SUPPLEMENTAL MATERIALS

Phenotypic adaption of *Pseudomonas aeruginosa* by hacking siderophores produced by other microorganisms

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Table 1SM. Strains and plasmids used in this study.

Strain	**Collection ID**	**Relevant characteristics**	**Source or references**
Pseudomonas aeruginosa			
PAO1	PAO1	Wild-type strain	(1)
Δ*pvdF*Δ*pchA*	PAS283	PAO1; *pvdF* and *pchA* chromosomally deleted	(2)
Δ*pvdF*Δ*pchA*Δ*pfeA*	PAS294	PAO1; *pvdF, pchA* and *pfeA* chromosomally deleted	(3)
Δ*pvdF*Δ*pchA*Δ*fiuA*Δ*foxA*	PAS536	PAO1; *pvdF, pchA, fiuA and foxA* chromosomally deleted	This study
Escherichia coli			
TOP10		*F− mcrA* Δ(*mrr-hsdRMS-mcrBC*) *φ80lacZ*Δ*M15* Δ*lacX74 nupG recA1 araD139* Δ(*ara-leu*)*7697 galE15 galK16 rpsL*(*Str*^{R}) *endA1 λ−*	Invitrogen
Plasmids	**Collection ID**	**Relevant characteristics**	**Source or references**
pEXG2	pEXG2	allelic exchange vector with pBR origin, gentamicin resistance, sacB	(4)
pEXG2 Δ*foxA*	pQUP8	pEXG2 carrying the sequence to delete *foxA*	This study
pEXG2 Δ*fiuA*	pQUP9	pEXG2 carrying the sequence to delete *fiuA*	This study

Table 2SM. TBDTs present in the *P. aeruginosa* PAO1 genome.

Gene	Name	Known ligand	Reference
PA4897	*optI*	-	(5)
PA4837	*cntO*	Pseudopaline	(6)
PA4710	*phuR*	Haem	(7)
PA4675	*chtA*	Aerobactin, Rhizobactin 1021, Schizokinen	(8)
PA4514	*piuA*	BAL30072, MC-1	(9)
PA4221	*fptA*	Pyochelin	(10)
PA4168	*fpvB*	Pyoverdine	(11)
PA4156	*fvbA*	Vibriobactin	(12)
PA3901	*fecA*	Citrate	(13)
PA3790	*oprC*	-	(14)
PA3408	*hasR*	HasA-haem	(7)
PA3268	-	-	(14)
PA2911	-	-	(14)
PA2688	*pfeA*	Enterobactin	(15)
PA2590	-	-	(14)
PA2466	*foxA*	Ferrioxamine	(16)
PA2398	*fpvA*	Pyoverdine	(17)
PA2335	*optO*	-	(14)
PA2289	-	-	(14)
PA2089	-	-	(14)
PA2070	-	-	(14)
PA2057	*sppR*	-	(18)
PA1922	*cirA*	-	(14)
PA1910	*femA*	Mycobactins, carboxymycobactins	(5)
PA1613	-	-	(14)
PA1365	-	-	(14)
PA1322	*pfuA*	-	(14)
PA1302	*hxuC*	-	(14)
PA1271	-	-	(14)
PA0931	*pirA*	Enterobactin, BAL30072, MC-1	(9, 19)
PA0781	-	-	(14)
PA0470	*fiuA*	Ferrichrome	(16)
PA0434	-	-	(14)
PA0192	-	-	(14)
PA0151	-	-	(14)

Table 3SM. Primers used for RT-qPCR analysis.

