Pyochelin potentiates the inhibitory activity of gallium on *Pseudomonas aeruginosa*

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SUPPLEMENTAL MATERIAL

TABLE S1. Strains, plasmids and oligonucleotides used in this study

Strains	Relevant characteristics	Reference or source
E. coli		
DH5α	*recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1* Δ(*lacZYA-argF*)U169 [ϕ80Δ*lacZ*ΔM15] F^- Nal^r	1
SM10	*thi-1 thr leu tonA lacY supE recA::RP4–2-Tc::Mu* Km^R	2
S17.1λpir	Tp^R Sm^R *recA, thi, pro, hsdR*-M+RP4*:* 2-Tc:Mu:K_mTn7λpir	3
HB101	*proA2 hsdS20 (*rb^- mb^-*) recA13 ara-14 lacY1 galK2 rpsL20 supE44 xyl-5 mtl-1* F^-	1
P. aeruginosa		
PAO1 (ATCC15692)	Wild type	4
PAO1Δ*pchD*	PAO1 carrying a deletion in *pchD*	This study
PAO1Δ*pvdA*	PAO1 carrying a deletion in *pvdA*	5
PAO1Δ*pvdA*Δ*pchD*	PAO1 carrying a deletion in *pvdA* and *pchD*	This study
PAO1Δ*fptX*	PAO1 carrying a deletion in *fptX*	This study
PAO1Δ*fptAX*	PAO1 carrying a deletion in *fptABCX*	This study
TR1	Clinical isolate	6
TR1Δ*pvdA*	TR1 carrying a deletion in *pvdA*	This study
Plasmids		
pRK2013	Helper plasmid; Tra^+ Km^R	7
pEX18Tc	Suicide vector for allelic replacement; Tc^R	8
pPS858	Source of Gm^R-GFP cassette; Ap^R	8
pME3087	Suicide vector for allelic replacement; Tc^R	9
pDM4	Suicide vector for allelic replacement; Cm^R	10
pMEΔ*fptX*	pME3087 derivative for *fptX* deletion; Tc^R	This study
pMEΔ*fptAX*	pME3087 derivative for *fptABCX* deletion; Tc^R	This study
pEXΔ*pvdA*	pEX18Tc derivative for *pvdA* deletion; Tc^R	5
pDM4Δ*pchD*	pDM4 derivative for *pchD* deletion; Cm^R	This study

Oligonucleotides	Sequence (5’ → 3’)[a]	Restriction site
pchDmutUPFW	GCTCTAGAGCCCGGGCGCTGCCG	XbaI
pchDmutUPRV	CGGAATTCGACGAAGGCGATGCCG	EcoRI
pchDmutDWFW	CGGAATTCCCCCAAACCGGCATCG	EcoRI
pchDmutDWRV	CCGCTCGAGTAGCTCGCTGGC	XhoI
fptAUPFW	CCCGAATTCCTGCTGGGTGGCGTG	EcoRI
fptAUPRV	AAGGGATCCGTTTTCATCGTTCGAAC	BamHI
fptAXDWFW	CCCGGATCCCTGAGGCGCTGCCGATA	BamHI
fptAXDWRV	GCCAAGCTTCCATTCGGCGGTCCGC	HindIII
fptXUPFW	GGGGAATTCGGCGAGCGCAGCTCG	EcoRI

fptXUPRV	CCCGGATCCAGCTCAAGCATGGTGGTC	BamHI
mutPA4687UP_FW	CCCAAGCTTCCGCATCGTCCTCG	HindIII
mutPA4687UP_RV	CCCGGATCCAGGGCGGCGTGGCG	BamHI
mutPA4687DOWN_FW	CCCGGATCCCTCAGCCTCGAACGCG	BamHI
mutPA4687DOWN_RV	CCCGAATTCGAACTCCTGGTAGATGG	EcoRI

[a] Restriction sites are underlined

Supplemental Experimental Procedures

Construction of plasmids and gene replacement *P. aeruginosa* mutants

For the generation of the *P. aeruginosa* PAO1Δ*pchD* mutant, site-specific excision of the entire *pchD* coding sequence was performed using a *sacB*-based strategy described by Milton *et al.* (1996). Briefly, two regions of approximately 500 bp upstream and downstream of the *pchD* gene were generated by PCR with primer pairs pchDmutUPFW/pchDmutUPRV and pchDmutDWFW/pchDmutDWRV, respectively (Table S1). Upstream and downstream fragments were digested with appropriate restriction enzymes, and directionally ligated to the pDM4 suicide vector (10). The resulting pDM4Δ*pchD* construct was introduced in *Escherichia coli* S17.1λpir and conjugally transferred into *P. aeruginosa* PAO1. Merodiploides were resolved as described (10). The deletion event was verified by PCR and DNA sequencing, and the resulting mutant was named PAO1Δ*pchD*.

