Supporting Information

Cordero et al. 10.1073/pnas.1213344109

SI Materials and Methods

Siderophore Screen of Wild Isolates. We screened a total of 1,710 Vibrionaceae isolates from seawater collected at the Plum Island Estuary, MA (1, 2). The isolates were chosen such that each ecologically cohesive population previously identified as part of these studies was represented. Siderophore production was assessed using the well-established Chrome-Azurol S (CAS) assay (3). Biological replicate measurements for both the liquid and solid versions of the assay were performed in high-throughput, using either 96-well 2-mL culture blocks or 96-well microtiter plates for absorbance measurements.

For the solid version of the CAS assay, strains were grown in duplicates overnight in 2216 marine broth (Difco, Becton Dickinson) at room temperature and stamped directly onto CAS agar plates. CAS agar plates were prepared by mixing a dye made of CAS, Fe, and hexadecyl-trimethyl-ammonium bromide (HDTMA) with M9-based growth media (see below) appropriate for Vibrionaceae. For 1 L of CAS-agar, 100 mL of CAS-Fe-HDTMA dye was mixed with 900 mL of freshly prepared growth media. The CAS-Fe-HDTMA dye was prepared in advance as follows, for 1L: 10 mL of a 10 mM ferric chloride ($FeCl_3$) in 100 mM hydrochloric acid (HCl) solution was mixed with 590 mL of a 1-mM aqueous solution of CAS. The Fe-CAS solution was then added to 400 mL of a 2-mM aqueous solution of HDTMA. The resulting CAS-Fe-HDTMA solution was autoclaved for 25 min in a polycarbonate bottle that had previously been soaked overnight in 10% (vol/vol) HCl then rinsed five times with MilliQ water. The CAS-Fe-HDTMA dye was stored at room temperature covered from light until use.

The growth media was prepared as follows, for 1L of CAS-agar: 30.24 g of 1,4-piperazine-diethanesulfonic acid (Pipes), together with 1 g of ammonium chloride (NH_4Cl), 3 g potassium phosphate (KH_2PO_4), and 20 g sodium chloride (NaCl) was dissolved into MilliQ water by adjusting the pH with 10 M NaOH to 6.8. Note that the commonly used phosphate buffer disodium phosphate (Na_2HPO_4-$_7H_2O$) was omitted because phosphate can chelate iron and lead to a discoloration of the CAS dye. As a solidifying agent, 9 g of agar noble (Difco) were added to the solution. We found that the more commonly used solidifying agents, agarose, and agar also led to a discoloration of the CAS dye, likely owing to higher phosphate content. The volume was adjusted to 860 mL, and the solution was autoclaved. After cooling, 30 mL of a sterile 10% (wt/vol) Casamino acids (Difco) aqueous solution and 10 mL of a sterile 20% (wt/vol) glucose aqueous solutions were added. Finally, the 100 mL of CAS-Fe-HDTMA were added to the growth media. The final concentrations of the CAS-agar components are as follows: 100 mM Pipes, 18 mM NH_4Cl, 22 mM KH_2PO_4, 2% (wt/vol) NaCl, 0.3% casamino acids, 0.2% (wt/vol) glucose, 10 μM $FeCl_3$, 58 μM CAS, and 80 μM HDTMA. Unless otherwise noted, all chemicals were obtained from Sigma-Aldrich.

For the liquid version of the assay, isolates were first grown overnight in duplicates in 2216 marine broth (Difco) at room temperature, then transferred into the Vibrionaceae growth media described above (without the solidifying agent or the CAS-Fe-HDTMA dye). After overnight growth at room temperature, isolates were transferred into iron-poor media, consisting of the Vibrionaceae growth media described above amended with 100 μM 2,2′-bipyridyl and 10 nM FeCl3 to induce siderophore production. After 48 h, cultures were centrifuged and 99 μL of supernatant were mixed in a 1:1 ratio with liquid CAS dye and 2 μL shuttle solution (3). The mixture was incubated in the dark for 15 min, after which absorbance at 630 nm was measured on a Synergy2 filter-based multimode plate reader (Biotek). In the presence of a siderophore, the absorbance of the dye at 630 nm is quenched. Siderophore production was considered positive for all absorbances <0.3. The liquid CAS dye and shuttle solution were prepared as previously described (3).