Name	**Gene**	**Sequence**
uvrD F	*uvrD*	CTACGGTAGCGAGACCTACAACAA
uvrD R	*uvrD*	GCGGCTGACGGTATTGGA
GAPDH F	*GAPDH*	TGCACCACCAACTGCTTAGC
GAPDH R	*GAPDH*	GGCATGGACTGTGGTCATGAG
fiuA F	*fiuA*	GCCGCGACAAGAAGTTCAG
fiuA R	*fiuA*	ACGACTCCGCATAGGAGATATAGG
foxA F	*foxA*	AAGGGCTCGGATACCCAGTT
foxA R	*foxA*	CGTTGGGATCGTGTTGCA
femA F	*femA*	CATTCCGAAGACCAATGAAACC
femA R	*femA*	TGACCTGCACGCGATCCT
pfeA F	*pfeA*	GCCGAGACCAGCGTGAAC
pfeA F	*pfeA*	GGCCGGATTCGATCTTGTT
fvbA F	*fvbA*	CCAGCTACCGCTTCAACGA
fvbA R	*fvbA*	CCGGTTTTCCTGCATGTAGGT
fpvA F	*fpvA*	AGCCGCCTACCAGGATAAGC
fpvA R	*fpvA*	TGCCGTAATAGACGCTGGTTT
fptA F	*fptA*	GCGCCTGGGCTACAAGATC
fptA R	*fptA*	CCGTAGCGGTTGTTCCAGTT
PA0434 F	*PA0434*	CGCTACGTGTCCTCGGTGTAC
PA0434 R	*PA0434*	GGAAAGTCCCGTAGACCGTGTA
piuA F	*piuA*	CAGCGCGGCTTATGTGTTC
piuA R	*piuA*	CCGAGATTCAGCTCCCATTG
hasR F	*hasR*	AGCGCCTGCAGTTCAGCTA
hasR R	*hasR*	GTTCTCGGTGTTGAGCATGTTG
phuR F	*phuR*	GGTCGAACTGCCCAACGA
phuR R	*phuR*	TACGATGTCCGGATCGACGTA
piv F	*piv*	GCGTGGGCCTGAAAACG
piv R	*piv*	CGATCCATTCGAGGGTTGTC
toxA F	*toxA*	CCCGGCGAAGCATGAC
toxA R	*toxA*	GGGAAATGCAGGCGATGA
aprA F	*aprA*	AACCAGAAGATCAACCTCAACGA
aprA R	*aprA*	TCGACACATTGCCCTTCAAC
exoY F	*exoY*	AATGGATGGCGGAGCCTATA
exoY R	*exoY*	CAAGGCGTTGCCGAGAGAT

Table 4SM. Primers used for the construction of the Δ*pvdF*Δ*pchA*Δ*fiuA*Δ*foxA* strain.

Name	Gene	Sequence
FiuAatg-672F	*fiuA*	AGCAGCGCGAGCTGACCCTCCCGGATGCC
FiuAoverlapR	*fiuA*	TGCGGGGCTCCTGGATGGAAACGAGTCTCAATGCC
FiuAoverlapF	*fiuA*	GGCATTGAGACTCGTTTCCATCCAGGAGCCCCGCAGCGACGCGTCACAGGTGCGTAACGGCGGATAAC
FiuAstop+639R	*fiuA*	TCGGCGACAGCCGGATCCTCGTCGAAGGCG
FoxAatg-694F	*foxA*	TGCGCTACGACGCCGGCCAGCGCCTGATC
FoxAoverlapR	*foxA*	GAACGGGATCCGTTGGAGTCGTTGAAGGGTGTTCAATCG
FoxAoverlapF	*foxA*	CGATTGAACACCCTTCAACGACTCCAACGGATCCCGTTCTTCTGACCGTCCACCCGGGCCCCGGCG
FoxAstop+661R	*foxA*	AGCGGTAGGCGTTGCCGCGCTTGATCTTCCAGG

