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		

 Table S1. Primers used in the construction of mutants of P. protegens Pf-5

Target gene and primers	Sequence 5'-3'					
Pf1A506_3090 (pvdD of A506)						
PflA506_3090 F	AGTACCTGTTCAACGGCTATG					
PflA506_3090 M1	TGCCGACGCATTTGATGTTC					
PflA506_3090 M2	CGAAGACGACCATGTGTTG					
PflA506_3090 R	CCAGAAGTGCTACCACCATTTC					
PFLU_2544 (pvdD of SBW25)						
PFLU_2544 F1	CTACCTGTTCAACGGCTAC					
PFLU_2544 R1	CAACTGCTCCTTGATCTGCT					
PFLU_2544 F2	CTCAAGCAGATCAAGGAGCA					
PFLU_2544 R2	GCCAACGACCATTTCAATCG					

 Table S2. Primers used to amplify *pvdD* of *P. fluorescens* A506, WCS374, and SBW25

Table S3. Growth of Fpv⁻ mutants of Pf-5 on an iron-limited medium in the presence of crossfeeding strains of *Pseudomonas* spp.

Crossfeeding Strain	Growth of Pf-5 Deletion Mutant ^a					
	fpvZ	fpvU	fpvY	fpvW	fpvX	fpvV
P. protegens Pf-5	-	+	+	+	+	+
P. aeruginosa PAO1	+	-	+	+	+	+
P. fluorescens SBW25	+	-	+	+	+	+
P. fluorescens ATCC 13525	+	-	+	+	+	+
P. chlororaphis ATCC 9446	+	-	+	+	+	+
P. rhodesiae CFML 92-104	+	+	-	+	+	+
P. fluorescens B10	+	+	+	-	+	+
P. putida CS111	+	+	+	+	-	+
P. putida BN7	+	+	+	+	+	-
P. fluorescens A506	+	+	+	+	+	+
P. fluorescens WCS374	+	+	+	+	+	+
P. aeruginosa LESB58	+	+	+	+	+	+

^a Growth of Fpv⁻ mutants in a $\Delta pvdI$ -pchA background of Pf-5 on an iron-limited medium (KMB amended with 600 μ M 2,2'-dipyridyl) in the presence of the crossfeeding strain: +, growth; –, no growth. The $\Delta pvdI$ -pchA mutant of Pf-5 did not grow on the iron-limited medium in isolation, but did grow in the presence of all crossfeeding strains listed.



Fig. S1. Homology models of FpvAI of *P. aeruginosa* **PAO1 and five proteins (FpvU, FpvY, FpvZ; FpvV; FpvX) of** *P. protegens* **Pf-5.** Structural components of Fpvs, with the β-barrel in green, plug in red, N-terminal signaling domain in blue, connecting loop in purple, and TonB box in brown. The homology models were constructed using a structure-based sequence alignment with the crystal structure of FpvAI from *P. aeruginosa* PAO1 (3) as a template. Locus tags corresponding to Fpvs of Pf-5 are FpvU (PFL_2391), FpvV (PFL_2527), FpvW (PFL_2293), FpvX (PFL_3315), FpvY (PFL_3485), and FpvZ (PFL_4092).