Bioinformatic Analysis of Siderophore Synthesis and Transport Gene Clusters. Gene finding and annotations were performed using the SEED subsystems (4) and the RAST server (5). Siderophore biosynthesis clusters were identified using a combination of annotation text searches and the software AntiSMASH developed to identify biosynthetic clusters of secondary metabolites (6). AntiSMASH was run on all of the contigs for all 61 genomes using default parameters. For the purpose of this study, we focused on the "nrps" and "siderophore" metabolite clusters identified by AntiSMASH. These matched well with the annotation text searches that were concurrently performed. Text searches were performed for the following expressions: "siderophore," "actin" (encompassing many of the names of known siderophores), "ferrin," "pyoverdin," "chelin," "ferrichrome," "heme," "hydroxamate," "catechol." In some cases, genes belonging to a siderophore synthesis cluster were missed by the RAST annotation software. The presence of those genes in the siderophore synthesis clusters was confirmed by performing a BLASTp (7) search against the National Center for Biotechnology Information nonredundant protein database. For the genomes in which a "truncated pyoverdin-like" cluster was found, only two nonribosomal peptide-synthetase (NRPS) enzymes, homologs of PvdI and PvdD, were found as opposed to three or more NRPS needed for the synthesis of pyoverdin (8).

Growth Enhancement Experiments. Growth enhancement experiments were performed for the following isolates: ZS139, ZF90, 12B01, and ZF264. Isolates were grown overnight at room temperature in 2216 marine broth (Difco). Cultures were pelleted (2 min at 10,000 rpm in a microcentrifuge), the supernatant was discarded, and pellets were washed once in modified M9 salts [42.2 mM dibasic sodium phosphate (Na_2HPO_4), 22 mM monobasic potassium phosphate (KH_2PO_4), 18.7 mM ammonium chloride (NH_4Cl), and 2% (wt/vol) sodium chloride (NaCl)] to remove any carry-over 2216 media. Washed cells were pelleted and resuspended in modified M9 salts. Cells were then inoculated (1:1,000) into growth media [modified M9 salts, 0.3% (wt/vol) casamino acids, 200 mM magnesium sulfate ($MgSO_4$), 20 μM calcium chloride ($CaCl_2$), and vitamins: 0.1 $\mu g \cdot L^{-1}$ vitamin B12, 2 $\mu g \cdot L^{-1}$ biotin, 5 $\mu g \cdot L^{-1}$ calcium pantothenate, 2 $\mu g \cdot L^{-1}$ folic acid, 5 $\mu g \cdot L^{-1}$ nicotinamide, 10 $\mu g \cdot L^{-1}$ pyridoxin hydrochloride, 5 $\mu g \cdot L^{-1}$ riboflavin, 5 $mg \cdot L^{-1}$ thiamin hydrochloride] with (iron-poor) or without (iron-replete) the iron-specific chelator EDDA (ethylenediamine-N,N′-diacetic acid). Cells were incubated at room temperature. Absorbance at 600 nm was measured every 3 h for 24 h. Purified ferric-aerobactin (EMC Microcollections) and vibrioferrin (generously provided by Carl Carrano, San Diego State University, San Diego, CA) were added at specified concentrations. Extracts from the aerobactin producer 5F-79 and the vibrioferrin producer 12B01 grown under iron-poor conditions were obtained by filtering cell-free supernatant through a 3-kDa membrane using an Amicon Ultra centrifugal unit (Millipore).