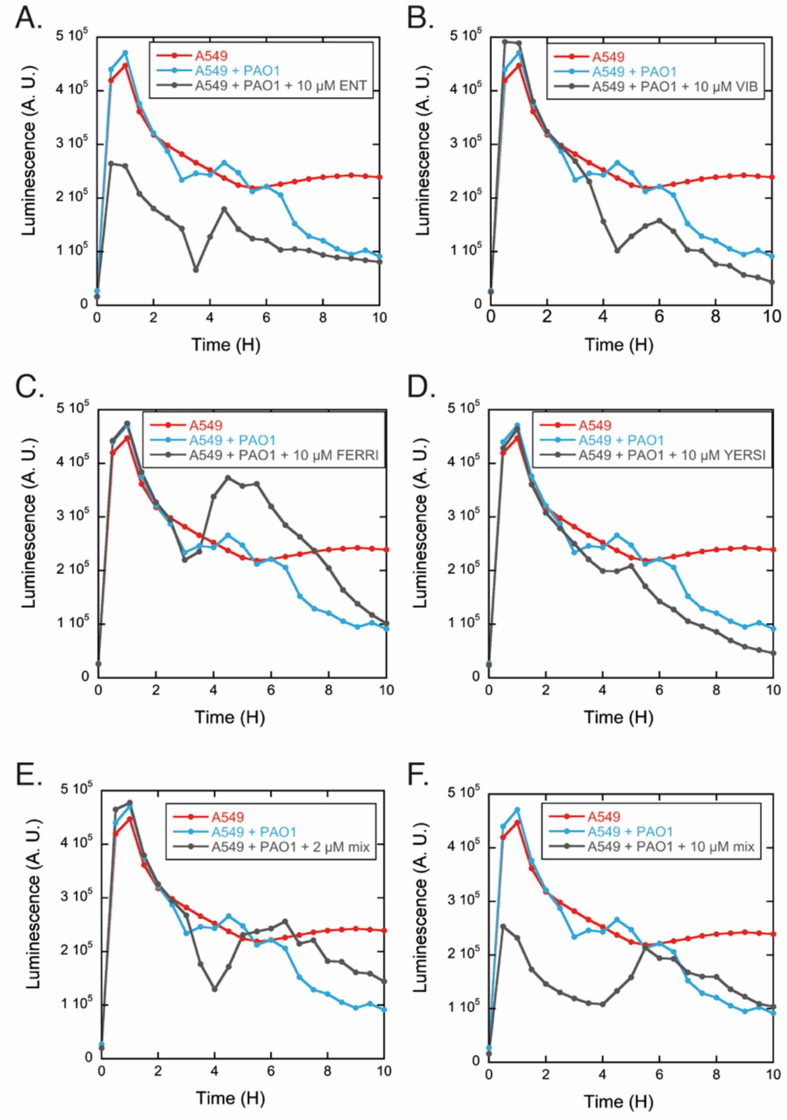

Figure 1SM. A549 cell viability in the presence or absence of *P. aeruginosa* and/or siderophores. The Real Time-Glo™ MT cell viability kit (Promega™) was used as described in Materials and Methods. Cell viability is represented as a function of luminescence signal (A. U., arbitrary unit). Viability of the A549 cells alone is represented by the red dots, infected cell viability by the blue dots, and the viability of infected cells in the presence of siderophore by grey dots.

