 | PseBG33_2364 | 77 | Pfl01_2232 | 84 | PFLU2365 | 82 | PFWH6_2249 | 76 |
| ORF02703 | PflQ8_3000 | PflQ2_4231 | 21 | PFL_3715 | 87 | PflA506_2346 | 84 | Pchl3084_3605 | 86 | PchlO6_0861 | 24 | PflSS101_3074 | 84 | PseBG33_0836 | 23 | Pfl01_4379 | 24 | PFLU3698 | 87 | PFWH6_0820 | 23 |
| ORF02716 | PflQ8_2987 | PflQ2_3112 | 23 | PFL_3835 | 27 | PflA506_2218 | 38 | Pchl3084_3046 | 78 | PchlO6_3307 | 79 | PflSS101_2109 | 38 | PseBG33_2000 | 37 | Pfl01_3798 | 41 | PFLU4093 | 86 | PFWH6_3849 | 81 |
| ORF02907 | PflQ8_2798 | PflQ2_2980 | 89 | PFL_0995 | 43 | PflA506_1001 | 42 | Pchl3084_3848 | 80 | PchlO6_4138 | 81 | PflSS101_1043 | 42 | PseBG33_1043 | 42 | Pfl01_0931 | 43 | PFLU1022 | 43 | PFWH6_1030 | 40 |
| ORF03272 | PflQ8_2430 | PflQ2_2925 | 28 | PFL_3620 | 30 | PflA506_3446 | 24 | Pchl3084_0224 | 23 | PchlO6_3255 | 27 | PflSS101_3463 | 24 | PseBG33_3630 | 24 | Pfl01_0216 | 24 | PFLU3218 | 73 | PFWH6_2193 | 78 |
| ORF03651 | PflQ8_2057 | PflQ2_1672 | 40 | PFL_2293 | 73 | PflA506_1983 | 57 | Pchl3084_3355 | 82 | PchlO6_3657 | 81 | PflSS101_1938 | 57 | PseBG33_2016 | 57 | Pfl01_1848 | 41 | PFLU5798 | 41 | PFWH6_1926 | 58 |
| ORF04510 | PflQ8_1239 | PflQ2_4231 | 85 | PFL_4627 | 76 | PflA506_4277 | 72 | Pchl3084_4498 | 81 | PchlO6_4739 | 81 | PflSS101_4358 | 73 | PseBG33_4466 | 73 | Pfl01_4379 | 85 | PFLU4968 | 73 | PFWH6_4738 | 72 |
| ORF04819 | PflQ8_0935 | PflQ2_4530 | 88 | PFL_0213 | 36 | PflA506_0217 | 35 | Pchl3084_0224 | 36 | PchlO6_0225 | 36 | PflSS101_0233 | 35 | PseBG33_2000 | 37 | Pfl01_0216 | 35 | PFLU0206 | 35 | PFWH6_2193 | 24 |
| ORF05160 | PflQ8_5164 | PflQ2_4902 | 85 | PFL_5511 | 69 | PflA506_4759 | 70 | Pchl3084_5255 | 79 | PchlO6_5540 | 79 | PflSS101_4814 | 69 | PseBG33_4946 | 69 | Pfl01_5008 | 81 | PFLU5463 | 75 | PFWH6_5214 | 69 |
| ORF05340 | PflQ8_5352 | PflQ2_0538 | 92 | PFL_5706 | 67 | PflA506_4932 | 65 | Pchl3084_5442 | 67 | PchlO6_5726 | 66 | PflSS101_4985 | 66 | PseBG33_5128 | 82 | Pfl01_5189 | 69 | PFLU5629 | 66 | PFWH6_5394 | 65 |

## E. *P. fluorescens* Q2-87

| Q2-87 | | A506 | % ID | 30-84 | % ID | O-6 | % ID | Q8r1-96 | % ID | BG33R | % ID | SS101 | % ID | PF-5 | % ID | SBW25 | % ID | Pf0-1 | % ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ORF | Locus tag | | | | | | | | | | | | | | | | | | |
| ORF04393 | PflQ2_0538 | PflA506_4932 | 64 | Pchl3084_5442 | 67 | PchlO6_5726 | 66 | PflQ8_5352 | 92 | PseBG33_5128 | 81 | PflSS101_4985 | 65 | PFL_5706 | 67 | PFLU5629 | 66 | Pfl01_5189 | 69.7 |
| ORF04245 | PflQ2_0688 | PflA506_2859 | 83 | Pchl3084_3950 | 49 | PchlO6_4229 | 49 | PflQ8_3550 | 85 | PseBG33_2658 | 83 | PflSS101_2880 | 83 | PFL_4092 | 50 | PFLU3378 | 82 | Pfl01_2462 | 84.7 |
| ORF03440 | PflQ2_1453 | PflA506_1861 | 80 | Pchl3084_1705 | 86 | PchlO6_1837 | 85 | PflQ8_4192 | 95 | PseBG33_1898 | 78 | PflSS101_1822 | 80 | PFL_1740 | 82 | PFLU1839 | 80 | Pfl01_4209 | 86.8 |
| ORF03223 | PflQ2_1672 | PflA506_1983 | 41 | Pchl3084_3950 | 39 | PchlO6_4229 | 39 | PflQ8_3969 | 87 | PseBG33_5305 | 52 | PflSS101_1938 | 41 | PFL_2293 | 39 | PFLU5798 | 52 | Pfl01_1848 | 54.8 |
| ORF03197 | PflQ2_1695 | PflA506_1995 | 77 | Pchl3084_3828 | 86 | PchlO6_0989 | 44 | PflQ8_0202 | 20 | PseBG33_2027 | 77 | PflSS101_1951 | 77 | PFL_4039 | 87 | PFLU1040 | 45 | Pfl01_3784 | 86 |
| ORF01916 | PflQ2_2925 | PflA506_3446 | 30 | Pchl3084_0273 | 32 | PchlO6_3255 | 90 | PflQ8_0935 | 26 | PseBG33_3630 | 30 | PflSS101_3463 | 30 | PFL_3176 | 30 | PFLU4085 | 32 | Pfl01_0250 | 32.4 |
| ORF01913 | PflQ2_2928 | PflA506_5184 | 36 | Pchl3084_2164 | 26 | PchlO6_3258 | 87 | PflQ8_5164 | 25 | PseBG33_3617 | 56 | PflSS101_3450 | 57 | PFL_1386 | 47 | PFLU4069 | 59 | Pfl01_2342 | 36.4 |
| ORF01860 | PflQ2_2980 | PflA506_1001 | 42 | Pchl3084_3848 | 81 | PchlO6_4138 | 82 | PflQ8_2798 | 89 | PseBG33_1043 | 42 | PflSS101_1043 | 42 | PFL_0995 | 43 | PFLU1022 | 43 | Pfl01_0931 | 43.3 |
| ORF01721 | PflQ2_3112 | PflA506_2242 | 78 | Pchl3084_3174 | 70 | PchlO6_4138 | 31 | PflQ8_3242 | 90 | PseBG33_2364 | 77 | PflSS101_2136 | 77 | PFL_0932 | 47 | PFLU2365 | 81 | Pfl01_2232 | 82.6 |
| ORF01583 | PflQ2_3242 | PflA506_3089 | 34 | Pchl3084_3355 | 33 | PchlO6_3657 | 32 | PflQ8_2057 | 32 | PseBG33_2445 | 32 | PflSS101_3096 | 35 | PFL_2391 | 35 | PFLU2545 | 35 | Pfl01_1848 | 33.2 |
| ORF00560 | PflQ2_4231 | PflA506_4277 | 70 | Pchl3084_4498 | 79 | PchlO6_4739 | 79 | PflQ8_1239 | 86 | PseBG33_4466 | 70 | PflSS101_4358 | 70 | PFL_4627 | 75 | PFLU4968 | 72 | Pfl01_4379 | 83.6 |
| ORF00254 | PflQ2_4530 | PflA506_0217 | 36 | Pchl3084_0224 | 38 | PchlO6_0225 | 38 | PflQ8_0935 | 90 | PseBG33_0251 | 36 | PflSS101_0233 | 35 | PFL_0213 | 37 | PFLU0206 | 36 | Pfl01_0216 | 36.5 |
| ORF04571 | PflQ2_4902 | PflA506_4759 | 75 | Pchl3084_5255 | 83 | PchlO6_5540 | 82 | PflQ8_5164 | 89 | PseBG33_4946 | 73 | PflSS101_4814 | 74 | PFL_5511 | 68 | PFLU5463 | 75 | Pfl01_5008 | 80.6 |
| ORF04750 | PflQ2_5073 | PflA506_0576 | 85 | Pchl3084_0629 | 87 | PchlO6_0635 | 87 | PflQ8_0637 | 95 | PseBG33_0612 | 84 | PflSS101_0606 | 84 | PFL_0648 | 86 | PFLU0595 | 86 | Pfl01_0596 | 86.9 |

