

Supplementary Information

FrpA is the outer membrane piscibactin transporter in *Vibrio anguillarum*: structural elements in synthetic piscibactin analogues required for transport

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Table S1: Strains and plasmids used in this work.

Strain	Relevant characteristics	Source
<i>V. anguillarum</i>		
MB14	RV22 with in-frame deletion of <i>vabF</i> gene RV22 $\Delta vabF$	Balado <i>et. al.</i> , 2006 ¹
MB67	RV22 with in-frame deletion of <i>vabD</i> gene RV22 $\Delta vabD$	Balado <i>et. al.</i> , 2008 ²
ML208	RV22 with in-frame deletion of <i>vabF</i> and <i>frpA</i> genes RV22 $\Delta vabF\Delta frpA$	This study
ML210	RV22 with in-frame deletion of <i>vabD</i> and <i>frpA</i> genes RV22 $\Delta vabD\Delta frpA$	This study
<i>Photobacterium damsela</i> subsp. <i>piscicida</i>		
DI21	Piscibactin producer strain	Toranzo <i>et. al.</i> ³
<i>E. coli</i>		
DH5 α	Cloning strain	Laboratory strain
S17-1- <i>λpir</i>	RP4 (Km::Tn7, Tc::Mu-1) <i>pro-82 λpir recA1 end A1 thiE1</i> <i>hsdR17 creC510</i>	Herrero <i>et. al.</i> ⁴
Plasmids		
pWKS30	Low-copy number cloning vector, Amp ^r	Wang and Kushner ⁵
pNidKan	Suicide vector derived from pCVD442, Kan ^r	Mouriño <i>et. al.</i> ⁶
pML257	S17 <i>λpir</i> pSEVA 651 <i>frpA</i> comp	This study

¹ Balado, M.; Osorio, C. R.; Lemos, M. L. A gene cluster involved in the biosynthesis of vanchrobactin, a chromosome-encoded siderophore produced by *Vibrio anguillarum*. *Microbiology* **2006**, *152*, 3517–3528. doi: 10.1099/mic.0.29298-0.

² Balado, M.; Osorio, C. R.; Lemos, M. L. Biosynthetic and regulatory elements involved in the production of the siderophore vanchrobactin in *Vibrio anguillarum*. *Microbiology* **2008**, *154*, 1400–1413. doi: 10.1099/mic.0.2008/016618-0.

³ Toranzo, A. E.; Barreiro, S.; Casal, J. F.; Figueras, A.; Magariños, B.; Barja, J. L. Pasteurellosis in cultured gilthead seabream (*Sparus aurata*): first report in Spain. *Aquaculture* **1991**, *99*, 1–15. doi: 10.1016/0044-8486(91)90284-E.

⁴ Herrero, M.; de Lorenzo, V.; and Timmis, K. N. Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in Gram-negative bacteria. *J. Bacteriol.* **1990**, *172*, 6557–6567. doi: 10.1128/jb.172.11.6557-6567.1990.

⁵ Wang, R. F.; Kushner, S. R. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* **1991**, *100*, 195–199. doi: 10.1016/0378-1119(91)90366-J.

⁶ Mourino, S.; Osorio, C. R.; Lemos, M. L. Characterization of heme uptake cluster genes in the fish pathogen *Vibrio anguillarum*. *J. Bacteriol.* **2004**, *186*, 6159–6167. doi: 10.1128/JB.186.18.6159-6167.2004

Table S2: Oligonucleotides used for construction of *frpA*-defective mutants by allelic exchange and oligonucleotides complementation. Underlined sequences denote restriction sites.

Oligonucleotide	Sequence (5' → 3')	Amplicon Size (bp)
<i>frpA</i> mutant construction		
1_FrpAang_XbaI	CGCTCTAGAGGCAGCACTCAACAACAAGG	750
2_FrpAang_BamHI	GCGGGATCCGAGTGCGAAAGCGGTTGAAG	
3_FrpAang_BamHI	CCGGGATCCAACCTATTCGTTGCAATGC	750
4_FrpAang_4_XhoI	GCGCTCGAGGCTTTCAGAGATGGATGGTG	
<i>frpA</i> complementation		
1_FrpAang_comp_F_XbaI	CGCTCTAGACTCAGCGCAGAAATCTACAC	2570
2_FrpAang_comp_R_BamHI	GGCGGATCCAGAGACGCCTTCTGAACGCA	