PA2398 PFL_2391 PFL_3485 PFL_4092 PFL_2527 PFL_3315 PFL_2293	MQIIPISYGTSMHUP - TITPARSILALAI - CLACNPVMAVEP - SLPLAGYVÇAQEVE MQIIPISYGTSMHUP - TITPARSILALAI - CLACNPVMAVEP - TTATD - NQATATYSF MHIRUT - PIA - ATMRPVIIG - SVASTBLPLLA - AETGAVTDHQQRDY MPAQHRUT - DITKA - LRQAFS - PKLASRTLGLAY - TLPLMAQVÇAQEIHF MSSAVTQRR - HRTF - SUKQNLAGAVAQG - VCLGASTATI - LPTWALAAEQAQVE MPSPRRSRLPFHTLDLCTA - DLCGAAPWAHAA - ADAGQNQSÇAERRSY M 8 - DVHKSKMAQAIKVGWGALAVYGAADLPLTAVAASECTAVRRF
PA2398 PFL 2391 PFL 3485 PFL 4092 PFL_2527 PFL_3315 PFL_2293	DIPEQALGSI QEFCROADIQUL TRPEEVRNERSAIKCKLEPNQL TEDERGEGASVDF ALARQSI ANALDQLST SGLQTALSI ATAQGIESACVSGRGAEQALGKLIAGTGIGFER ALAPON DQVIGIFQQSASMAIDAN SAGKESTGLNGE SVAEGLQRD KPLGLQAVA NYVSQSMSALQEFCROANMQVL MPDDVQKESNALSGSPERATAAMINGTGVAYTL DIAPON ASALTRFQQSAHLLSIPASITECESSPELCERGHDVSGLALDISGTGLQAV STOPPVTVINRFAESAVFAGINDIAACKQSPCLNGETSVAEALQLIANSGLQAQA DTAACDUTEVISRTASAAGAAISDARQTAGLESAGLKCETGVQEGFARTAAGSGWQAEP
PA2398 PFL_2391 PFL 3485 PFL 4092 PFL 2527 PFL 3315 PFL_2293	QGN-AITISVAEAADSSVDLCAIMITSNOLGTITEDSGSVTPCTIATATELVLTPRETPC NGANAVLLTRLPQSSQAVELEATOIVSNOLGTVTEGSGSVTPCTIATATELVLTPRETPC EGAG-YRVIQ-ASGGERVELGAITVTNOLGTVTEGSSSVTPGTIATATELVLTPRETPC KD-NSVTI-RHEGNGASLELGTITGGGLGTTEDTGSVTGCANGTASELSLTARETPC GANG-DYSLQTRGNGASLELSPVSISGKAPGSTTEGTGEYTTVSSSSTENLTPRETPC VSG-GYVLKVLPATSCPLQLGTIGISAGGLGSVTEGSQSVTTCAASASATGLNLSLERETPC QSNGSFVLRPVPQGSGALELGTIQVQGGELGATTEYSGSVTTCAAV-TIGEGQHSLEETPC
PA2398 PFL_2391 PFL_3485 PFL_4092 PFL_2527 PFL_3315 PFL_2293	SITVVTRCNIDD FGLNNIDDVMRHTPGITVSA - VDTDRNNIVARGFSINN - FQYDGIPS SITVVTRCHEDFGLNNVDDVMRHTPGITVSA - VDTDRNNIVARGFSINN - FQYDGIPS SISVVRCAMDFGLNSIDEVMRHTPGITVSA - VDTDRINIVARGFSINN - FQYDGIPS SISVVRCAMDFGLNSIDEVMRHTPGITVAT - VDSDRISIYARGFAION - FQYDGIPI SVTVIRCRIDDONNRSLEDVLKATPGISITK - DGPQRPTYRCFAION - FQYDGIPI SITVMTRCRIDDONLSTLTDTLEATPGISITK - DGPQRPTYRCFAION - VEIDGPT TVTVIRCQUDDGATSIADTLRAPGVSVQN - VDSRWEFSCRCLPIN - FQYDGVA SVTVIRCRMIDDONLNTIDQVMEKTPGITLYD - SPMGGKYFYSRGFAION FQYDGVDGVPL ** **