The *P. aeruginosa* TR1 strain deleted of the *pvdA* gene was obtained using plasmid pEXΔ*pvdA*, as described (5).

For the deletion of *fptX* and the *fptABCX* operon in the *P. aeruginosa* PAO1 chromosome, fragments of approximately 700 pb upstream and downstream of the *fptX* gene or the *fptABCX* operon were amplified by PCR using the primer couples fptXUPFW/fptXUPRV and fptAXDWFW/fptAXDWRV or fptAUPFW/fptAUPRV and fptAXDWFW/fptAXDWRV,

respectively (Table S1). The PCR products were digested with the suitable restriction enzymes and cloned into the suicide vector pME3087 (9), yielding plasmid pMEΔ*fptX* and pMEΔ*fptAX*, respectively. These plasmids were then introduced into *P. aeruginosa* PAO1 by triparental mating, using the helper strain *E. coli* HB101(pRK2013). Merodiploids were resolved as previously described (11). The deletion events were verified by PCR and DNA sequencing, and the resulting strains were named PAO1Δ*fptX* and PAO1Δ*fptAX*.

Supplemental References

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11. **Ye RW, Haas D, Ka JO, Krishnapillai V, Zimmermann A, Baird C, Tiedje JM.** 1995. Anaerobic activation of the entire denitrification pathway in *Pseudomonas aeruginosa* requires Anr, an analog of Fnr. J. Bacteriol. **177:**3606-3609.

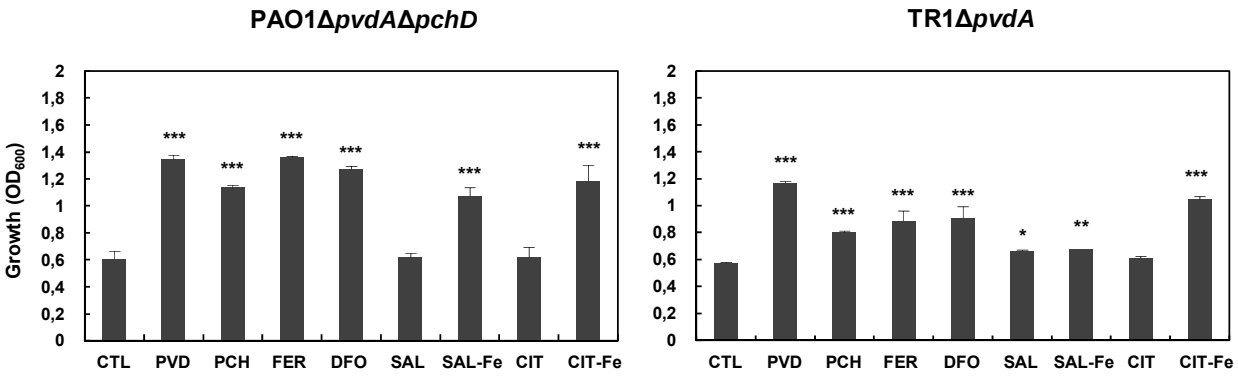

Fig S1. Effect of different siderophores and iron chelators on *P. aeruginosa* growth under iron depletion. Bacteria were grown for 24 h at 37°C in M9-succinate minimal medium supplemented with 400 µM 2,2'-dipyridyl and 5 µM $FeCl_3$ (CTL). Where indicated, the siderophore/iron chelator was supplemented at 20 µM. Abbreviations: pyoverdine (PVD), pyochelin (PCH) ferrichrome (FER), desferrioxamine (DFO), salycilate (SAL), citrate (CIT), citrate-iron complex (CIT-Fe; 2:1 ratio), salycilate-iron complex (SAL-Fe; 3:1 ratio). Growth was measured as OD_{600} (y axis). Mean values of three independent experiments ± the standard deviation are shown. Statistically significant differences (ANOVA) relative to the CTL are indicated. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