## F. *P.* sp. BG33R

| BG33R | | A506 | | 30-84 | | O-6 | | Q8r1-96 | | Q2-87 | | SS101 | | Pf-5 | | Pf0-1 | | SBW25 | | WH6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ORF | Locus tag | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | % ID | | %ID |
| ORF00025 | PseBG33_0163 | PflA506_0139 | 96 | PchI3084_0129 | 38 | PchIO6_0126 | 37 | PflQ8_5352 | 39 | PflQ2_0538 | 38 | PflSS101_0139 | 96 | PFL_0147 | 72 | Pfl01_5189 | 38 | PFLU2598 | 42 | PFWH6_5394 | 36 |
| ORF00111 | PseBG33_0251 | PflA506_3446 | 91 | PchI3084_0273 | 86 | PchIO6_3255 | 86 | PflQ8_0935 | 34 | PflQ2_4530 | 35 | PflSS101_3463 | 91 | PFL_0213 | 91 | Pfl01_0216 | 88 | PFLU0206 | 95 | PFWH6_2193 | 21 |
| ORF00191 | PseBG33_0325 | PflA506_0292 | 94 | PchI3084_0320 | 78 | PchIO6_0324 | 78 | PflQ8_4827 | 32 | PflQ2_3112 | 28 | PflSS101_0311 | 94 | PFL_0310 | 80 | Pfl01_0293 | 79 | PFLU0295 | 89 | PFWH6_0295 | 87 |
| ORF00362 | PseBG33_0489 | PflA506_0460 | 86 | PchI3084_5442 | 22 | PchIO6_5726 | 22 | PflQ8_5352 | 23 | PflQ2_0538 | 22 | PflSS101_0492 | 88 | PFL_5706 | 22 | Pfl01_0931 | 27 | PFLU0473 | 91 | PFWH6_5394 | 22 |
| ORF00488 | PseBG33_0610 | PflA506_3017 | 30 | PchI3084_0627 | 75 | PchIO6_0633 | 74 | PflQ8_3242 | 25 | PflQ2_3112 | 25 | PflSS101_2915 | 31 | PFL_0646 | 77 | Pfl01_0594 | 77 | PFLU0593 | 90 | PFWH6_0295 | 25 |
| ORF00490 | PseBG33_0612 | PflA506_0576 | 95 | PchI3084_0629 | 85 | PchIO6_0635 | 85 | PflQ8_0637 | 85 | PflQ2_5073 | 84 | PflSS101_0606 | 96 | PFL_0648 | 85 | Pfl01_0596 | 85 | PFLU0595 | 93 | PFWH6_0621 | 94 |
| ORF01166 | PseBG33_3630 | PflA506_3446 | 97 | PchI3084_0273 | 97 | PchIO6_0225 | 32 | PflQ8_0935 | 30 | PflQ2_4530 | 31 | PflSS101_3463 | 97 | PFL_0255 | 89 | Pfl01_0250 | 87 | PFLU4085 | 94 | PFWH6_2193 | 24 |
| ORF01180 | PseBG33_3617 | PflA506_5184 | 37 | PchI3084_2164 | 37 | PchIO6_3258 | 57 | PflQ8_5164 | 25 | PflQ2_2928 | 55 | PflSS101_3450 | 91 | PFL_1386 | 47 | Pfl01_2342 | 37 | PFLU4069 | 94 | PFWH6_3831 | 89 |
| ORF01533 | PseBG33_3265 | PflA506_3446 | 86 | PchI3084_0273 | 59 | PchIO6_2626 | 59 | PflQ8_1239 | 24 | PflQ2_1672 | 35 | PflSS101_3463 | 88 | PFL_2772 | 65 | Pfl01_2318 | 64 | PFLU3750 | 91 | PFWH6_3525 | 81 |
| ORF01583 | PseBG33_3219 | PflA506_2222 | 89 | PchI3084_3046 | 77 | PchIO6_3307 | 77 | PflQ8_2987 | 27 | PflQ2_2980 | 26 | PflSS101_2113 | 93 | PFL_3835 | 74 | Pfl01_3798 | 28 | PFLU2562 | 92 | PFWH6_2397 | 86 |
| ORF01737 | PseBG33_3069 | PflA506_3008 | 91 | PchI3084_2783 | 72 | PchIO6_3015 | 73 | PflQ8_2057 | 35 | PflQ2_0688 | 34 | PflSS101_2361 | 91 | PFL_3315 | 40 | Pfl01_2583 | 71 | PFLU2688 | 89 | PFWH6_3086 | 33 |
| ORF01976 | PseBG33_2840 | PflA506_2771 | 62 | PchI3084_3046 | 38 | PchIO6_3307 | 39 | PflQ8_2987 | 40 | PflQ2_2980 | 24 | PflSS101_2153 | 42 | PFL_3835 | 28 | Pfl01_3798 | 36 | PFLU4093 | 39 | PFWH6_2186 | 40 |
| ORF02159 | PseBG33_2658 | PflA506_2859 | 88 | PchI3084_3950 | 45 | PchIO6_4229 | 46 | PflQ8_3550 | 79 | PflQ2_0688 | 80 | PflSS101_2880 | 88 | PFL_4092 | 48 | Pfl01_2462 | 82 | PFLU3378 | 85 | PFWH6_3086 | 80 |
| ORF02163 | PseBG33_2654 | PflA506_2859 | 49 | PchI3084_3950 | 43 | PchIO6_4229 | 43 | PflQ8_3550 | 52 | PflQ2_0688 | 51 | PflSS101_2880 | 50 | PFL_4092 | 43 | Pfl01_2462 | 50 | PFLU3378 | 51 | PFWH6_3086 | 50 |
| ORF02313 | PseBG33_2513 | PflA506_3017 | 94 | PchI3084_2407 | 81 | PchIO6_2626 | 81 | PflQ8_3242 | 25 | PflQ2_3112 | 24 | PflSS101_2915 | 93 | PFL_2604 | 80 | Pfl01_3318 | 83 | PFLU2593 | 90 | PFWH6_0820 | 25 |
| ORF02381 | PseBG33_2445 | PflA506_3089 | 74 | PchI3084_2488 | 42 | PchIO6_4229 | 42 | PflQ8_3550 | 38 | PflQ2_0688 | 38 | PflSS101_3096 | 60 | PFL_2391 | 70 | Pfl01_2583 | 38 | PFLU2545 | 90 | PFWH6_1926 | 36 |
| ORF02466 | PseBG33_2364 | PflA506_2242 | 92 | PchI3084_3174 | 70 | PchIO6_4138 | 30 | PflQ8_3242 | 78 | PflQ2_3112 | 77 | PflSS101_2136 | 91 | PFL_0932 | 44 | Pfl01_2232 | 86 | PFLU2365 | 91 | PFWH6_2274 | 89 |
| ORF02475 | PseBG33_2355 | PflA506_0217 | 24 | PchI3084_0273 | 25 | PchIO6_0225 | 24 | PflQ8_0935 | 33 | PflQ2_2925 | 23 | PflSS101_3463 | 24 | PFL_0255 | 25 | Pfl01_0250 | 26 | PFLU4085 | 27 | PFWH6_1926 | 24 |
| ORF02476 | PseBG33_2354 | PflA506_3446 | 28 | PchI3084_3840 | 28 | PchIO6_0225 | 28 | PflQ8_0935 | 25 | PflQ2_4530 | 27 | PflSS101_0233 | 27 | PFL_0213 | 28 | Pfl01_0216 | 27 | PFLU0206 | 27 | PFWH6_2193 | 25 |
| ORF02799 | PseBG33_2027 | PflA506_1995 | 91 | PchI3084_3828 | 77 | PchIO6_0989 | 43 | PflQ8_0202 | 22 | PflQ2_1695 | 75 | PflSS101_1951 | 91 | PFL_4039 | 81 | Pfl01_3784 | 81 | PFLU1040 | 44 | PFWH6_1047 | 42 |
| ORF02812 | PseBG33_2016 | PflA506_1983 | 93 | PchI3084_3355 | 57 | PchIO6_3657 | 56 | PflQ8_2057 | 56 | PflQ2_1672 | 39 | PflSS101_1938 | 92 | PFL_2293 | 57 | Pfl01_1848 | 43 | PFLU5798 | 43 | PFWH6_1926 | 77 |
| ORF02828 | PseBG33_2000 | PflA506_2222 | 38 | PchI3084_3659 | 39 | PchIO6_3952 | 39 | PflQ8_2987 | 38 | PflQ2_3112 | 23 | PflSS101_2109 | 95 | PFL_3835 | 26 | Pfl01_3798 | 61 | PFLU3566 | 59 | PFWH6_3849 | 39 |
| ORF02914 | PseBG33_0782 | PflA506_0745 | 93 | PchI3084_3174 | 43 | PchIO6_0126 | 29 | PflQ8_4827 | 83 | PflQ2_3112 | 46 | PflSS101_0775 | 93 | PFL_0932 | 81 | Pfl01_0874 | 83 | PFLU0757 | 91 | PFWH6_2914 | 89 |
| ORF02965 | PseBG33_0836 | PflA506_0798 | 92 | PchI3084_0855 | 83 | PchIO6_0861 | 82 | PflQ8_3242 | 26 | PflQ2_3112 | 25 | PflSS101_0832 | 89 | PFL_0864 | 84 | Pfl01_0798 | 66 | PFLU0295 | 30 | PFWH6_0820 | 88 |
| ORF02976 | PseBG33_0847 | PflA506_0808 | 94 | PchI3084_2974 | 27 | PchIO6_5540 | 24 | PflQ8_1239 | 24 | PflQ2_4231 | 24 | PflSS101_0843 | 94 | PFL_2663 | 53 | Pfl01_5008 | 25 | PFLU3643 | 28 | PFWH6_4738 | 21 |
| ORF03178 | PseBG33_1043 | PflA506_1001 | 93 | PchI3084_3848 | 42 | PchIO6_4138 | 42 | PflQ8_2798 | 43 | PflQ2_2980 | 43 | PflSS101_1043 | 92 | PFL_0995 | 79 | Pfl01_0931 | 75 | PFLU1022 | 86 | PFWH6_1030 | 83 |
| ORF03196 | PseBG33_1061 | PflA506_1022 | 95 | PchI3084_0981 | 87 | PchIO6_0989 | 87 | PflQ8_2987 | 23 | PflQ2_1695 | 43 | PflSS101_1061 | 96 | PFL_0982 | 89 | Pfl01_0923 | 89 | PFLU1040 | 96 | PFWH6_1047 | 91 |
| ORF03204 | PseBG33_1069 | PflA506_1030 | 93 | PchI3084_3950 | 21 | PchIO6_0633 | 21 | PflQ8_3242 | 24 | PflQ2_2980 | 25 | PflSS101_1069 | 93 | PFL_0992 | 79 | Pfl01_2232 | 25 | PFLU1022 | 26 | PFWH6_1796 | 56 |
| ORF03236 | PseBG33_1099 | PflA506_1059 | 92 | PchI3084_4710 | 84 | PchIO6_4957 | 85 | PflQ8_1239 | 25 | PflQ2_4902 | 25 | PflSS101_1097 | 92 | PFL_4912 | 88 | Pfl01_0594 | 41 | PFLU1087 | 94 | PFWH6_1047 | 21 |
| ORF03673 | PseBG33_1530 | PflA506_4277 | 91 | PchI3084_4498 | 65 | PchIO6_4739 | 64 | PflQ8_1239 | 24 | PflQ2_4231 | 23 | PflSS101_4358 | 92 | PFL_1371 | 67 | Pfl01_4379 | 26 | PFLU1405 | 74 | PFWH6_1417 | 85 |
| ORF04054 | PseBG33_1898 | PflA506_0808 | 91 | PchI3084_2974 | 79 | PchIO6_0225 | 80 | PflQ8_4192 | 76 | PflQ2_1453 | 76 | PflSS101_0843 | 91 | PFL_1740 | 80 | Pfl01_4209 | 77 | PFLU1839 | 93 | PFWH6_1770 | 87 |
| ORF04083 | PseBG33_1930 | PflA506_1893 | 96 | PchI3084_0855 | 22 | PchIO6_0861 | 22 | PflQ8_2798 | 25 | PflQ2_2980 | 26 | PflSS101_1855 | 96 | PFL_0992 | 57 | Pfl01_0931 | 25 | PFLU1022 | 28 | PFWH6_1796 | 88 |
| ORF04362 | PseBG33_4466 | PflA506_4277 | 95 | PchI3084_4498 | 74 | PchIO6_4739 | 75 | PflQ8_1239 | 73 | PflQ2_4231 | 71 | PflSS101_4358 | 95 | PFL_4627 | 78 | Pfl01_4379 | 74 | PFLU4968 | 86 | PFWH6_4738 | 88 |
| ORF04667 | PseBG33_4831 | PflA506_4277 | 27 | PchI3084_4498 | 27 | PchIO6_4739 | 27 | PflQ8_1239 | 25 | PflQ2_4231 | 25 | PflSS101_4708 | 88 | PFL_5378 | 75 | Pfl01_4379 | 26 | PFLU5361 | 52 | PFWH6_4738 | 26 |
| ORF04786 | PseBG33_4946 | PflA506_4759 | 93 | PchI3084_5255 | 73 | PchIO6_5540 | 73 | PflQ8_5164 | 72 | PflQ2_4902 | 72 | PflSS101_4814 | 93 | PFL_5511 | 70 | Pfl01_5008 | 76 | PFLU5463 | 90 | PFWH6_5214 | 86 |
| ORF04959 | PseBG33_5128 | PflA506_4932 | 67 | PchI3084_5442 | 68 | PchIO6_5726 | 67 | PflQ8_5352 | 83 | PflQ2_0538 | 82 | PflSS101_4985 | 69 | PFL_5706 | 68 | Pfl01_5189 | 69 | PFLU5629 | 67 | PFWH6_5394 | 67 |
| ORF05140 | PseBG33_5305 | PflA506_1983 | 41 | PchI3084_3355 | 41 | PchIO6_3657 | 41 | PflQ8_3969 | 51 | PflQ2_1672 | 52 | PflSS101_1938 | 42 | PFL_2293 | 40 | Pfl01_1848 | 56 | PFLU5798 | 94 | PFWH6_1926 | 41 |
| ORF05242 | PseBG33_5403 | PflA506_5184 | 96 | PchI3084_2164 | 29 | PchIO6_3670 | 81 | PflQ8_5164 | 24 | PflQ2_2928 | 36 | PflSS101_5234 | 97 | PFL_3177 | 39 | Pfl01_2342 | 41 | PFLU5895 | 96 | PFWH6_3831 | 40 |
| ORF02656 truncated | PseBG33_2167 | | | | | | | | | | | | | | | | | | | | |