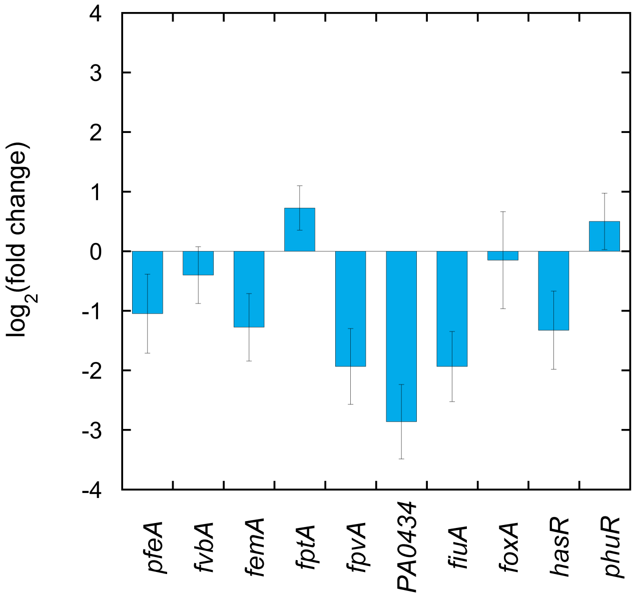

Figure 2SM: Analysis of changes in the transcription of TBDT genes involved in iron-uptake pathways in *P. aeruginosa* cells in RPMI medium in the presence of A549 epithelial cells during 3 h *versus* those of bacteria grown as in figure 4, i.e. for 8 h in RPMI medium. The data were normalized relative to the reference gene *uvrD* and are representative of three independent experiments. Results are given as the ratio between the values obtained in RPMI in the presence of epithelial cells and those for bacteria grown as in Figure 3 and 4, for 8 h in RPMI without epithelial cells.

REFERENCES

1. Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S., Hufnagle, W. O., Kowalik, D. J., Lagrou, M., Garber, R. L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L. L., Coulter, S. N., Folger, K. R., Kas, A., Larbig, K., Lim, R., Smith, K., Spencer, D., Wong, G. K., Wu, Z., Paulsen, I. T., Reizer, J., Saier, M. H., Hancock, R. E., Lory, S., and Olson, M. V. (2000) Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406, 959–64
2. Gasser, V., Baco, E., Cunrath, O., August, P. S., Perraud, Q., Zill, N., Schleberger, C., Schmidt, A., Paulen, A., Bumann, D., Mislin, G. L. A., and Schalk, I. J. (2016) Catechol siderophores repress the pyochelin pathway and activate the enterobactin pathway in *Pseudomonas aeruginosa*: an opportunity for siderophore-antibiotic conjugates development. *Environ. Microbiol.* 18, 819–832
3. Paulen, A., Gasser, V., Hoegy, F., Perraud, Q., Pesset, B., Schalk, I. J., and Mislin, G. L. A. (2015) Synthesis and antibiotic activity of oxazolidinone-catechol conjugates against *Pseudomonas aeruginosa*. *Org. Biomol. Chem.* 13, 11567–11579
4. Rietsch, A., Vallet-Gely, I., Dove, S. L., and Mekalanos, J. J. (2005) ExsE, a secreted regulator of type III secretion genes in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U.S.A.* 102, 8006–8011
5. Llamas, M. A., Mooij, M. J., Sparrius, M., Vandenbroucke-Grauls, C. M., Ratledge, C., and Bitter, W. (2008) Characterization of five novel *Pseudomonas aeruginosa* cell-surface signalling systems. *Molecular microbiology* 67, 458–72
6. Lhospice, S., Gomez, N. O., Ouerdane, L., Brutesco, C., Ghssein, G., Hajjar, C., Liratni, A., Wang, S., Richaud, P., Bleves, S., Ball, G., Borezée-Durant, E., Lobinski, R., Pignol, D., Arnoux, P., and

Voulhoux, R. (2017) *Pseudomonas aeruginosa* zinc uptake in chelating environment is primarily mediated by the metallophore pseudopaline. *Sci Rep* 7, 17132