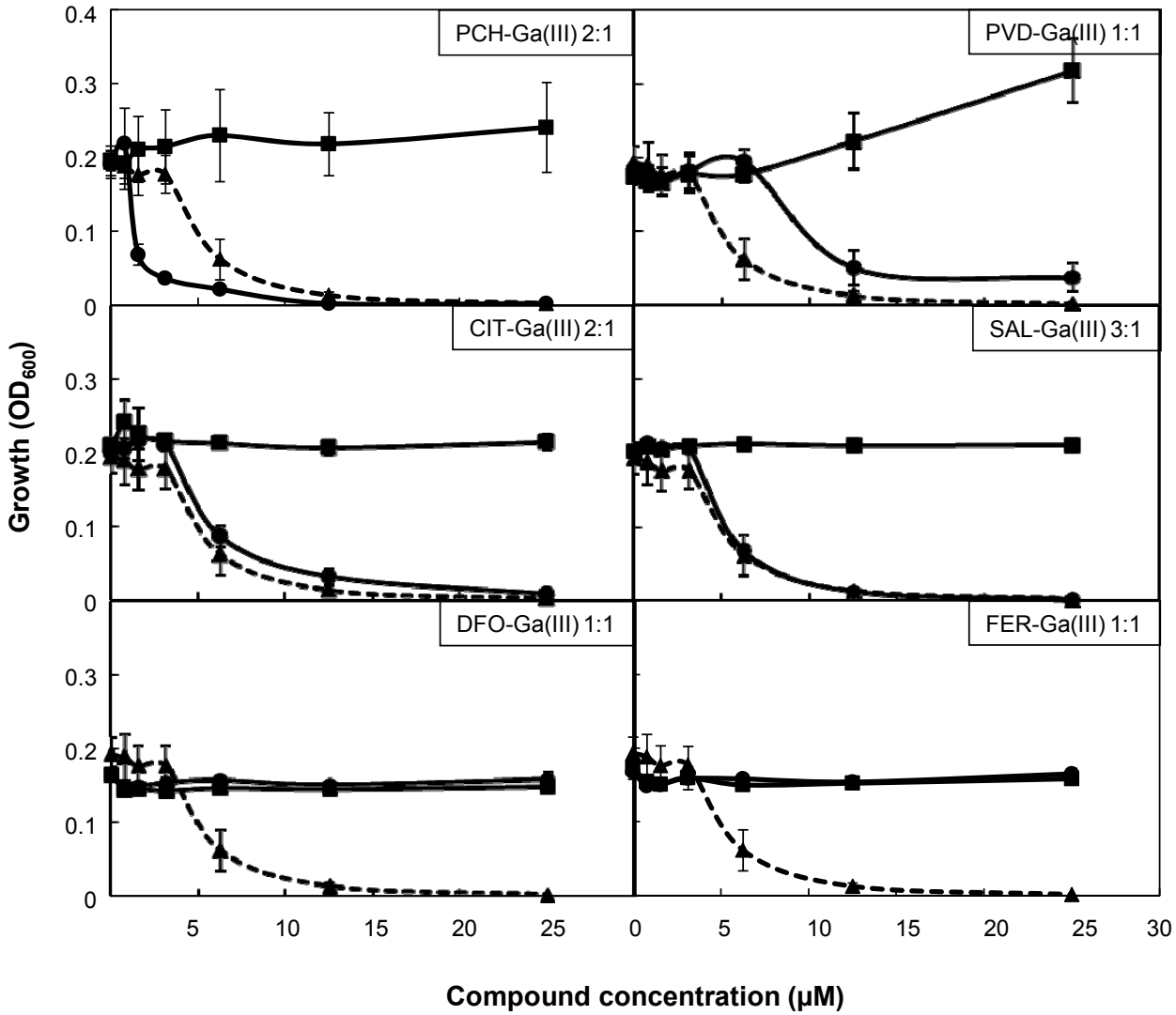

FIG S2. Effect of $Ga(NO_3)_3$ and Ga(III)-complexes on *P. aeruginosa* TR1 growth. Growth (OD_{600}) of *P. aeruginosa* TR1 in microtiter plates containing (per well) 200 µl DCAA supplemented with different concentrations of $Ga(NO_3)_3$ (triangles, dashed lines), the indicated Ga(III)-chelator complex (circles, solid lines) or the chelator alone as control (squares, solid lines), after 24 h at 37°C. The stoichiometry (binding ratio) of each Ga(III)-chelator complex is indicated in the inset of each panel. Values are the mean of at least two independent experiments ± the standard deviation.