## G. *P. fluorescens* SBW25

| SBW25 | A506 | % ID | 30-84 | % ID | O-6 | % ID | Q8r1-96 | % ID | Q2-87 | % ID | BG33R | % ID | SS101 | % ID | Pf0-1 | % ID | Pf-5 | %ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus tag | | | | | | | | | | | | | | | | | | |
| PFLU0473 | PflA506_0460 | 85 | Pchl3084_5442 | 21 | PchlO6_5726 | 22 | PflQ8_5352 | 23 | PflQ2_0538 | 22 | PseBG33_0489 | 91 | PflSS101_0492 | 87 | Pfl01_5189 | 22.8 | PFL_5706 | 22.6 |
| PFLU0593 | PflA506_3017 | 31 | Pchl3084_0627 | 75 | PchlO6_0633 | 76 | PflQ8_2798 | 26 | PflQ2_2980 | 26 | PseBG33_0610 | 88 | PflSS101_2915 | 32 | Pfl01_0593 | 78 | PFL_0646 | 77 |
| PFLU1022 | PflA506_1001 | 82 | Pchl3084_3848 | 41 | PchlO6_4138 | 41 | PflQ8_2798 | 42 | PflQ2_2980 | 42 | PseBG33_1043 | 82 | PflSS101_1043 | 82 | Pfl01_0931 | 78 | PFL_0995 | 78 |
| PFLU1040 | PflA506_1022 | 93 | Pchl3084_0981 | 87 | PchlO6_0989 | 87 | PflQ8_2987 | 23 | PflQ2_1695 | 43 | PseBG33_1061 | 93 | PflSS101_1061 | 94 | Pfl01_0923 | 89 | PFL_0982 | 89 |
| PFLU1087 | PflA506_1059 | 93 | Pchl3084_4710 | 86 | PchlO6_4957 | 86 | PflQ8_0637 | 21 | PflQ2_5073 | 22 | PseBG33_1099 | 92 | PflSS101_1097 | 93 | Pfl01_0594 | 41.38 | PFL_4912 | 87 |
| PFLU1405 | PflA506_1363 | 72 | Pchl3084_1354 | 67 | PchlO6_1431 | 67 | PflQ8_1239 | 22 | PflQ2_4231 | 22 | PseBG33_1530 | 72 | PflSS101_1406 | 73 | Pfl01_3784 | 22 | PFL_1371 | 68 |
| PFLU1839 | PflA506_1861 | 89 | Pchl3084_1705 | 78 | PchlO6_1837 | 78 | PflQ8_4192 | 75 | PflQ2_1453 | 76 | PseBG33_1898 | 88 | PflSS101_1822 | 89 | Pfl01_4209 | 79 | PFL_1740 | 81 |
| PFLU0206 | PflA506_0217 | 89 | Pchl3084_0224 | 85 | PchlO6_0225 | 85 | PflQ8_0935 | 34 | PflQ2_4530 | 35 | PseBG33_0251 | 89 | PflSS101_0233 | 89 | Pfl01_0216 | 88 | PFL_0213 | 91 |
| PFLU2202 | PflA506_5184 | 37 | Pchl3084_2164 | 24 | PchlO6_3258 | 43 | PflQ8_2798 | 22 | PflQ2_2928 | 44 | PseBG33_3617 | 40 | PflSS101_3450 | 41 | Pfl01_2342 | 33 | PFL_1386 | 44 |
| PFLU2216 | PflA506_5184 | 40 | Pchl3084_2164 | 34 | PchlO6_3258 | 41 | PflQ8_5164 | 28 | PflQ2_2928 | 42 | PseBG33_3617 | 45 | PflSS101_3450 | 44 | Pfl01_2342 | 37 | PFL_1386 | 46 |
| PFLU2365 | PflA506_2242 | 87 | Pchl3084_3174 | 71 | PchlO6_4138 | 30 | PflQ8_3242 | 79 | PflQ2_3112 | 78 | PseBG33_2364 | 87 | PflSS101_2136 | 87 | Pfl01_2232 | 86 | PFL_0932 | 45 |
| PFLU2509 | PflA506_1022 | 21 | Pchl3084_0981 | 22 | PchlO6_0989 | 21 | PflQ8_3242 | 22 | PflQ2_1695 | 20 | PseBG33_0489 | 23 | PflSS101_0492 | 24 | Pfl01_0923 | 23 | PFL_0982 | 26 |
| PFLU2545 | PflA506_3089 | 73 | Pchl3084_2488 | 43 | PchlO6_4229 | 41 | PflQ8_3550 | 38 | PflQ2_1672 | 34 | PseBG33_2445 | 88 | PflSS101_3096 | 59 | Pfl01_2583 | 39 | PFL_2391 | 74.5 |
| PFLU2562 | PflA506_2311 | 87 | Pchl3084_3659 | 76 | PchlO6_3952 | 76 | PflQ8_2987 | 27 | PflQ2_2980 | 26 | PseBG33_3219 | 90 | PflSS101_2208 | 90 | Pfl01_3798 | 27.71 | PFL_3835 | 74 |
| PFLU2593 | PflA506_3017 | 91 | Pchl3084_2407 | 80 | PchlO6_2626 | 80 | PflQ8_3242 | 25 | PflQ2_3112 | 24 | PseBG33_2513 | 90 | PflSS101_2915 | 90 | Pfl01_3318 | 82.14 | PFL_2604 | 81.6 |
| PFLU2598 | PflA506_0139 | 40 | Pchl3084_5442 | 39 | PchlO6_5726 | 39 | PflQ8_5352 | 39 | PflQ2_0538 | 38 | PseBG33_0163 | 39 | PflSS101_0139 | 40 | Pfl01_0121 | 39 | PFL_5706 | 41 |
| PFLU2688 | PflA506_3008 | 88 | Pchl3084_2486 | 74 | PchlO6_3015 | 74 | PflQ8_3550 | 33 | PflQ2_1672 | 32 | PseBG33_3069 | 89 | PflSS101_2361 | 88 | Pfl01_2583 | 72 | PFL_3315 | 41 |
| PFLU2941 | PflA506_1022 | 21 | Pchl3084_0981 | 22 | PchlO6_0989 | 26 | PflQ8_3242 | 21 | PflQ2_1695 | 22 | PseBG33_1034 | 22 | PflSS101_0492 | 24 | Pfl01_0923 | 22.94 | PFL_0982 | 26.6 |
| PFLU2948 | PflA506_5184 | 36 | Pchl3084_2164 | 33 | PchlO6_3258 | 40 | PflQ8_5164 | 24 | PflQ2_2928 | 41 | PseBG33_3617 | 40 | PflSS101_3450 | 41 | Pfl01_3121 | 34 | PFL_1386 | 42 |
| PFLU0295 | PflA506_0292 | 88 | Pchl3084_0320 | 79 | PchlO6_0324 | 78 | PflQ8_4827 | 32 | PflQ2_3112 | 28 | PseBG33_0325 | 87 | PflSS101_0311 | 88 | Pfl01_0293 | 81 | PFL_0310 | 81 |
| PFLU3218 | PflA506_3446 | 24 | Pchl3084_0224 | 24 | PchlO6_3255 | 26 | PflQ8_2430 | 71 | PflQ2_2925 | 27 | PseBG33_3630 | 24 | PflSS101_3463 | 24 | Pfl01_0216 | 23.9 | PFL_3620 | 29 |
| PFLU3378 | PflA506_2859 | 82 | Pchl3084_3950 | 48 | PchlO6_4229 | 49 | PflQ8_3550 | 87 | PflQ2_1672 | 37 | PseBG33_2658 | 87 | PflSS101_2880 | 83 | Pfl01_2462 | 86 | PFL_4092 | 49 |
| PFLU3566 | PflA506_2218 | 38 | Pchl3084_3046 | 42 | PchlO6_3307 | 42 | PflQ8_2987 | 41 | PflQ2_0538 | 25 | PseBG33_2000 | 59 | PflSS101_1922 | 58 | Pfl01_3798 | 68 | PFL_3835 | 28 |
| PFLU3633 | PflA506_3089 | 43 | Pchl3084_2488 | 55 | PchlO6_3015 | 42 | PflQ8_3550 | 37 | PflQ2_0538 | 25 | PseBG33_2445 | 41 | PflSS101_3096 | 41 | Pfl01_2583 | 41 | PFL_3315 | 74 |
| PFLU3643 | PflA506_0808 | 28 | Pchl3084_2974 | 85 | PchlO6_5540 | 26 | PflQ8_1239 | 23 | PflQ2_4231 | 23 | PseBG33_0847 | 28 | PflSS101_0843 | 28 | Pfl01_5008 | 25 | PFL_5169 | 34 |
| PFLU3698 | PflA506_2346 | 89 | Pchl3084_3605 | 86 | PchlO6_0861 | 24 | PflQ8_3000 | 85 | PflQ2_4231 | 23 | PseBG33_0836 | 22 | PflSS101_3074 | 89 | Pfl01_4379 | 23.34 | PFL_3715 | 87.9 |
| PFLU3750 | PflA506_3139 | 86 | Pchl3084_2519 | 62 | PchlO6_2744 | 62 | PflQ8_2798 | 21 | PflQ2_4231 | 21 | PseBG33_3265 | 88 | PflSS101_3147 | 88 | Pfl01_2318 | 64.6 | PFL_2772 | 64.4 |
| PFLU4069 | PflA506_5184 | 38 | Pchl3084_2164 | 24 | PchlO6_3258 | 57 | PflQ8_5164 | 26 | PflQ2_2928 | 56 | PseBG33_3617 | 92 | PflSS101_3450 | 92 | Pfl01_2342 | 36 | PFL_1386 | 47 |
| PFLU4085 | PflA506_3446 | 90 | Pchl3084_0273 | 87 | PchlO6_0225 | 32 | PflQ8_0935 | 31 | PflQ2_4530 | 32 | PseBG33_3630 | 90 | PflSS101_3463 | 91 | Pfl01_0250 | 91.6 | PFL_0255 | 90.1 |
| PFLU4093 | PflA506_2218 | 37 | Pchl3084_3046 | 83 | PchlO6_3307 | 83 | PflQ8_2987 | 83 | PflQ2_2980 | 24 | PseBG33_2000 | 39 | PflSS101_2109 | 37 | Pfl01_3798 | 43 | PFL_3835 | 28 |
| PFLU4968 | PflA506_4277 | 81 | Pchl3084_4498 | 74 | PchlO6_4739 | 73 | PflQ8_1239 | 72 | PflQ2_4231 | 70 | PseBG33_4466 | 83 | PflSS101_4358 | 81 | Pfl01_4379 | 71 | PFL_4627 | 78 |
| PFLU5361 | PflA506_4277 | 24 | Pchl3084_2211 | 24 | PchlO6_2435 | 24 | PflQ8_1239 | 24 | PflQ2_4231 | 24 | PseBG33_4831 | 52 | PflSS101_4708 | 51 | Pfl01_4379 | 24 | PFL_5378 | 51 |
| PFLU5463 | PflA506_4759 | 89 | Pchl3084_5255 | 76 | PchlO6_5540 | 75 | PflQ8_5164 | 74 | PflQ2_4902 | 75 | PseBG33_4946 | 89 | PflSS101_4814 | 88 | Pfl01_5008 | 75 | PFL_5511 | 69 |
| PFLU5629 | PflA506_4932 | 89 | Pchl3084_5442 | 89 | PchlO6_5726 | 88 | PflQ8_5352 | 65 | PflQ2_0538 | 66 | PseBG33_5128 | 67 | PflSS101_4985 | 91 | Pfl01_5189 | 81 | PFL_5706 | 89 |
| PFLU5798 | PflA506_1983 | 42 | Pchl3084_3355 | 42 | PchlO6_3657 | 41 | PflQ8_3969 | 50 | PflQ2_1672 | 52 | PseBG33_5305 | 94 | PflSS101_1938 | 43 | Pfl01_1848 | 57 | PFL_2293 | 41 |
| PFLU5895 | PflA506_5184 | 94 | Pchl3084_2164 | 29 | PchlO6_3670 | 81 | PflQ8_5164 | 28 | PflQ2_2928 | 36 | PseBG33_5403 | 94 | PflSS101_5234 | 94 | Pfl01_2342 | 41 | PFL_3177 | 39 |
| PFLU0595 | PflA506_0576 | 93 | Pchl3084_0629 | 84 | PchlO6_0635 | 84 | PflQ8_0637 | 85 | PflQ2_5073 | 85 | PseBG33_0612 | 93 | PflSS101_0606 | 92 | Pfl01_0596 | 85 | PFL_0648 | 84 |
| PFLU6132 | PflA506_5433 | 92 | Pchl3084_0129 | 36 | PchlO6_0126 | 36 | PflQ8_0202 | 36 | PflQ2_0538 | 35 | PseBG33_5128 | 35 | PflSS101_4985 | 34 | Pfl01_0121 | 38 | PFL_0125 | 38 |
| PFLU0757 | PflA506_0745 | 89 | Pchl3084_3174 | 44 | PchlO6_0324 | 31 | PflQ8_4827 | 83 | PflQ2_3112 | 47 | PseBG33_0782 | 89 | PflSS101_0775 | 89 | Pfl01_0874 | 83 | PFL_0932 | 82 |