1. Hunt DE, et al. (2008) Resource partitioning and sympatric differentiation among closely related bacterioplankton. *Science* 320(5879):1081–1085.
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8. Ravel J, Cornelis P (2003) Genomics of pyoverdine-mediated iron uptake in pseudomonads. *Trends Microbiol* 11(5):195–200.

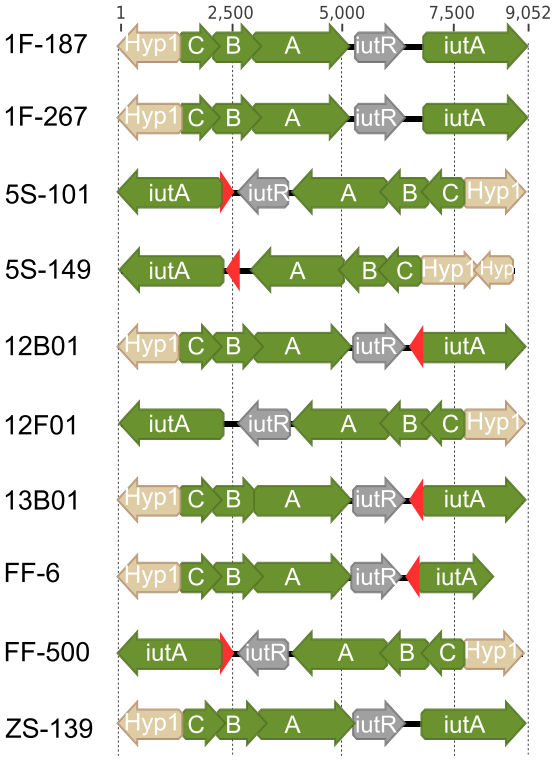

Fig. S1. List of aerobactin clusters in nonproducers. Genes in orange correspond to biosynthesis and genes in green to transport. Other colors correspond to genes annotated as hypothetical or unknown function. With the exception of 5S-149, there is basically only one cluster variant present across nonproducers, suggesting that the cluster was created once and shared by recombination.

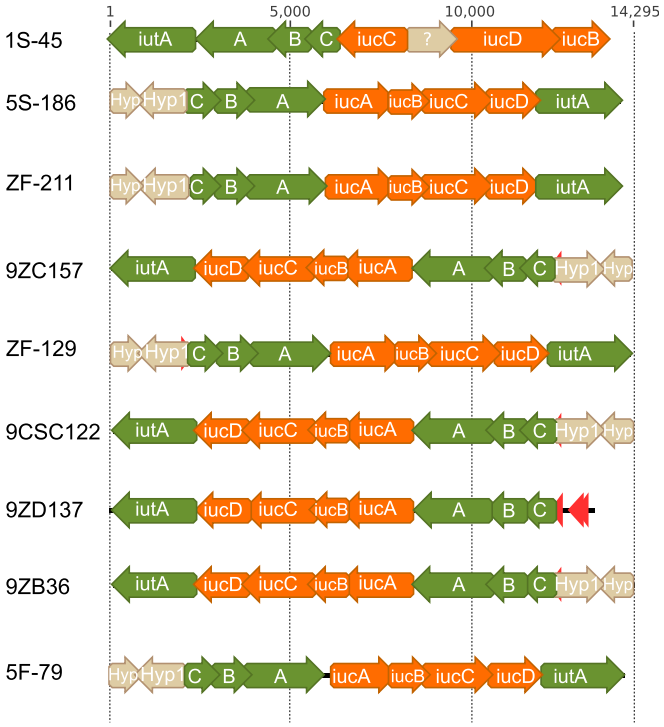

Fig. S2. Aerobactin biosynthesis and transport gene clusters present in producers. Genes in orange correspond to biosynthesis and genes in green to transport. Other colors correspond to genes annotated as hypothetical or unknown function. The figure shows that across the studied *Vibrio* populations there is essentially one version of the biosynthesis gene cluster.