PA2398 PFL_2391 PFL_3485 PFL_4092 PFL_2527 PFL_3315 PFL_2293	TARNVG TSAGNT LSDMAIYDRVEVLEGATGLLTGAGSLGATINLTRK PTHEFKGHWELG TVRNVA SAGNTLSDMAIYDRVEVLEGATGLLTGAGSLGATINLVRK PTAG PGGHABLG - LQDAQISSGHTLTDTVIYDRVETLEGATGLLTGAGCFGGTINNVRK PTAFAGHIDLG DETHYLSRDMASSADMAIFDHVEVVEGATGMNGGAGNPSAINEVRK PTATPRVTVTGS NTRLDNYSQSMANDBWEVVEGATGLISGMGNPSAINEVRK PTSEAQASITG TYDGV-DYGTTSTDMATFDRVEIGAAGMLKGAGGTAGSVNFVRR RCQATPHTELTMS
PA2398 PFL_2391 PFL_3485 PFL_4092 PFL_2527 PFL_3315 PFL_2293	AGSWDNYRSELDVSGPLTESGNVRGRAVAAYQDKHSYMDIYERKTSVYGILEIDLNPDT MGSWDNYRSELDVSGPLTETGWRGRAVAAYQDKGSFLDHYGFKSYYGILEIDLSPDT AGSWDNYRSEUVSGPLTBGRWRGRWVAAYQDKKSFLDHYGFUSCULEIDLSPDT AGSWDNYRSEUDSGNYGGPLTBGRWRGRUVAAYQDKKSFLDHYGFUSCUNVYGILEIDLSPDT AGSWDNYRSEGDTSGPLTBGRWRGRLVADYKTESAWYDRKQQLQLLYGISETDLSEDT VGSWDNYRSEGDTSGPLTBGRWRGRLVADYKTESAWYDRKQQLQLLYGISETDLSEDT AGSWDNYRSEGDTSGPLTBGRWRGRLVADYKTESAWYDRKQQLQLLYGISETDLSEDT AGSWDNYRSEGDTSGPLTBGRWRGRLVADYKTESAWYDRKQQLQLLYGISETDLSEDT
PA2398 PFL 2391 PFL 3485 PFL 4092 PFL_2527 PFL_3315 PFL_2293	MITYCADYODNDPKCSCSCSCPLEDEQCNRNDVSRSFINCAKIESTEOYTRVYANLEH LLTYCDYODNIPKCSSSCTPLUINATGCHNSMSRSFINCATUSCTEOYTRAFAMLEH LLTYCDYODNDPKCSSSSSSLTDSKONAISTSRSFINCASUSRSSYTRAFTLEH TFTFOASNOSCR-NNTSNCC-L V-AADCSDLHEKRSTYLCSKEYDONNTTAPSRLY LLTFCLOONTRSRCATUCC-L PTFFSCERTDFKRSTITSPHEYDDEYDHQQTSYASIQ LLTFCLOONTRSRCATUCC-PHYFDCSRTFFKRSTITSPHEYDHQQTSYASIQ TICLOMAYEDVDSR-PCNCC-L PRI-SDCSDLKLGRSTCLNTANNSRSKRATYFDTRQ * *
PA2398 PFL_2391 PFL 3485 PFL 4092 PFL 2527 PFL 3315 PFL_2293	NFANCT VGTVQLDEKINGTHAPIGATMCDWP APDNSAKTVAQTYTGETKSNSLDIVIT DIGDGNVTXLQLDEKINSTHAELGSIQFDEP QTDGTAKVNAQXTGDTTSDSADIVE SFANGNVARAQYNEQINGTNAPLGEIMSP-N AETGIASLITRKYTGETVSDSGDIVA RFANSLKMLSASKSWSDLNM-LGEIPER - MGANYDEFGONIGRYDYEDQQNSYGGVT OLGNCNSGTVEFTEAENQEDELFNFAMGSV- NKDGSGLTQFVPEGTPRQDNLDIVIT OLANDTILVYSYDELRRQHDTLGESASGNPDQASGDGWFYMGGFKGDQRQDNLDIVIT OLANDTILVYSYDELRRQHDTLGESASGNPDQASGDGWFYMGFKGDQRQDNLDIN OLNDDALKVAGVYTRNTQDIEYAFPSGSVPVGASRSSTLMLGSIYDYDQVDYGFDATVD
PA2398 PFL_2391 PFL_3485 PFL_4092 PFL_2527 PFL_3315 PFL_2293	CPTQFLCREHELVVCTSASFEHWEGKSTWNLRNYD-NTTD-DFINWDGDIG P CPTNLFCREHELVLCGSIAN RWTGKGTWSPDFPGGK-GNVV-DFINWHCKLER CPTDLCREHOLVVCASISNSHWKGRDFTSAT-NH-NNFY-DVFNWDGHSPXP CPTSLFCRTHELVVCASRRDLTKGKGL-FIDLETH-TNIYKPSG-IPXP CPTSLFCRTHELIGGMTLSQYKERTPSWGCWRDYAG-SP-AGPIDNLFDWNGHSPXP CPTSLFCREHELIACFMLMNAKQDIPVHGSVYP-PVGG-SIYDWRGFAXP CHTQAECREHELIACFMLMNAKQDIPVHGSVYP-QRQ-NVLDPDHH