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| A506 | | 30-84 | | O-6 | | Q8r1-96 | | Q2-87 | | BG33R | | SS101 | | Pf-5 | | PF0-1 | | SBW25 | | WH6 | |
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| ORF00072 | PflA506_1995 | Pchl3084_3828 | 78 | PchlO6_0989 | 43 | PflQ8_0202 | 22 | PflQ2_1695 | 76 | PseBG33_2027 | 93 | PflSS101_1951 | 96 | PFL_4039 | 80 | Pfl01_3784 | 80.6 | PFLU1040 | 45 | PFWH6_1047 | 43 |
| ORF00294 | PflA506_2218 | Pchl3084_3046 | 39 | PchlO6_3307 | 39 | PflQ8_2987 | 39 | PflQ2_0538 | 24 | PseBG33_2840 | 42 | PflSS101_2109 | 95 | PFL_3835 | 29 | Pfl01_3798 | 37.8 | PFLU4093 | 39 | PFWH6_2186 | 82 |
| ORF00298 | PflA506_2222 | Pchl3084_3046 | 42 | PchlO6_3307 | 42 | PflQ8_2987 | 41 | PflQ2_2980 | 27 | PseBG33_2840 | 43 | PflSS101_2113 | 100 | PFL_3835 | 27 | Pfl01_3798 | 41.1 | PFLU4093 | 41 | PFWH6_2190 | 93 |
| ORF00319 | PflA506_2242 | Pchl3084_3174 | 72 | PchlO6_4138 | 30 | PflQ8_3242 | 78 | PflQ2_3112 | 77 | PseBG33_2364 | 92 | PflSS101_2136 | 94 | PFL_0932 | 45 | Pfl01_2232 | 86 | PFLU2365 | 91 | PFWH6_2249 | 88 |
| ORF00395 | PflA506_2311 | Pchl3084_3659 | 75 | PchlO6_3952 | 75 | PflQ8_2987 | 27 | PflQ2_2980 | 26 | PseBG33_3219 | 89 | PflSS101_2208 | 90 | PFL_3835 | 75 | Pfl01_3798 | 27.3 | PFLU2562 | 90 | PFWH6_2397 | 88 |
| ORF00435 | PflA506_2346 | Pchl3084_3605 | 84 | PchlO6_0861 | 24 | PflQ8_3000 | 84 | PflQ2_4231 | 23 | PseBG33_0836 | 23 | PflSS101_3074 | 95 | PFL_3715 | 86 | Pfl01_4379 | 23 | PFLU3698 | 92 | PFWH6_0820 | 23 |
| ORF00877 | PflA506_2772 | Pchl3084_3046 | 37 | PchlO6_3307 | 37 | PflQ8_2987 | 38 | PflQ2_3112 | 25 | PseBG33_2840 | 62 | PflSS101_2109 | 39 | PFL_3835 | 26 | Pfl01_3798 | 36.2 | PFLU4093 | 39 | PFWH6_2186 | 38 |
| ORF00968 | PflA506_2860 | Pchl3084_3950 | 48 | PchlO6_4229 | 48 | PflQ8_3550 | 83 | PflQ2_0688 | 82 | PseBG33_2658 | 88 | PflSS101_2880 | 93 | PFL_4092 | 49 | Pfl01_2462 | 84.6 | PFLU3378 | 84 | PFWH6_3086 | 84 |
| ORF01128 | PflA506_3009 | Pchl3084_2783 | 72 | PchlO6_3015 | 71 | PflQ8_2057 | 35 | PflQ2_0688 | 33 | PseBG33_3069 | 91 | PflSS101_2361 | 96 | PFL_3315 | 41 | Pfl01_2583 | 71 | PFLU2688 | 88 | PFWH6_3086 | 33 |
| ORF01138 | PflA506_3018 | Pchl3084_2407 | 82 | PchlO6_2626 | 82 | PflQ8_4827 | 25 | PflQ2_3112 | 24 | PseBG33_2513 | 94 | PflSS101_2915 | 97 | PFL_2604 | 81 | Pfl01_3318 | 85.2 | PFLU2593 | 91 | PFWH6_0820 | 25 |
| ORF01212 | PflA506_3090 | Pchl3084_2488 | 43 | PchlO6_4229 | 40 | PflQ8_3550 | 36 | PflQ2_0688 | 36 | PseBG33_2445 | 72 | PflSS101_3096 | 59 | PFL_2391 | 73 | Pfl01_2583 | 40.2 | PFLU2545 | 75 | PFWH6_1926 | 33 |
| ORF01265 | PflA506_3140 | Pchl3084_2519 | 57 | PchlO6_2744 | 57 | PflQ8_1239 | 28 | PflQ2_1453 | 33 | PseBG33_3265 | 84 | PflSS101_3147 | 85 | PFL_2772 | 66 | Pfl01_2318 | 65.2 | PFLU3750 | 90 | PFWH6_3525 | 79 |
| ORF01571 | PflA506_3447 | Pchl3084_0707 | 87 | PchlO6_0225 | 32 | PflQ8_0935 | 30 | PflQ2_4530 | 31 | PseBG33_3630 | 95 | PflSS101_3463 | 97 | PFL_0255 | 89 | Pfl01_0250 | 88.6 | PFLU4085 | 94 | PFWH6_2193 | 24 |
| ORF01922 | PflA506_3812 | Pchl3084_4498 | 21 | PchlO6_4739 | 21 | PflQ8_1239 | 20 | PflQ2_4231 | 20 | PseBG33_4466 | 20 | PflSS101_3832 | 96 | PFL_3154 | 31 | Pfl01_2318 | 65.2 | PFLU4968 | 23 | PFWH6_4738 | 20 |
| ORF02404 | PflA506_4278 | Pchl3084_4498 | 73 | PchlO6_4739 | 74 | PflQ8_1239 | 73 | PflQ2_4231 | 71 | PseBG33_4466 | 95 | PflSS101_4358 | 98 | PFL_4627 | 77 | Pfl01_4379 | 73 | PFLU4968 | 86 | PFWH6_4738 | 87 |
| ORF02895 | PflA506_4759 | Pchl3084_5255 | 77 | PchlO6_5540 | 76 | PflQ8_5164 | 76 | PflQ2_4902 | 76 | PseBG33_4946 | 95 | PflSS101_4814 | 97 | PFL_5511 | 71 | Pfl01_5008 | 77.5 | PFLU5463 | 89 | PFWH6_5214 | 86 |
| ORF03061 | PflA506_4932 | Pchl3084_5442 | 88 | PchlO6_5726 | 88 | PflQ8_5352 | 66 | PflQ2_0538 | 65 | PseBG33_5128 | 68 | PflSS101_4985 | 93 | PFL_5706 | 87 | Pfl01_5189 | 80.1 | PFLU5629 | 90 | PFWH6_5394 | 87 |
| ORF03319 | PflA506_5184 | Pchl3084_2164 | 26 | PchlO6_3670 | 82 | PflQ8_5164 | 25 | PflQ2_2928 | 36 | PseBG33_5403 | 98 | PflSS101_5234 | 99 | PFL_3177 | 38 | Pfl01_2342 | 40.5 | PFLU5895 | 96 | PFWH6_3831 | 40 |
| ORF03577 | PflA506_5433 | Pchl3084_0129 | 35 | PchlO6_0126 | 35 | PflQ8_0202 | 35 | PflQ2_0538 | 34 | PseBG33_5128 | 34 | PflSS101_5488 | 96 | PFL_0125 | 38 | Pfl01_0121 | 36.3 | PFLU6132 | 92 | PFWH6_5931 | 91 |
| ORF03717 | PflA506_0139 | Pchl3084_5442 | 37 | PchlO6_5726 | 37 | PflQ8_5352 | 39 | PflQ2_0538 | 39 | PseBG33_0163 | 94 | PflSS101_0139 | 96 | PFL_0147 | 73 | Pfl01_5189 | 38.3 | PFLU2598 | 43 | PFWH6_5394 | 36 |
| ORF03796 | PflA506_0217 | Pchl3084_0224 | 86 | PchlO6_0225 | 85 | PflQ8_0935 | 34 | PflQ2_4530 | 35 | PseBG33_0251 | 90 | PflSS101_0233 | 90 | PFL_0213 | 90 | Pfl01_0216 | 87.8 | PFLU0206 | 95 | PFWH6_2193 | 21 |
| ORF03877 | PflA506_0292 | Pchl3084_0320 | 76 | PchlO6_0324 | 75 | PflQ8_4827 | 33 | PflQ2_3112 | 28 | PseBG33_0325 | 91 | PflSS101_0311 | 95 | PFL_0310 | 80 | Pfl01_0293 | 80 | PFLU0295 | 90 | PFWH6_0295 | 84 |
| ORF04052 | PflA506_0460 | Pchl3084_5442 | 22 | PchlO6_5726 | 22 | PflQ8_5352 | 21 | PflQ2_0538 | 22 | PseBG33_0489 | 86 | PflSS101_0492 | 85 | PFL_5706 | 23 | Pfl01_0931 | 26.2 | PFLU0473 | 85 | PFWH6_5394 | 22 |
| ORF04175 | PflA506_0576 | Pchl3084_0629 | 90 | PchlO6_0635 | 90 | PflQ8_0637 | 89 | PflQ2_5073 | 89 | PseBG33_0612 | 93 | PflSS101_0606 | 94 | PFL_0648 | 89 | Pfl01_0596 | 88.8 | PFLU0595 | 93 | PFWH6_0621 | 88 |
| ORF04338 | PflA506_0745 | Pchl3084_3174 | 44 | PchlO6_5726 | 29 | PflQ8_4827 | 82 | PflQ2_3112 | 46 | PseBG33_0782 | 93 | PflSS101_0775 | 97 | PFL_0932 | 81 | Pfl01_0874 | 83.5 | PFLU0757 | 91 | PFWH6_0766 | 90 |
| ORF04388 | PflA506_0798 | Pchl3084_0855 | 84 | PchlO6_0861 | 83 | PflQ8_3242 | 27 | PflQ2_3112 | 26 | PseBG33_0836 | 92 | PflSS101_0832 | 89 | PFL_0864 | 84 | Pfl01_0798 | 66 | PFLU0295 | 30 | PFWH6_0820 | 88 |
| ORF04399 | PflA506_0808 | Pchl3084_2974 | 27 | PchlO6_0989 | 21 | PflQ8_1239 | 24 | PflQ2_4231 | 24 | PseBG33_0847 | 92 | PflSS101_0843 | 94 | PFL_2663 | 53 | Pfl01_2342 | 23.5 | PFLU3643 | 28 | PFWH6_4738 | 21 |
| ORF04601 | PflA506_1001 | Pchl3084_3848 | 42 | PchlO6_4138 | 42 | PflQ8_2798 | 43 | PflQ2_2980 | 43 | PseBG33_1043 | 93 | PflSS101_1043 | 92 | PFL_0995 | 77 | Pfl01_0931 | 74.6 | PFLU1022 | 85 | PFWH6_1030 | 82 |
| ORF04622 | PflA506_1022 | Pchl3084_0981 | 87 | PchlO6_0989 | 87 | PflQ8_2987 | 23 | PflQ2_1695 | 42 | PseBG33_1061 | 93 | PflSS101_1061 | 94 | PFL_0982 | 89 | Pfl01_0923 | 88.6 | PFLU1040 | 96 | PFWH6_1047 | 89 |
| ORF04630 | PflA506_1030 | Pchl3084_3848 | 22 | PchlO6_4138 | 21 | PflQ8_3242 | 24 | PflQ2_2980 | 25 | PseBG33_1069 | 89 | PflSS101_1069 | 94 | PFL_0992 | 78 | Pfl01_2232 | 25 | PFLU1022 | 26 | PFWH6_1796 | 55 |
| ORF04662 | PflA506_1059 | Pchl3084_4710 | 86 | PchlO6_4957 | 86 | PflQ8_5164 | 26 | PflQ2_1695 | 21 | PseBG33_1099 | 94 | PflSS101_1097 | 95 | PFL_4912 | 88 | Pfl01_0596 | 23.2 | PFLU1087 | 95 | PFWH6_4738 | 24 |
| ORF04972 | PflA506_1363 | Pchl3084_1354 | 65 | PchlO6_1431 | 64 | PflQ8_1239 | 25 | PflQ2_4231 | 25 | PseBG33_1530 | 92 | PflSS101_1406 | 97 | PFL_1371 | 67 | Pfl01_4379 | 25.9 | PFLU1405 | 74 | PFWH6_1417 | 86 |
| ORF05452 | PflA506_1861 | Pchl3084_1705 | 77 | PchlO6_1837 | 77 | PflQ8_4192 | 74 | PflQ2_1453 | 75 | PseBG33_1898 | 89 | PflSS101_1822 | 93 | PFL_1740 | 79 | Pfl01_4209 | 77.8 | PFLU1839 | 94 | PFWH6_1770 | 84 |
| ORF05482 | PflA506_1893 | Pchl3084_0855 | 23 | PchlO6_0861 | 23 | PflQ8_2798 | 26 | PflQ2_2980 | 27 | PseBG33_1930 | 96 | PflSS101_1855 | 99 | PFL_0992 | 57 | Pfl01_0798 | 20.2 | PFLU1022 | 28 | PFWH6_1796 | 90 |