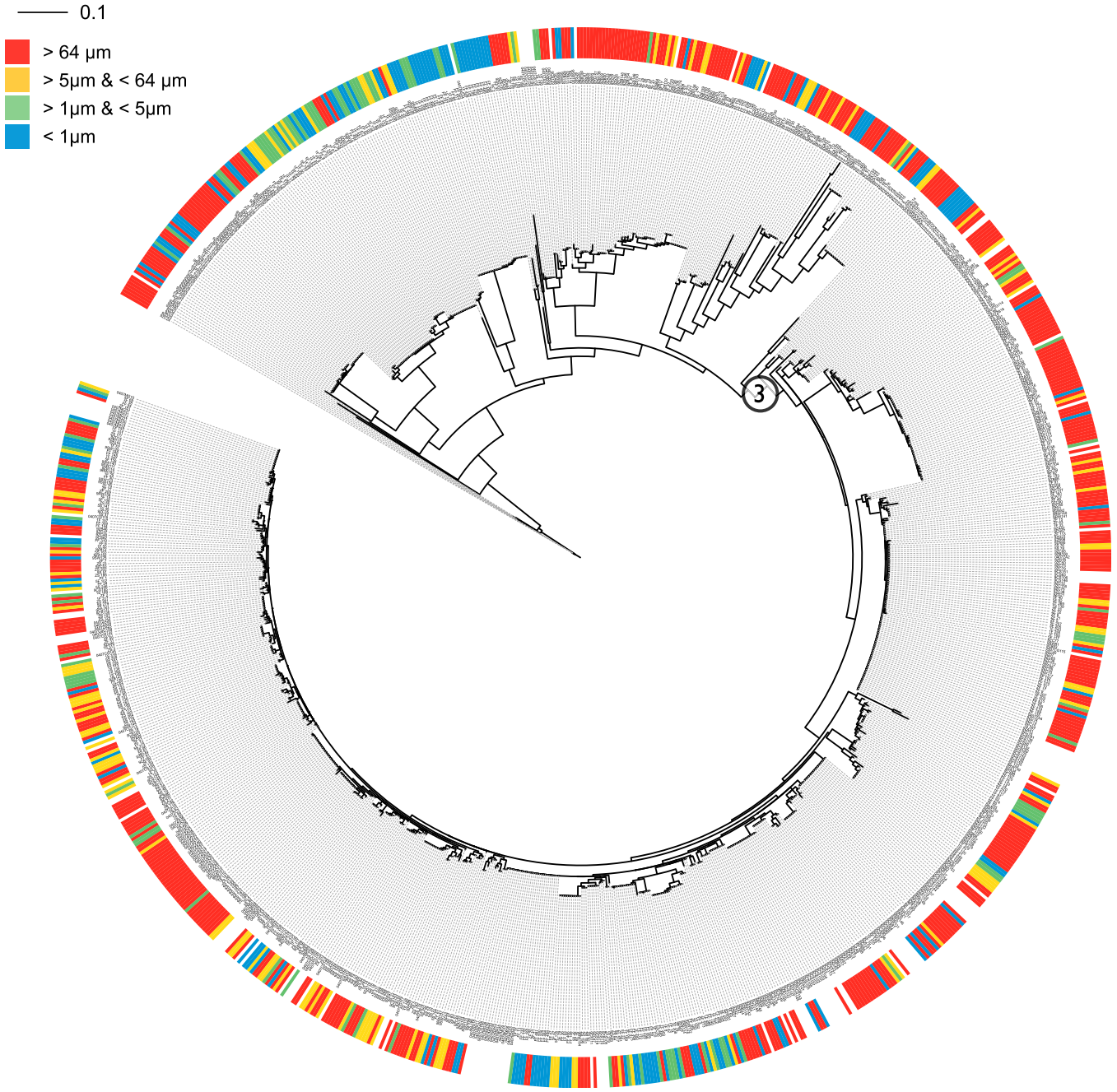

Fig. S3. Hsp60 phylogeny of vibrios indicating the corresponding size fractions for those strains isolated via the size fractionation scheme. The figure shows that in the *Vibrio splendidus*-like group (under node 3), the majority of strains are particle attached and that transitions to nonattached lifestyle have occurred multiple times on different parts of the phylogeny. Approximately 70% of the strains in the figure are found in particles larger than 5 µm, and the remainder in particles smaller than 5 µm.