7. Smith, A. D., and Wilks, A. (2015) Differential contributions of the outer membrane receptors PhuR and HasR to heme acquisition in *Pseudomonas aeruginosa*. *J. Biol. Chem.* 290, 7756–7766
8. Cuiv, P. O., Clarke, P., and O'Connell, M. (2006) Identification and characterization of an iron-regulated gene, *chtA*, required for the utilization of the xenosiderophores aerobactin, rhizobactin 1021 and schizokinen by *Pseudomonas aeruginosa*. *Microbiology* 152, 945–54
9. Moynié, L., Luscher, A., Rolo, D., Pletzer, D., Tortajada, A., Weingart, H., Braun, Y., Page, M. G. P., Naismith, J. H., and Köhler, T. (2017) Structure and Function of the PiuA and PirA Siderophore-Drug Receptors from *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 61,
10. Ankenbauer, R. G., and Quan, H. N. (1994) FptA, the Fe(III)-pyochelin receptor of *Pseudomonas aeruginosa*: a phenolate siderophore receptor homologous to hydroxamate siderophore receptors. *J Bacteriol* 176, 307–19
11. Ghysels, B., Dieu, B. T., Beatson, S. A., Pirnay, J. P., Ochsner, U. A., Vasil, M. L., and Cornelis, P. (2004) FpvB, an alternative type I ferripyoverdine receptor of *Pseudomonas aeruginosa*. *Microbiology* 150, 1671–80
12. Elias, S., Degtyar, E., and Banin, E. (2011) FvbA is required for vibriobactin utilization in *Pseudomonas aeruginosa*. *Microbiology* 157, 2172–80
13. Marshall, B., Stintzi, A., Gilmour, C., Meyer, J.-M., and Poole, K. (2009) Citrate-mediated iron uptake in *Pseudomonas aeruginosa*: involvement of the citrate-inducible FecA receptor and the FeoB ferrous iron transporter. *Microbiology (Reading, Engl.)* 155, 305–315
14. Cornelis, P., and Bodilis, J. (2009) A survey of TonB-dependent receptors in fluorescent pseudomonads. *Environmental microbiology reports* 1, 256–62
15. Poole, K., Young, L., and Neshat, S. (1990) Enterobactin-mediated iron transport in *Pseudomonas aeruginosa*. *J Bacteriol* 172, 6991–6
16. Llamas, M. A., Sparrius, M., Kloet, R., Jimenez, C. R., Vandenbroucke-Grauls, C., and Bitter, W. (2006) The heterologous siderophores ferrioxamine B and ferrichrome activate signaling pathways in *Pseudomonas aeruginosa*. *Journal of bacteriology* 188, 1882–91
17. Poole, K., Neshat, S., Krebes, K., and Heinrichs, D. E. (1993) Cloning and nucleotide sequence analysis of the ferripyoverdine receptor gene *fpvA* of *Pseudomonas aeruginosa*. *J Bacteriol* 175, 4597–604
18. Pletzer, D., Braun, Y., and Weingart, H. (2016) Swarming motility is modulated by expression of the putative xenosiderophore transporter SppR-SppABCD in *Pseudomonas aeruginosa* PA14. *Antonie Van Leeuwenhoek* 109, 737–753
19. Ghysels, B., Ochsner, U., Mollman, U., Heinisch, L., Vasil, M., Cornelis, P., and Matthijs, S. (2005) The *Pseudomonas aeruginosa pirA* gene encodes a second receptor for ferrienterobactin and synthetic catecholate analogues. *FEMS microbiology letters* 246, 167–74
20. Raymond, K. N., Dertz, E. A., and Kim, S. S. (2003) Enterobactin: an archetype for microbial iron transport. *Proceedings of the National Academy of Sciences of the United States of America* 100, 3584–8
21. Albrecht-Gary, A. M., Blanc, S., Rochel, N., Ocacktan, A. Z., and Abdallah, M. A. (1994) Bacterial iron transport: coordination properties of pyoverdin PaA, a peptidic siderophore of *Pseudomonas aeruginosa*. *Inorg. Chem.* 33, 6391–6402
22. Anderegg, G., L'Eplattenier, F., and Schwarzenbach, G. (1963) Hydroxamatkomplexe III. Eisen(III)-Austausch zwischen Sideraminen und Komplexonen. Diskussion der Bildungskonstanten der Hydroxamatkomplexe. *Helvetica Chimica Acta* 46, 1409–1422
23. Brandel, J., Humbert, N., Elhabiri, M., Schalk, I. J., Mislin, G. L. A., and Albrecht-Garry, A.-M. (2012) Pyochelin, a siderophore of *Pseudomonas aeruginosa*: Physicochemical characterization of the iron(III), copper(II) and zinc(II) complexes. *Dalton Trans* 41, 2820–34