## I. *P. fluorescens* SS101

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| ORF00242 | PflSS101_4814 | PflA506_4759 | 97 | Pchl3084_5255 | 76 | PchlO6_5540 | 75 | PflQ8_5164 | 74 | PflQ2_4902 | 75 | PseBG33_4946 | 95 | PFL_5511 | 70 | PFLU5463 | 89 | Pfl01_5008 | 77 |
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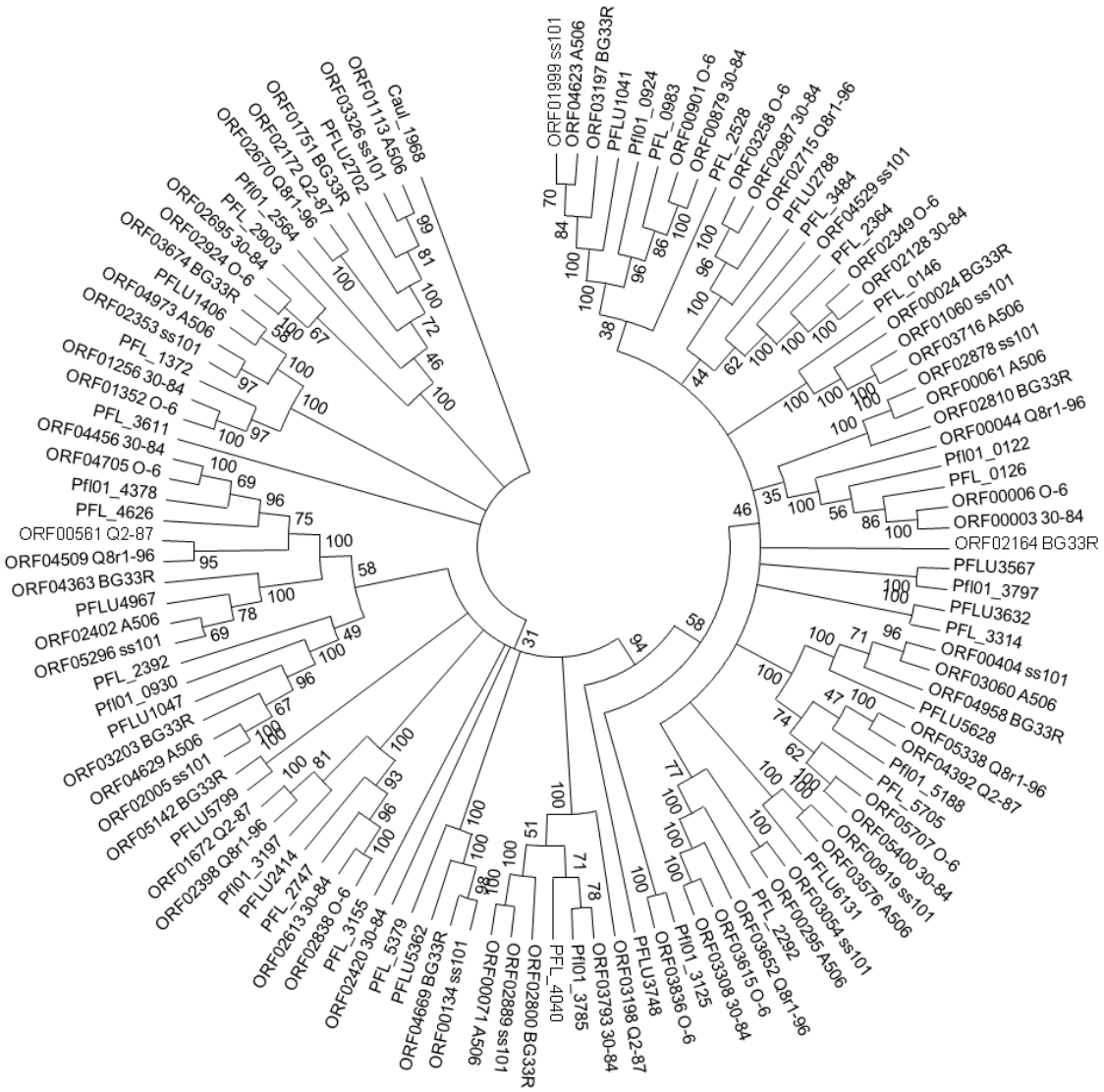
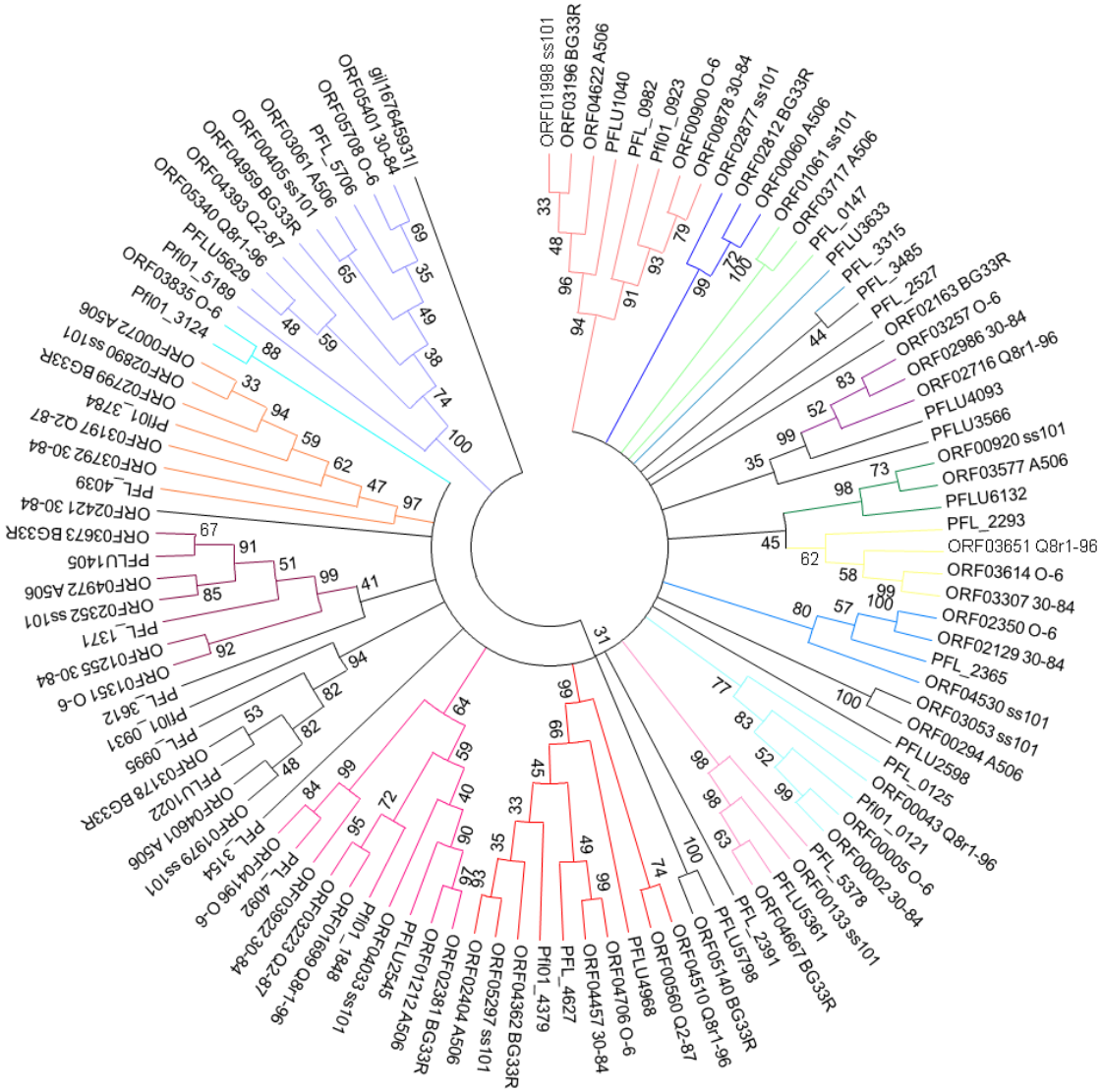
| ORF00660 | PflSS101_5234 | PflA506_5184 | 99 | Pchl3084_2164 | 26 | PchlO6_3670 | 82 | PflQ8_4192 | 24 | PflQ2_2928 | 37 | PseBG33_5403 | 98 | PFL_3177 | 39 | PFLU5895 | 96 | Pfl01_2342 | 41 |
| ORF00920 | PflSS101_5488 | PflA506_5433 | 96 | Pchl3084_0129 | 36 | PchlO6_0126 | 36 | PflQ8_0202 | 36 | PflQ2_0538 | 34 | PseBG33_5128 | 33 | PFL_0125 | 38 | PFLU6132 | 92 | Pfl01_0121 | 37 |
| ORF01061 | PflSS101_0139 | PflA506_0139 | 96 | Pchl3084_5442 | 37 | PchlO6_5726 | 37 | PflQ8_5352 | 40 | PflQ2_0538 | 39 | PseBG33_0163 | 94 | PFL_0147 | 73 | PFLU2598 | 42 | Pfl01_5189 | 38 |
| ORF01155 | PflSS101_0233 | PflA506_0217 | 92 | Pchl3084_0224 | 84 | PchlO6_0225 | 84 | PflQ8_0935 | 33 | PflQ2_4530 | 34 | PseBG33_0251 | 91 | PFL_0213 | 90 | PFLU0206 | 95 | Pfl01_0216 | 88 |
| ORF01237 | PflSS101_0311 | PflA506_0292 | 95 | Pchl3084_0320 | 76 | PchlO6_0324 | 75 | PflQ8_4827 | 33 | PflQ2_3112 | 28 | PseBG33_0325 | 91 | PFL_0310 | 80 | PFLU0295 | 90 | Pfl01_0293 | 79 |
| ORF01428 | PflSS101_0492 | PflA506_0460 | 85 | Pchl3084_5442 | 21 | PchlO6_5726 | 21 | PflQ8_5352 | 21 | PflQ2_0538 | 21 | PseBG33_0489 | 88 | PFL_5706 | 23 | PFLU0473 | 87 | Pfl01_0931 | 27 |
| ORF01546 | PflSS101_0606 | PflA506_0576 | 94 | Pchl3084_0629 | 90 | PchlO6_0635 | 90 | PflQ8_0637 | 89 | PflQ2_5073 | 88 | PseBG33_0612 | 93 | PFL_0648 | 89 | PFLU0595 | 93 | Pfl01_0596 | 88 |
| ORF01711 | PflSS101_0775 | PflA506_0745 | 97 | Pchl3084_3174 | 44 | PchlO6_5726 | 29 | PflQ8_4827 | 82 | PflQ2_3112 | 46 | PseBG33_0782 | 93 | PFL_0932 | 81 | PFLU0757 | 91 | Pfl01_0874 | 83 |
| ORF01765 | PflSS101_0832 | PflA506_0798 | 89 | Pchl3084_0855 | 83 | PchlO6_0861 | 83 | PflQ8_3242 | 26 | PflQ2_3112 | 26 | PseBG33_0836 | 89 | PFL_0864 | 85 | PFLU0295 | 31 | Pfl01_0798 | 65 |
| ORF01777 | PflSS101_0843 | PflA506_0808 | 94 | Pchl3084_2974 | 27 | PchlO6_5540 | 23 | PflQ8_1239 | 24 | PflQ2_4231 | 24 | PseBG33_0847 | 93 | PFL_2663 | 53 | PFLU3643 | 28 | Pfl01_5008 | 25 |
| ORF01979 | PflSS101_1043 | PflA506_1001 | 92 | Pchl3084_3848 | 42 | PchlO6_4138 | 42 | PflQ8_2798 | 43 | PflQ2_2980 | 43 | PseBG33_1043 | 92 | PFL_0995 | 77 | PFLU1022 | 85 | Pfl01_0931 | 74 |
| ORF01998 | PflSS101_1061 | PflA506_1022 | 97 | Pchl3084_0981 | 87 | PchlO6_0989 | 87 | PflQ8_2987 | 23 | PflQ2_1695 | 43 | PseBG33_1061 | 96 | PFL_0982 | 89 | PFLU1040 | 96 | Pfl01_0923 | 89 |
| ORF02006 | PflSS101_1069 | PflA506_1030 | 94 | Pchl3084_3848 | 23 | PchlO6_4138 | 21 | PflQ8_3242 | 24 | PflQ2_2980 | 25 | PseBG33_1069 | 90 | PFL_0992 | 78 | PFLU1022 | 26 | Pfl01_2232 | 25 |
| ORF02035 | PflSS101_1097 | PflA506_1059 | 95 | Pchl3084_4710 | 86 | PchlO6_4957 | 86 | PflQ8_1239 | 23 | PflQ2_5073 | 21 | PseBG33_1099 | 94 | PFL_4912 | 88 | PFLU1087 | 95 | Pfl01_0594 | 41 |
| ORF02352 | PflSS101_1406 | PflA506_1363 | 96 | Pchl3084_1354 | 64 | PchlO6_1431 | 63 | PflQ8_1239 | 25 | PflQ2_4231 | 24 | PseBG33_1530 | 92 | PFL_1371 | 67 | PFLU1405 | 74 | Pfl01_4379 | 26 |
| ORF02769 | PflSS101_1822 | PflA506_1861 | 93 | Pchl3084_1705 | 77 | PchlO6_1837 | 77 | PflQ8_4192 | 75 | PflQ2_1453 | 75 | PseBG33_1898 | 89 | PFL_1740 | 80 | PFLU1839 | 94 | Pfl01_4209 | 78 |
| ORF02799 | PflSS101_1855 | PflA506_1893 | 99 | Pchl3084_0855 | 23 | PchlO6_0861 | 23 | PflQ8_2798 | 26 | PflQ2_2980 | 27 | PseBG33_1930 | 96 | PFL_0992 | 57 | PFLU1022 | 28 | Pfl01_0931 | 27 |