Fig. S2. Alignment of PA2398 (FpvAI) from *P. aeruginosa* PAO1 with the six Fpv outermembrane proteins from Pf-5. The colored lines above the alignment delineate domains and conserved regions based on the characterized residues of FpvAI: N-terminal signaling domain, blue; connecting loop, purple; TonB box, brown; plug domain, red; and β-strands of the β-barrel, green. Amino acid residues of FpvAI (PA2398) involved in pyoverdine binding are indicated with an asterisk. Residues are highlighted to show levels of similarity: identical residues present in all proteins, black; identical residues present in a subset of proteins, pink; similar residues, green. Locus tags correspond to FpvU (PFL_2391), FpvV (PFL_2527), FpvW (PFL_2293), FpvX (PFL_3315), FpvY (PFL_3485), and FpvZ (PFL_4092).



Fig. S3. Low-Res-ESI-MS spectrum of the pyoverdine of *P.protegens* Pf-5.



Fig. S4. HR-ESI-MS spectrum of the pyoverdine of *P.protegens* Pf-5.

Text S1. Identification of purified pyoverdines including co-occuring isoforms by high resolution electron spray ionization mass spectroscopy (HR-ESI-MS). HR-ESI-MS analysis of the pyoverdines of P. aeruginosa PAO1 yielded $[M+H]^+$ ions at m/z 1334.5938 and 1362.5906, which agrees with values obtained in previous studies (5) and the molecular formula of pyoverdine D ([M+H]⁺: C₅₅H₈₄N₁₇O₂₂, *m/z* 1334.5971) and C ([M+H]⁺: C₅₆H₈₄N₁₇O₂₃, *m/z* 1362.5920), respectively (1). The mass difference (Δ 28 Da) corresponds to the side chains α ketoglutaric acid in pyoverdine C and succinic acid in pyoverdine D. Mass analysis of free pyoverdines of *P. fluorescens* B10 gave quasi molecular ions $[M+H]^+$ at m/z 1035.4381, 1019.4475 and 990.4166. The latter ion is consistent with the formula of pseudobactin ([M+H]⁺: $C_{42}H_{60}N_{11}O_{17}$, m/z 990.4163), which is known to be produced by P. fluorescens B10 (7). The occurrence of the other two pyoverdines was reported (5-6) but, to the best of our knowledge, their structures have not been described previously. Empirically, the pyoverdines of one strain differ most likely in the nature of the dicarboxylic acid side chain bound via the amino group of the chromophore or in the chromophore itself. Therefore, we suggest that the derivative with M=1018 Da possibly represents the glutamate isoform of pseudobactin $([M+H]^+: C_{43}H_{63}N_{12}O_{17},$ m/z 1019.4429). The remaining derivative with M=1034 shows a difference of 16 Da to B10-1018 which points towards a hydroxylated congener of B10-1018 (e.g. OH-glutamate, 5-OHchromophore, exchange of Ala with Ser). HR-MS analysis of the pyoverdines of P. putida Bn7 revealed three isoforms with $[M+H]^+$ ions at m/z 1163.4956, 1047.441 and 1134.4751. The structures of these pyoverdines are currently unknown and further structure elucidation was not attempted. However, the mass difference of 29 mass units between Bn7-1133 and Bn7-1162 might be attributable to an exchange of succinic acid with glutamic acid regarding the acyl side chain. In the pyoverdine fractions of Pseudomonas sp. SB8.3 and P. rhodesiae CFML92-104, isoforms were absent and only one major pyoverdine was detectable. Peaks at m/z 1047.4787 and 1438.6061 confirmed the known pyoverdines of P. putida SB83 (Suc-Chr-Ala-Lys-Thr-Ser-AcOHOrn-cOHOrn; $[M+H]^+$: C₄₅H₆₇N₁₂O₁₇, *m/z* 1047, 4742)¹ and *P. rhodesiae* CFML92-104 (Mal-Chr-Ser-Lys-FOHOrn-Ser-Ser-Gly-(Lys-FOHOrn-Ser-Ser)²; C₅₈H₈₈N₁₇O₂₆, m/z 1438.6081), respectively (2, 4).

¹ Underline denotes D-configurated amino acids. ² Absolute configuration unknown.

REFERENCES

- 1. **Briskot, G., K. Taraz, and H. Budzikiewicz.** 1989. Pyoverdin-type siderophores from *Pseudomonas aeruginosa*. Liebigs Ann. Chem. **1989**:375-384.
- 2. **Budzikiewicz, H.** 2004. Siderophores of the Pseudomonadaceae *sensu stricto* (fluorescent and non-fluorescent *Pseudomonas* spp.). Fortschr. Chem. Org. Naturst. **87:**81-237.
- 3. **Cobessi, D., H. Celia, N. Folschweiller, I. J. Schalk, M. A. Abdallah, and F. Pattus.** 2005. The crystal structure of the pyoverdine outer membrane receptor FpvA from *Pseudomonas aeruginosa* at 3.6 angstrom resolution. J. Mol. Biol. **347:**121-134.
- Meyer, J. M. 2007. Siderotyping and bacterial taxonomy: A siderophore bank for a rapid identification at the species level of fluorescent and non-fluorescent *Pseudomonas*, p. 43-65. *In* A. Varma and S. B. Chincholkar (ed.), Soil Biology: Microbial Siderophores, vol. 12. Springer-Verlag, Berlin Heidelberg, Germany.
- 5. **Meyer, J. M., C. Gruffaz, V. Raharinosy, I. Bezverbnaya, M. Schafer, and H. Budzikiewicz.** 2008. Siderotyping of fluorescent *Pseudomonas*: molecular mass determination by mass spectrometry as a powerful pyoverdine siderotyping method. Biometals **21**:259-271.
- 6. **Nowak-Thompson, B., and S. J. Gould.** 1994. A simple assay for fluorescent siderophores produced by *Pseudomonas* species and an efficient isolation of pseudobactin. Biometals **7:**20-24.
- 7. **Teintze, M., M. B. Hossain, C. L. Barnes, J. Leong, and D. van der Helm.** 1981. Structure of ferric pseudobactin, a siderophore from a plant growth promoting *Pseudomonas*. Biochemistry **20:**6446-6457.