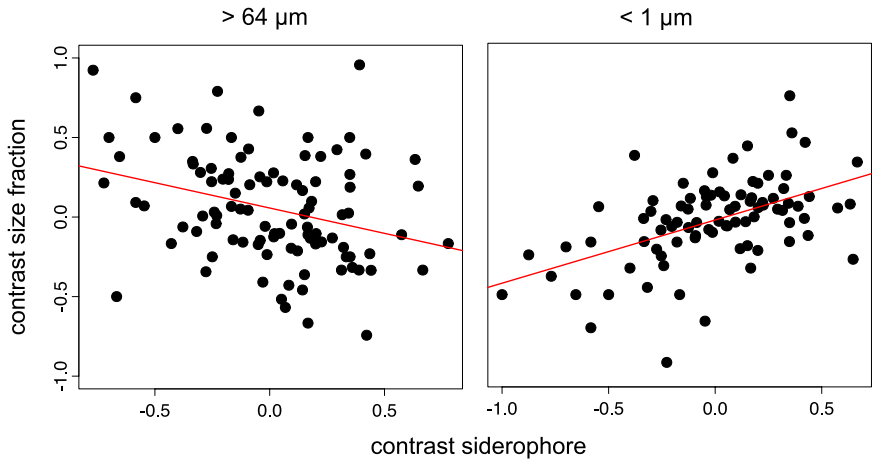

Fig. S4. Scatter plot of the independent contrasts between the siderophore trait and the two extreme size fractions in a randomly chosen bootstrap tree. The figure illustrates that the measured correlations are supported by multiple phylogenetic transitions. Accordingly, correlations calculated on these data are significant at *P* value 0.01.

Table S1. List of 61 isolates with full genome sequences

Strain	Production measured	Siderophore anNotated
ZF-55	No	No
FF-50	No	No
1A06	No	No
ZF-29	No	No
1S-45	Yes	Yes
5S-186	Yes	Yes
ZF-211	Yes	Yes
FF-238	No	No
FF-167	No	Yes
FF-93	Yes	Yes
FS-238	Yes	Yes
FS-144	Yes	Yes
12B09	Yes	Yes
9ZC157	Yes	Yes
ZF-129	Yes	Yes
9CSC122	Yes	Yes
9ZD137	Yes	Yes
9ZB36	No	Yes
12G01	Yes	Yes
5S-149	Yes*	Yes
12E03	Yes	Yes
ZF-57	No	No
9ZC77	No	No
9ZC13	Yes	Yes
9CS106	No	No
ZF-91	No	No
9ZC88	No	No
ZS-139	No	No
1F187	Yes	Yes
1F-155	No	No
1F-267	No	No
5F-79	Yes	Yes
ZS-17	No	No†
FF-500	Yes	Yes
ZS-63	Yes	Yes
ZF-90	No	No†
5S-101	No	No
FF-6	Yes	Yes
1F-157	Yes	Yes
0407ZC148	Yes	Yes
1S-124	No	Yes
13B01	Yes	Yes
12F01	Yes	Yes
12B01	Yes	Yes
FF-75	No	No†
ZF-99	No	No†
1F-289	No	No†
1F-53	No	No†
ZF-205	No	No†
ZF-255	No	No†
ZF-28	No	No†
ZF-270	No	No†
ZF-264	No	No†
ZF-207	No	No†
ZF-30	No	No†
ZF-65	No	No†
ZF-170	No	No†
ZF-14	No	No†
5F-59	No	No†
1F-97	No	No†
1F-111	No	No†

*Siderophore biosynthesis genes identified on plasmid sequence.
†Truncated pyoverdin-like cluster.