| ORF02861 | PflSS101_1922 | PflA506_2218 | 38 | Pchl3084_3046 | 39 | PchlO6_3307 | 39 | PflQ8_2987 | 38 | PflQ2_2980 | 23 | PseBG33_2000 | 92 | PFL_3835 | 26 | PFLU3566 | 59 | Pfl01_3798 | 60 |
| ORF02877 | PflSS101_1938 | PflA506_1983 | 90 | Pchl3084_3355 | 56 | PchlO6_3657 | 56 | PflQ8_2057 | 56 | PflQ2_1672 | 39 | PseBG33_2016 | 90 | PFL_2293 | 58 | PFLU5798 | 44 | Pfl01_1848 | 43 |
| ORF02890 | PflSS101_1951 | PflA506_1995 | 96 | Pchl3084_3828 | 78 | PchlO6_0989 | 43 | PflQ8_0202 | 22 | PflQ2_1695 | 76 | PseBG33_2027 | 93 | PFL_4039 | 81 | PFLU1040 | 44 | Pfl01_3784 | 81 |
| ORF02973 | PflSS101_2030 | PflA506_4759 | 22 | Pchl3084_3848 | 23 | PchlO6_5540 | 22 | PflQ8_2798 | 23 | PflQ2_2980 | 23 | PseBG33_4946 | 22 | PFL_2240 | 75 | PFLU5463 | 23 | Pfl01_5008 | 23 |
| ORF03053 | PflSS101_2109 | PflA506_2218 | 95 | Pchl3084_3046 | 39 | PchlO6_3307 | 39 | PflQ8_2987 | 39 | PflQ2_0538 | 24 | PseBG33_2840 | 42 | PFL_3835 | 29 | PFLU4093 | 39 | Pfl01_3798 | 38 |
| ORF03057 | PflSS101_2113 | PflA506_2222 | 100 | Pchl3084_3046 | 42 | PchlO6_3307 | 42 | PflQ8_2987 | 41 | PflQ2_2980 | 27 | PseBG33_2840 | 43 | PFL_3835 | 27 | PFLU4093 | 41 | Pfl01_3798 | 40 |
| ORF03080 | PflSS101_2136 | PflA506_2242 | 94 | Pchl3084_3174 | 71 | PchlO6_4138 | 29 | PflQ8_3242 | 78 | PflQ2_3112 | 77 | PseBG33_2364 | 91 | PFL_0932 | 44 | PFLU2365 | 91 | Pfl01_2232 | 85 |
| ORF03155 | PflSS101_2208 | PflA506_2311 | 90 | Pchl3084_3659 | 76 | PchlO6_3952 | 77 | PflQ8_2987 | 27 | PflQ2_2980 | 26 | PseBG33_3219 | 93 | PFL_3835 | 74 | PFLU2562 | 92 | Pfl01_3798 | 28 |
| ORF03312 | PflSS101_2361 | PflA506_3008 | 97 | Pchl3084_2783 | 72 | PchlO6_3015 | 72 | PflQ8_2057 | 35 | PflQ2_0688 | 34 | PseBG33_3069 | 91 | PFL_3315 | 40 | PFLU2688 | 88 | Pfl01_2583 | 72 |
| ORF03808 | PflSS101_2880 | PflA506_2859 | 93 | Pchl3084_3950 | 47 | PchlO6_4229 | 47 | PflQ8_3550 | 83 | PflQ2_0688 | 82 | PseBG33_2658 | 88 | PFL_4092 | 48 | PFLU3378 | 86 | Pfl01_2462 | 85 |
| ORF03842 | PflSS101_2915 | PflA506_3017 | 97 | Pchl3084_2407 | 82 | PchlO6_2626 | 82 | PflQ8_3242 | 23 | PflQ2_3112 | 23 | PseBG33_2513 | 93 | PFL_2604 | 81 | PFLU2593 | 91 | Pfl01_3318 | 86 |
| ORF04009 | PflSS101_3074 | PflA506_2346 | 96 | Pchl3084_3605 | 86 | PchlO6_0861 | 24 | PflQ8_3000 | 85 | PflQ2_4231 | 23 | PseBG33_0836 | 23 | PFL_3715 | 86 | PFLU3698 | 92 | Pfl01_4379 | 23 |
| ORF04033 | PflSS101_3096 | PflA506_3089 | 57 | Pchl3084_2488 | 40 | PchlO6_4229 | 36 | PflQ8_2057 | 33 | PflQ2_0688 | 34 | PseBG33_2445 | 57 | PFL_3485 | 62 | PFLU2545 | 61 | Pfl01_1848 | 37 |
| ORF04087 | PflSS101_3147 | PflA506_3139 | 87 | Pchl3084_2519 | 60 | PchlO6_2744 | 60 | PflQ8_1239 | 23 | PflQ2_1695 | 22 | PseBG33_3265 | 89 | PFL_2772 | 68 | PFLU3750 | 91 | Pfl01_2318 | 67 |
| ORF04397 | PflSS101_3450 | PflA506_5184 | 38 | Pchl3084_2164 | 38 | PchlO6_3258 | 58 | PflQ8_5164 | 26 | PflQ2_2928 | 57 | PseBG33_3617 | 91 | PFL_1386 | 47 | PFLU4069 | 94 | Pfl01_2342 | 36 |
| ORF04412 | PflSS101_3463 | PflA506_3446 | 97 | Pchl3084_3950 | 47 | PchlO6_3957 | 47 | PflQ8_0935 | 30 | PflQ2_4530 | 31 | PseBG33_3630 | 96 | PFL_0255 | 90 | PFLU4085 | 95 | Pfl01_0250 | 88 |
| ORF04530 | PflSS101_3593 | PflA506_1363 | 28 | Pchl3084_2211 | 80 | PchlO6_2435 | 80 | PflQ8_1239 | 29 | PflQ2_4231 | 33 | PseBG33_4831 | 28 | PFL_2365 | 80 | PFLU5361 | 29 | Pfl01_4379 | 33 |
| ORF04772 | PflSS101_3832 | PflA506_3811 | 96 | Pchl3084_4498 | 20 | PchlO6_4739 | 21 | PflQ8_1239 | 18 | PflQ2_4231 | 21 | PseBG33_4466 | 21 | PFL_3154 | 30 | PFLU4968 | 23 | Pfl01_4379 | 22 |
| ORF05297 | PflSS101_4358 | PflA506_4277 | 97 | Pchl3084_4498 | 71 | PchlO6_4739 | 72 | PflQ8_1239 | 71 | PflQ2_4231 | 69 | PseBG33_4466 | 92 | PFL_4627 | 76 | PFLU4968 | 86 | Pfl01_4379 | 73 |