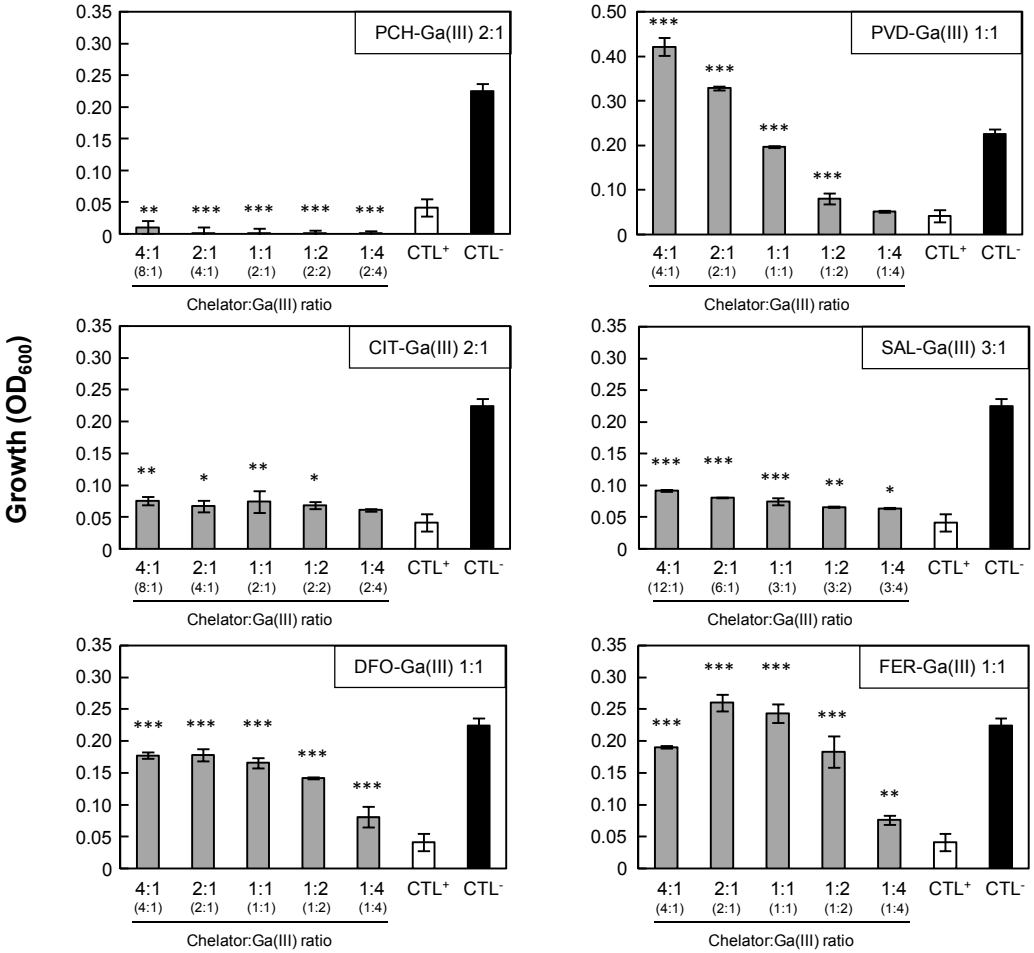

FIG S3. Effect of different chelator:Ga(III) ratios on *P. aeruginosa* TR1 growth. *P. aeruginosa* TR1 was grown for 24 h at 37°C in microtiter plates containing (per well) 200 µl DCAA supplemented with 12.5 µM $Ga(NO_3)_3$ and various concentrations of each chelator to obtain different chelator:Ga(III) ratios. The chelator:Ga(III) ratio (x axis) takes into account the binding stoichiometry, as indicated in the inset of each panel. The actual chelator:Ga(III) ratio is given in brackets on the x axis. CTL^+ indicates growth in the presence of 12.5 µM $Ga(NO_3)_3$, while CTL^- indicates growth without chelators or $Ga(NO_3)_3$. Growth was measured as OD_{600} (y axis). Each value is the mean of three independent experiments ± the standard deviation. Statistically significant differences compared to CTL^+ are indicated (ANOVA). * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.