**Appendix 3. Neighbor Joining analysis of the anti-ECF sigma factors of the *P. fluorescens* group.** Branches with bootstrap values less than 30 are collapsed.



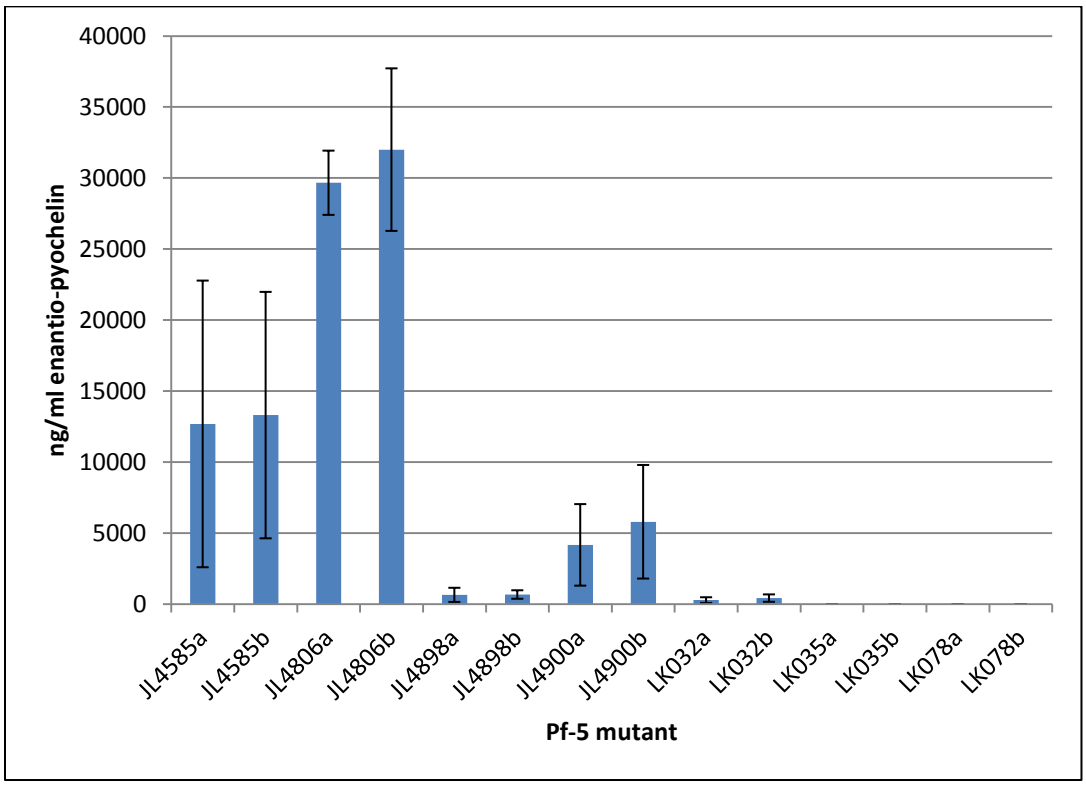
**Appendix 4. Neighbor Joining analysis of the N-terminal signaling domain of the TBDTs of the *P. fluorescens* group.** Branches are colored to indicate related TBDTs. Branches with bootstrap values less than 30 are collapsed.



**Appendix 5. HPLC analysis of enantio-pyochelin production by Pf-5 siderophore mutants.**

| Lab # | phenotype, mutant | Enantio-pyochelin (ng/ml) | | | Average | St dev |
|---|---|---|---|---|---|---|
| | | 10/6/2010 | 10/21/2010 | 11/17/2010 | | |
| JL4585a | Wt | 23665.1 | 3813.3 | 10583.9 | 12687.433 | 10091.69 |
| JL4585b | Wt | 20697.1 | 3755.9 | 15472.7 | 13308.567 | 8675.464 |
| JL4806a | pvd-, *pvdI* | 28302.4 | 32278 | 28421.3 | 29667.233 | 2261.772 |
| JL4806b | pvd-, *pvdI* | | 27953.1 | 36047 | 32000.05 | 5723.252 |
| JL4898a | reduced epch, *pchC* | 602.2 | 182.3 | 1174 | 652.83333 | 497.7851 |
| JL4898b | reduced epch, *pchC* | 752.4 | 345.3 | 931 | 676.23333 | 300.1868 |
| JL4900a | pvd- reduced epch, *pvdI pchC* | 5865.9 | 856.3 | 5791.7 | 4171.3 | 2871.114 |
| JL4900b | pvd- reduced epch, *pvdI pchC* | 8565.6 | 1214.6 | 7614.5 | 5798.2333 | 3997.927 |
| LK032a | pvd- reduced epch, *pvdI pchA* | 374.6 | 56.7 | 423.5 | 284.93333 | 199.1624 |
| LK032b | pvd- reduced epch, *pvdI pchA* | 240.2 | present | 608.7 | 424.45 | 260.5688 |
| LK035a | pvd- pch-, *pvdI-pchA pchC* | not run | 0 | 0 | 0 | 0 |
| LK035b | pvd- pch-, *pvdI- pchA pchC* | not run | 0 | 0 | 0 | 0 |
| LK078a | pch-, *pchA* | 0 | 0 | 0 | 0 | 0 |
| LK078b | pch-, *pchA* | 0 | 0 | 0 | 0 | 0 |

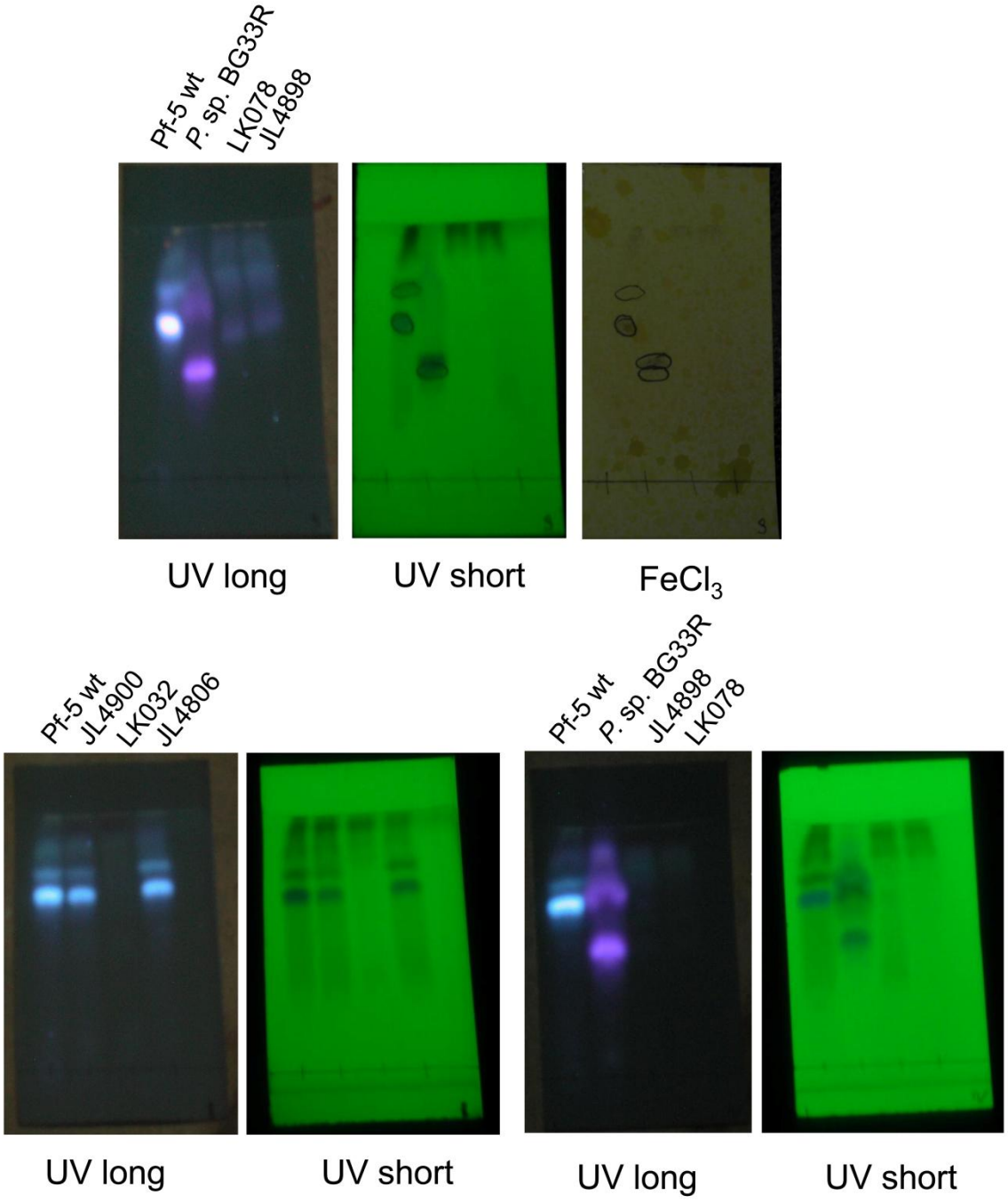
**Appendix 5. Continued. Graph of enantio-pyochelin production of Pf-5 siderophore mutants detected by HPLC.**



## Appendix 6. Secondary metabolite production in Pf-5 siderophore mutants detected by HPLC

| Lab # | phenotype, mutant | Pyoluteorin (ng/ml) | | | Phizoxin (ng/ml) | | Orfamide (ng/ml) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10/6/2010 | 10/21/2010 | 11/17/2010 | 10/6/2010 | 10/21/2010 | 10/6/2010 | 10/21/2010 | 11/17/2010 |
| JL4585a | Wt | 701.6 | 288.7 | 863.4 | 171.5 | not reported | 18874.7 | 33508.1 | 23692.3 |
| JL4585b | Wt | 804 | 309.9 | 807.6 | 174.7 | not reported | 16861 | 27887 | 20307 |
| JL4806a | pvd-, *pvdI* | 1470.1 | 642.4 | 1332.5 | 124.9 | not reported | 11721.4 | 18717.5 | 16717.3 |
| JL4806b | pvd-, *pvdI* | | 544.4 | 1483.3 | | not reported | | 19126.8 | 14254.6 |
| JL4898a | reduced epch, *pchC* | 1381 | 900.6 | 1126.1 | 60.9 | not reported | 15235.4 | 19162.3 | 15161.7 |
| JL4898b | reduced epch, *pchC* | 1526.6 | 1127.8 | 950.7 | 41.1 | not reported | 16934.6 | 21832.5 | 19822 |
| JL4900a | pvd- reduced epch, *pvdI-pchC* | 1802.3 | 1706.1 | 2891.2 | 119 | not reported | 14818.1 | 20044.3 | 12756.1 |
| JL4900b | pvd- reduced epch, *pvdI-pchC* | 1944.4 | 2983.9 | 3228.1 | 139.9 | not reported | 13188.6 | 16725.9 | 9134.2 |
| LK032a | pvd- reduced epch, *pvdI-pchA* | 5204.8 | 928.9 | 2714.5 | 59.5 | not reported | 10792 | 18424.4 | 4145.5 |
| LK032b | pvd- reduced epch, *pvdI-pchA* | 4565.6 | 1144.6 | 2854.9 | 41 | not reported | 7309.7 | 14159.3 | 3508.2 |
| LK035a | pvd- epch-, *pvdI-pchA-pchC* | not run | 782 | 782 | not run | not reported | not run | 15935.1 | 15935.1 |
| LK035b | pvd- epch-, *pvdI pchA pchC* | not run | 844.3 | 3040.8 | not run | not reported | not run | 16667.2 | 1717.5 |
| LK078a | epch-, *pchA* | 1050.1 | 457.4 | 1136.3 | 103.1 | not reported | 14601.8 | 24105.1 | 19173.5 |
| LK078b | epch-, *pchA* | 964.9 | 670.4 | 1205.4 | 87.6 | not reported | 16686.6 | 23090 | 13391 |

**Appendix 7. Thin layer chromatography of extracts from Pf-5, Pf-5 siderophore mutants and *P.* sp. BG33R for the detection of enantio-pyochelin in Pf-5 and the secondary siderophore in BG33R.** LK078 (Δ*pchA*), JL4898 (Δ*pchC*), LK032 (Δ*pvdI-pchA*), JL4806 (Δ*pvdI*).



## Appendix 8. Crossfeeding analysis of double *fpv* mutants in the Δ*pvdI-pchC* background of Pf-5.

| **Feeding Strains** | **Pf-5 mutants**[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *fpvV, fpvY* | *fpvZ, fpvV* | *fpvZ, fpvY* | *fpvU, fpvV* | *fpvU, fpvY* | *fpvU, fpvZ* | *fpvX, fpvY* | *fpvX, fpvZ* | *fpvX, fpvU* | *fpvX, fpvV* | *fpvW, fpvZ* | *fpvW, fpvV* | *fpvW, fpvX* | *fpvW, fpvU* | *fpvW, fpvY* |
| *P. fluorescens* A506 | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + |
| *P. fluorescens* WCS374 | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + |
| *P. fluorescens* DSM50106 | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + |
| *P. aeruginosa* LESB58 | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. fluorescens* CLR711 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. fluorescens* CTRp112 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. costantinii* CFBP5705 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. fluorescens* A6 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. putida* CFML90-40 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. fluorescens* Pf0-1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. aeruginosa* ATCC 27853 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. aeruginosa* Pa6 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *P. aeruginosa* 7NSK2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |