Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

Genetic, structural, and functional diversity of low- and high-affinity siderophores in strains of nitrogen fixing *Azotobacter chroococcum*

Xinning Zhang [1,2¶*], Oliver Baars [1,3¶*], François M. M. Morel[1]

[1]Department of Geosciences, Princeton University
[2]Princeton Environmental Institute, Princeton University
[3]Department of Entomology & Plant Pathology, North Carolina State University

¶These authors contributed equally to this work.

*Corresponding authors: xinningz@princeton.edu, obaars@ncsu.edu

TABLES

Table S1. General characteristics of *Azotobacter chroococcum* genomes.

Organism	**Size**	**% GC**	**Protein coding genes (CDS)**	**rRNA genes (No. operons)**	**tRNA genes**	**sequence identity %** [1]	**Accession**
A. chroococcum str.-B3 (chromosome, circular)	4,575,910	66.5	3937	18 (6)	64	99.5% (16s rRNA [2]), 98% (chromosome [3])	CP011835
pacx50fB3 (plasmid, linear)	306,103	62.7	259	0	0	99% [3]	CP011836
pacx50dB3a (plasmid, linear)	74,783	59.3	44	0	0	-	CP011837
pacx50fB3b (plasmid, linear)	66,259	61.7	45	0	0	-	CP011838
Ac-B3 (total draft genome)	5,023,055	66.1%	4,285	18 (6)	64		
A. chrococcum str. NCIMB 8003 (chromosome, circular)	4,591,803	66.3	3,959	18 (6)	66	reference	CP010415
pAcX50f (plasmid, circular)	311,724	62.7	292	0	0	-	CP010421
pAcX50e (plasmid, circular)	132,372	61.9	111	0	0	-	CP010420
pAcX50d (plasmid, circular)	69,317	59.2	55	0	0	-	CP010419
pAcX50c (plasmid, circular)	62,783	56.7	49	0	0	-	CP010418
pAcX50b (plasmid, circular)	13,852	55.3	10	0	0	-	CP010417
pAcX50a (plasmid, circular)	10,435	57.4	9	0	0	-	CP010416
Ac-8003 (total)	5,192,291	65.7	4485	18 (6)	66		

[1] Blast nr pairwise identity

[2] relative to AC-8003 16SrRNA in operon 1 (CP010415_185351_186886)

[3] query coverage: AC-B3 chromosome relative to reference chromosome, 87%; AC-B3 pacx50fB3 megaplasmid relative to reference megaplasmid, 47%

Table S2. Siderophore biosynthesis genes in *A. chroococcum* NCIMB 8003 and *Azotobacter chroococcum* B3. NRPS domains and adenylation domain specificity predicted using the software Antismash v. 3.0.2 (Weber et al. 2015). Domain names: C, condensation; A, adenylation; E, epimerization; T, thiolation; TE, thioesterase termination, KS ketosynthase, AT, acyltransferase, KR, ketoreductase, ACP.

Siderophore gene cluster	**Locus Tag (Accession)**	**Annotation**
AC-8003 vibrioferrin	Achr_32370 (AJE22645.1)	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase
	Achr_32380 (AJE22646.1)	Vibrioferrin receptor pvuA
	Achr_32390 (AJE22647.1)	Vibrioferrin ligase/carboxylase protein PvsA
	Achr_32400 (AJE22648.1)	Vibrioferrin amide bond forming protein PvsB
	Achr_32410 (AJE22649.1)	Vibrioferrin membrane-spanning transport protein PvsC
	Achr_32420 (AJE22650.1)	Vibrioferrin amide bond forming protein PvsD
	Achr_32430 (AJE22651.1)	Vibrioferrin decarboxylase protein PvsE
AC-8003 amphibactin	Achr_f2130 (AJE23907.1)	PvdS, sigma factor controlling pyoverdin biosynthesis
	Achr_f2140 (AJE23908.1)	Putative ATP-binding component of a transport system
	Achr_f2150 (AJE23909.1)	MbtH-like protein
	Achr_f2160 (AJE23910.1)	Non-ribosomal peptide synthetase (C-A_{orn}-T-E-C-A_{orn}-T-C-A_{ser}-T domains)
	Achr_f2170 (AJE23911.1)	Non-ribosomal peptide synthetase (C-A_{orn}-T-E-TE domains)
	Achr_f2180 (AJE23912.1)	Ferric hydroxamate outer membrane receptor, FhuA
	Achr_f2190 (AJE23913.1)	Acetyltransferase
	Achr_f2200 (AJE23914.1)	L-ornithine 5-monooxygenase, PvdA like

AC-8003 crochelin		
	Achr_39010 (AJE23287.1)	MbtH-like protein
	Achr_39000 (AJE23286.1)	Non-ribosomal peptide pseudogene (E domain)
	Achr_38990 (AJE23285.1)	L-ornithine 5-monooxygenase, PvdA-like
	Achr_38980 (AJE23284.1)	PvdS-like, sigma factor controlling pyoverdine biosynthesis
	Achr_38970 (AJE23283.1)	outer membrane factor (OMF) lipoprotein
	Achr_38960 (AJE23282.1)	RND family efflux transporter, membrane fusion protein subunit; pyoverdine efflux pump membrane fusion protein PvdR-like
	Achr_38950 (AJE23281.1)	CroA (MbtH-like protein)
	Achr_38940 (AJE23280.1)	CroB (Thioesterase, PvdG-like)
	Achr_38930 (AJE23279.1)	CroC
	Achr_38920 (AJE23278.1)	CroD Non-ribosomal peptide synthetase (CAL$_{NH2}$ -T-C-Aser-T)
	Achr_38910 (AJE23277.1)	CroE Polyketide synthase Type I (KS –AT- KR-ACP)
	Achr_38900 (AJE23276.1)	CroF Non-ribosomal peptide synthetase (C-Aser-T)
	Achr_38890 (AJE23275.1)	CroG Non-ribosomal peptide synthetase (C-Aasp-T-E-C-A$_{?}$-T)
	Achr_38880 (AJE23274.1)	CroH Non-ribosomal peptide synthetase (C*^{1}-A$_{?}$-T-TE)
	Achr_38870(AJE23273.1)	CroI (TonB-dependent ferric siderophore receptor, FhuA-like)
	Achr_38860 (AJE23272.1)	CroJ (L-2,4-diaminobutyrate:2-oxoglutarate aminotransferase, PvdH-like)
	Achr_38850 (AJE23271.1)	CroK (N5-hydroxyornithine formyltransferase, PvdF-like)
	Achr_38840 (AJE23270.1)	CroL Deacetylase, Acetylpolyamine aminohydrolase
	Achr_38830 (AJE23269.1)	CroM (Acyl-homoserine lactone acylase, PvdQ-like)
	Achr_38820 (AJE23268.1)	CroN (ATP binding cassette transporter, PvdE-like, FhuB like)

	Achr_38810 (AJE23267.1)	CroO (iron-regulated membrane protein, FhuC like)
	Achr_38800 (AJE23266.1)	CroP (ferric-siderophore ABC transporter, ATP-binding protein, FhuC-like)
AC-B3 vibrioferrin	ACG10_16850 (ASL27784.1)	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase
	ACG10_16855 (ASL27785.1)	Vibrioferrin receptor PvuA
	ACG10_16860 (ASL27786.1)	Vibrioferrin ligase/carboxylase protein PvsA
	ACG10_16865 (ASL27787.1)	Vibrioferrin amide bond forming protein PvsB
	ACG10_16870 (ASL27788.1)	Vibrioferrin membrane-spanning transport protein PvsC
	ACG10_16875 (ASL27789.1)	Vibrioferrin amide bond forming protein PvsD
	ACG10_16880 (ASL27790.1)	Vibrioferrin decarboxylase protein PvsE
	ACG10_16885 (ASL27791.1)	Acyl-CoA dehydrogenase
AC-B3 amphibactin	ACG10_19975 (ASL28769.1)	PvdS-like, sigma factor controlling pyoverdine biosynthesis
	ACG10_19970 (ASL28339.1)	Putative ATP-binding component of a transport system
	ACG10_19965 (ASL28338.1)	MbtH-like protein
	ACG10_19960 (ASL28768.1)	Non-ribosomal peptide synthetase (C-A_{orn}-T-E-C-A_{orn}-T-C-A_{ser}-T)
	ACG10_19955 (ASL28337.1)	Non-ribosomal peptide synthetase (C-A_{orn}-T-E-TE)
	ACG10_19950 (ASL28336.1)	Ferric hydroxamate outer membrane receptor FhuA
	ACG10_19945 (ASL28335.1)	Acetyltransferase
	ACG10_19940 (ASL28334.1)	L-ornithine 5-monooxygenase, PvdA like

Table S3. Genome accession information for *Azotobacter genomes.*

Organism	**Replicon Accession**
A. chroococcum str.-B3	CP011835-CP011838
A. chrococcum str. NCIMB 8003	CP010415-CP010421
A. vinelandii str. CA	CP005094
A. beijerinckii str. DSM 1041	NZ_FNYO01000001.1 - NZ_FNYO01000241.1
A. beijerinckii str. DSM 282	NZ_FOKJ01000001.1 - NZ_FOKJ01000239.1
A. beijerinckii str. DSM 373	NZ_FNYQ01000001. - NZ_FNYQ01000249.1
A. beijerinckii str. DSM 378	NZ_FOFJ01000001.1 - NZ_FOFJ01000179.1
A. beijerinckii str. DSM 381	NZ_FOSX01000001.1 - NZ_FOSX01000247.1

Table S4. Vibrioferrin gene cluster loci and accession information.

Organism	Vibrioferrin receptor, PvuA	Vibrioferrin biosynthesis, PvsA	Vibrioferrin biosynthesis, PvsB	Vibrioferrin biosynthesis, PvsC	Vibrioferrin biosynthesis, PvsD	Vibrioferrin biosynthesis, pvsE	locus information for psuA-pvsABCDE cluster
Azotobacter chroococcum B3	ASL27785.1	ASL27786.1	ASL27787.1	ASL27788.1	ASL27789.1	ASL27790.1	ACG10_16855 - ACG10_16880
Azotobacter chroococcum NCIMB 8003	AJE22646.1	AJE22647.1	AJE22648.1	AJE22649.1	AJE22650.1	AJE22651.1	ACHR_32380-ACHR_32430
Azotobacter vinelandii CA	AGK17151.1	AGK17152.1	AGK17153.1	AGK17154.1	AGK17155.1	AGK17156.1	AvCA_09350-AVCA_09300
Azotobacter beijerinckii 282	WP_090939916.1	WP_090939919.1	WP_090939922.1	WP_090939925.1	WP_090939928.1	WP_090939932.1	BM263_RS11710 to BM263_RS11685
Azotobacter beijerinckii 1041	WP_090897687.1	WP_090897685.1	WP_090897682.1	WP_090620949.1	WP_090897678.1	WP_090897675.1	BMZ11_RS04800 to BMZ11_RS04775
Azotobacter beijerinckii 373	WP_090731225.1	WP_090731226.1	WP_090731227.1	WP_090731228.1	WP_090731229.1	WP_090731230.1	BMW56_RS08840 to BMW56_RS08865
Azotobacter beijerinckii 378	WP_090620954.1	WP_090620952.1	WP_090620951.1	WP_090620949.1	WP_090620948.1	WP_090620946.1	BMY22_RS07840 to BMY22_RS07820
Azotobacter beijerinckii 381	WP_090939916.1	WP_090939919.1	WP_090939922.1	WP_090939925.1	WP_090939928.1	WP_090939932.1	BM240_RS11750 to BM240_RS11770
Pseudomonas fluorescens UK4	AIG01281.1	AIG01282.1	AIG01283.1	AIG01284.1	AIG01285.1	AIG01286.1	HZ99_03505 to HZ99_03530
Pseudomonas taeanensis MS-3	KFX68245.1	KFX68246.1	KFX68247.1	KFX68248.1	KFX68249.1	KFX68250.1	TMS3_0118550-TMS3_0118570
Pseudomonas aeruginosa PA7	ABR83810.1	ABR81139.1	ABR81664.1	ABR80780.1	ABR81509.1	ABR82594.1	PSPA7_3092-PSPA7_3095
Vibrio parahaemolyticus RIMD 2210633	BAC63000.1	BAC63001.1	BAC63002.1	BAC63003.1	BAC63004.1	BAC63005.1	VPA1658-VPA1662
Vibrio alginolyticus E0666	WP_005395581.1	WP_005395580.1	WP_005395578.1	WP_005395576.1	WP_031780477.1	WP_005395574.1	C408_RS20505 - C408_RS20485

Vibrio harveyi VHJR7	WP_047515630.1	WP_047515628.1	WP_047515626.1	WP_051177507.1	WP_047515624.1	WP_009700852.1	QZ23_RS16140 - QZ23_RS16110
Xanthomonas oryzae BLS256	WP_014504205.1	WP_014504206.1	WP_014504207.1	WP_014504208.1	WP_014504209.1	WP_014504210.1	XOC_RS15030 - XOC_RS15055
Xanthomonas vasicola NCPPB 206	WP_010366993.1	WP_017115120.1	WP_017117959.1	WP_017117958.1	WP_017117957.1	WP_017117956.1	KWI_RS0106545 - KWI_RS0106565
Xanthomonas citri	WP_011052081.1	WP_076605163.1	WP_040244739.1	WP_011052084.1	WP_011052085.1	WP_011052086.1	AMD03_RS17160 - AMD03_RS17185
Marinobacter algicola DG893		WP_007151737.1	WP_007151736.1	WP_007151735.1	WP_083797637.1	WP_007151733.1	MDG893_RS00495- MDG893_RS00475
Marinobacter nitratireducens str. AK21	WP_081849591.1	WP_036134067.1	WP_051669166.1	WP_036134069.1	WP_051669145.1	WP_036134071.1	D777_RS15640 - D777_RS15660
Marinobacter salarius str. R9SW1	WP_082040557.1	WP_041334587.1	WP_041334589.1	WP_052479503.1	WP_052479504.1	WP_041334594.1	AU15_RS13765 - AU15_RS13785

Table S5. Amphibactin gene cluster loci and accession information.

Organism	Locus information for ABO2093, ABO2092-like NRPS homologs	Loci for entire cluster	mbtH-like	ABO2093-like NRPS	ABO2092-like NRPS	Ferric siderophore receptor (FhuA)	acetyl transferase (IucB)	L-ornithine monooxygenase (pvdA)
Azotobacter chroococcum B3	ACG10_19960 , ACG10_19955	ACG10_19965- ACG10_19940	ASL28338.1	ASL28768.1	ASL28337.1	ASL28336.1	ASL28335.1	ASL28334.1
Azotobacter chroococcum NCIMB 8003	Achr_f2160 , Achr_f2170	Achr_f2150- Achr_f2200	AJE23909.1	AJE23910.1	AJE23911.1	AJE23912.1	AJE23913.1	AJE23914.1
Azotobacter beijerinckii 282	BM263_RS14315, BM263_RS14320	BM263_RS14310- BM263_RS14335	WP_090940695.1	WP_091013700.1	WP_090940739.1	WP_090940702.1	WP_090940705.1	WP_090940708.1
Azotobacter beijerinckii 1041	BBMZ11_RS02470, BBMZ11_RS02465	BMZ11_RS02475 - BMZ11_RS02450	WP_090621531.1	WP_090896840.1	WP_090896987.1	WP_090896837.1	WP_090896834.1	WP_090896830.1
Azotobacter beijerinckii 373	BMW56_RS00370, BMW56_RS00375	BMW56_RS00365 - BMW56_RS00390	WP_090729034.1	WP_090729036.1	WP_090729087.1	WP_090729038.1	WP_090621535.1	WP_090621537.1
Azotobacter beijerinckii 378	BMY22_RS09630, BMY22_RS09635	BMY22_RS09625 - BMY22_RS09650	WP_090621531.1	WP_090621532.1	WP_090621559.1	WP_090621534.1	WP_090621535.1	WP_090621537.1
Azotobacter beijerinckii 381	BM240_RS13475, BM240_RS13480	BM240_RS13470 - BM240_RS13495	WP_090940695.1	WP_090940698.1	WP_090940739.1	WP_090940702.1	WP_090940705.1	WP_090940708.1
Alcanivorax burkomensis SK2	ABO_2093, ABO_2092	ABO_2094- ABO_2089	WP_011589372.1	WP_011589371.1	WP_011589370.1	WP_011589369.1	WP_011589368.1	WP_011589367.1
Alcanivorax sp. HIO33	Q672_RS17510, Q672_RS17505	Q672_RS17515- Q672_RS17490	WP_022983689.1	WP_022983688.1	WP_022983687.1	WP_022983686.1	WP_022983685.1	WP_031225681.1
Vibrio coralliilyticus strain OCN014	JV59_RS06415, JV59_RS06420	JV59_30610 to JV59_RS06435	WP_006962378.1	WP_038511202.1	WP_038511204.1	WP_038511207.1	WP_038511209.1	WP_038511212.1
Vibrio sp. VPAP30	ZX61_RS18370, ZX61_RS18375	ZX61_RS18365- ZX61_RS18390	WP_049846048.1	WP_049846049.1	WP_049846050.1	WP_049846051.1	WP_049846052.1"	WP_049846053.1
Pseudomonas alcaligenes NBRC 14159	PA6_RS05435, PA6_RS05440	PA6_RS05425- PA6_RS05455	WP_021699878.1	WP_021699880.1	WP_052520116.1	WP_021699882.1	WP_021699883.1	WP_021699884.1
Pseudomonas sp. ML96	IP89_RS02245, IP89_RS02240	IP89_RS02255 to IP89_RS02225	WP_043307335.1	WP_043307333.1	WP_052080930.1	WP_052080921.1	WP_043307330.1	WP_043307329.1

Table S6. 16S rRNA sequence accession numbers.

Organism	16s rRNA Genbank Accession
Azotobacter chroococcum strain NCIMB 8003	CP010415: 185351_186886
Azotobacter chroococcum strain B3	CP011835: 187010_188553
Azotobacter vinelandii strain CA	CP005094: 1377983_1379520
Azotobacter beijerinckii strain DSM 373	FNYQ01000183.1:1-829 , contig: Ga0061119_scaffold00183.183
Azotobacter beijerinckii strain DSM 1041	FNYO01000148.1:1-827, contig: Ga0061142_scaffold00148.148
Azotobacter beijerinckii strain DSM 282	FOKJ01000164.1:c4461-3657, contig: Ga0061118_scaffold00164.164
Azotobacter beijerinckii strain DSM 381	FOSX01000169.1:c4460-3657, contig: Ga0061121_scaffold00169.169
Azotobacter beijerinckii strain DSM 378	FOFJ01000125.1:1-410 , contig: Ga0061120_scaffold00125.125
Azotobacter beijerinckii strain ICMP 8673	NR_042071.1
Azotobacter salinestris strain NBRC 102611	NR_114165.1
Azotobacter armeniacus strain DSM 2284	NR_041037.1

Table S8. Observed and calculated *m/z* values and retention times for siderophores in *A. chroococcum* supernatants (see also chromatograms and structures in **Fig. 4** and **Fig. 5**). Two retention times are given for each siderophore, one using a formic acid mobile phase buffer and one with a heptafluorobutyric acid (HFBA) buffer which was required for the analysis of crochelin A.

		#	***m/z* observed**	***m/z* calculated**	*z*	**Ret. Time Formic Acid buffer (min)**	**Ret. Time HFBA buffer (min)**
Vibrioferrin A			435.1231	435.1246	1	6.8,7.5	6.2,6.8
Amphibactins							
R							
H	Amphibactin Headgroup	**2**	622.3021	622.3042	1	9.10	10.55
C8H15O (C8:0)		**3**	748.4069	748.4087	1	13.10	13.10
C10H19O (C10:0)	Amphibactin ACB	**6**	776.4380	776.4400	1	14.33	14.28
C10H17O (C10:1)		**5**	774.4223	774.4244	1	14.00	14.00
C10H19O2 (OH-C10:0)	Amphibactin ACA	**4**	792.4329	792.4349	1	13.60	13.60
C12H23O (C12:0)	Amphibactin T	**10**	804.4694	804.4713	1	15.61	15.59
C12H21O (C12:1)		**9**	802.4537	802.4557	1	15.03	15.00
C12H23O (OH-C12:0)		**8**	820.4643	820.4662	1	14.84	14.81
C12H21O2 (OH-C12:1)		**7**	818.4486	818.4506	1	14.38	14.35
C14H25O (C14:1)		**11**	830.4840	830.4870	1	16.16	16.10
Crochelins							
R							
H	Crochelin A	**12**	348.6791	348.6798	2	-	11.36
C10H19O (C10:0)	Crochelin E	**14**	425.7469	425.7477	2	12.11	13.69
C10H17O (C10:1)	Crochelin D	**13**	424.7382	424.7399	2	11.77	13.44
C12H23O (C12:0)	Crochelin B	**17**	439.7618	439.7633	2	13.16	14.58
C12H21O (C12:1)	Crochelin C	**16**	438.7543	438.7555	2	12.70	14.18
C12H23O (OH-C12:0)		**15**	447.7593	447.7603	2	12.52	14.00
	C12:0-ADPA	**20**	332.2421	332.2431	1	16.88	16.89
	C12:0-ADPA-Ser	**19**	419.2736	419.2750	1	15.91	15.88
	C12:0-ADPA-Ser-OHAsp	**18**	550.2946	550.2970	1	15.45	15.30

Table S9. Observed and calculated *m/z* values for Amphibactins in cell-pellet extracts of AC-B3 and AC-8003. The table also indicates relative peak heights, normalized to the most abundant amphibactin analog in each cell-pellet extract. Shown in bold are amphibactins that were only observed in cell pellets extracts.

R	**ID**	***m/z* observed**	***m/z* calculated**	**AC-B3 relative abundance**	**AC-8003 relative abundance**
H	Amphibactin Headgroup		622.3042	n/d	n/d
C8H15O (C8:0)		748.4073	748.4087	0	1
C10H19O (C10:0)	Amphibactin ACB	776.4400	776.4400	2	32
C10H17O (C10:1)		774.4243	774.4244	0	2
C10H19O2 (OH-C10:0)	Amphibactin ACA	792.4329	792.4349	2	42
C12H23O (C12:0)	Amphibactin T	804.4694	804.4713	83	100
C12H21O (C12:1)		802.4537	802.4557	20	92
C12H23O (OH-C12:0)		820.4643	820.4662	9	92
C12H21O2 (OH-C12:1)		818.4486	818.4506	2	48
C14H25O (C14:1)		830.4840	830.4870	100	48
C14H27O (C14:0)		**832.5030**	**832.5026**	**48**	**25**
C16H29O (C16:1)		**858.5180**	**858.5183**	**13**	**43**
C22H41O3		**974.6010**	**974.6020**	**20**	**0**
C24H43O3		**1000.6200**	**1000.6176**	**16**	**0**
C24H45O3		**1002.6330**	**1002.6333**	**10**	**0**

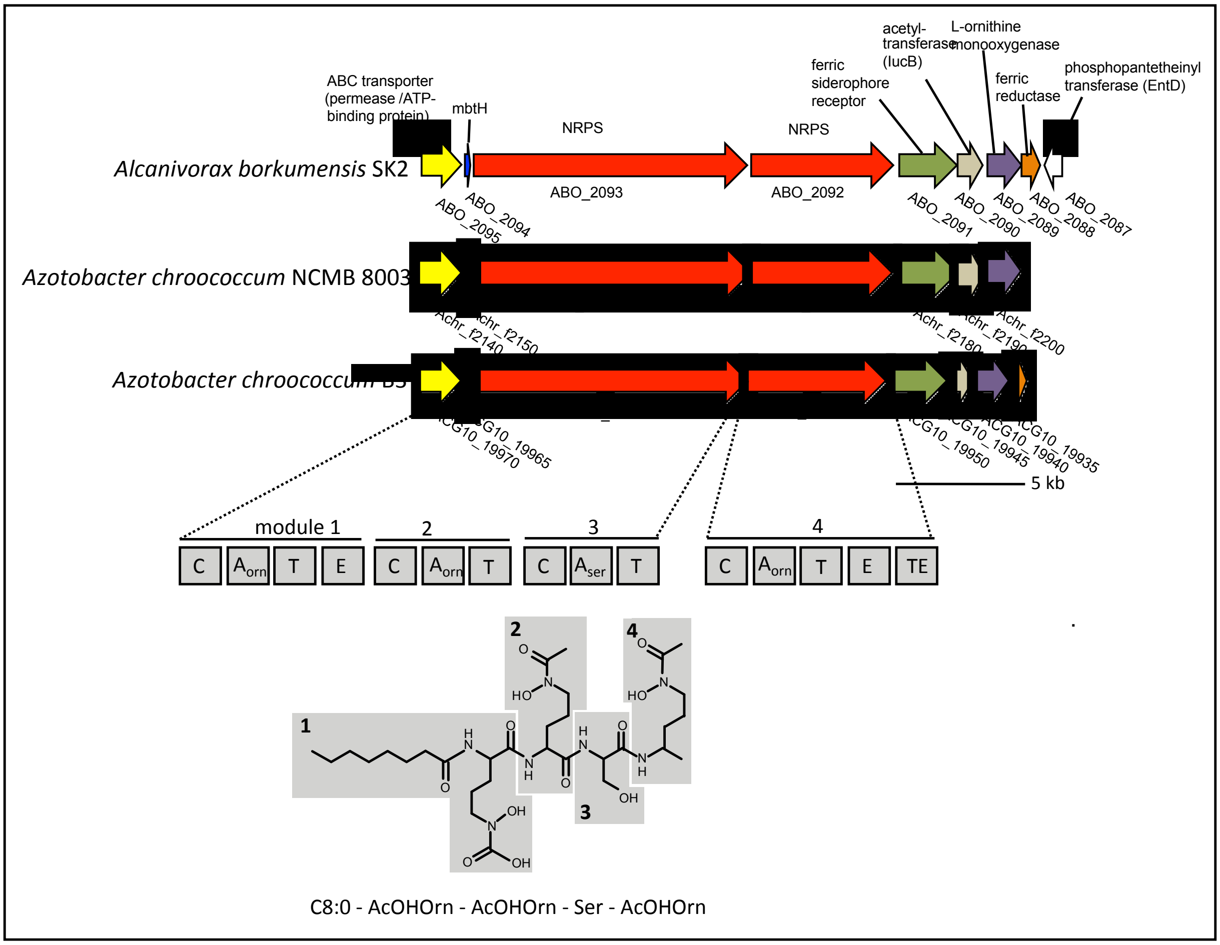
Table S10. Observed and calculated MS/MS fragmentation for each amphibactin analog.

R	**Siderophore**	**Fragment ions observed (calculated)**				
		b4	**b3**	**b2**	**y3**	**y2**
H	Amphibactin Headgroup	-	432.2079 (432.2089)	345.1759 (345.1769)	450.2183 (450.220)	278.1339 (278.135)
C8H15O (C8:0)		558.3112 (558.3134)	471.2781 (471.2814)	299.1946 (299.1966)	450.2176	278.1336
C10H19O (C10:0)	Amphibactin ACB	586.3432 (586.3447)	499.3112 (499.3127)	327.2269 (327.2279)	450.2184	278.134
C10H17O (C10:1)		584.3284 (584.329)	497.296 (497.297)	325.2116 (325.2122)	450.219	278.1343
C10H19O2 (OH-C10:0)	Amphibactin ACA	602.3370 (602.3396)	515.3052 (515.3076)	343.2212 (343.2228)	450.2177	278.1332
C12H23O (C12:0)	Amphibactin T	614.3738 (614.3760)	527.3425 (527.3440)	355.2581 (355.2592)	450.2181	278.1342
C12H21O (C12:1)		612.3579 (612.3603)	525.3253 (525.3283)	353.2417 (353.2435)	450.2181	278.1339
C12H23O2 (OH-C12:0)		630.3683 (630.3709)	543.3352 (543.3389)	371.2522 (371.2541)	450.2174	278.1417
C12H21O2 (OH-C12:1)		628.3527 (628.3553)	541.3206 (541.3233)	369.2372 (369.2385)	450.2176	278.1331
C14H25O (C14:1)		640.3896 (640.3916)	553.3562 (553.3596)	381.2740 (381.2748)	450.2181	278.1327
C14H27O (C14:0)*		642.4065 (642.4073)	555.3719 (555.3753)		450.2189	
C16H29O (C16:1)*		668.4210 (668.4229)	581.3903 (581.3909)		450.2215	
C22H41O3*		784.5015 (784.5066)	697.4718 (697.4746)	525.3915 (525.3898)	450.2188	
C24H43O3*		810.5236 (810.5223)	723.4887 (723.4903)	551.4040 (551.4055)	450.2184	
C24H45O3*		812.5361 (812.5379)	725.5030 (725.5059)	553.4242 (553.4211)	450.2198	

*Data collected on Q-TOF LC-MS with MS/MS scan starting at m/z=450; all other data collected on Orbitrap XL LC-MS

FIGURES

Figure S1. Amphibactin biosynthetic gene clusters in the marine isolate Alcanivorax borkumensis SK2 (Kem et al. 2014) and AC strains. Genes for NRPS ABO_2094 and ABO_2092 encode the stepwise assembly of amino acids (Orn – Orn – Ser – Orn) into the peptidic amphibactin headgroup**.** Genes for the tailoring enzymes, L-ornithine-monoxygenase and acetyltransferase, which generate the N-acyl-N-hydroxyornithine, substrate for NRPS incorporation at Orn-specific adenylation domains also appear in each cluster. Homologous genes are represented by the same color.



Reference: Kem MP et al. 2014. Amphiphilic siderophore production by oil-associating microbes. Metallomics **6:**1150-1155

Figure S2. High-resolution LC-MS data was mined for the characteristic Fe stable isotope pattern (^{54}Fe-^{56}Fe) and the occurrence of corresponding apo siderophore species. Shown is an exemplary mass spectrum for the Amphibactin S – Fe complex and free amphibactin S (R = C14:1; compound **11**)

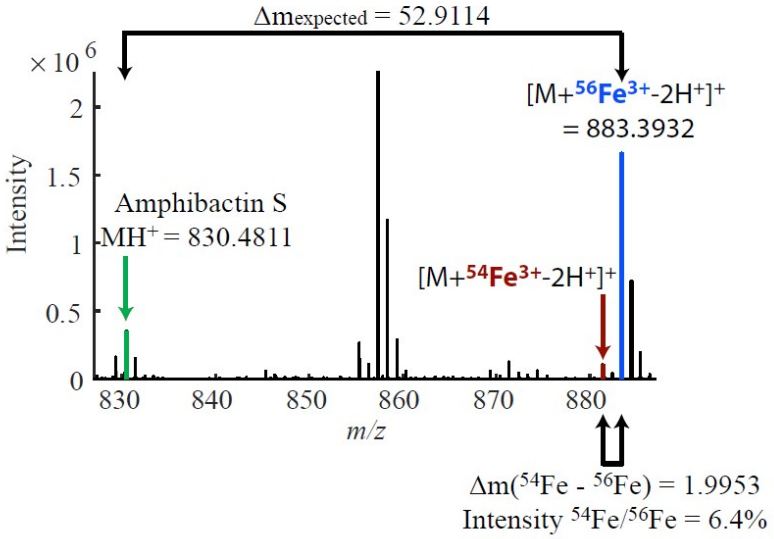


Figure S3. Vibrioferrin was identified by comparison of MS/MS pattern, precursor mass, and retention time to a standard. Shown is the MS/MS fragmentation pattern of vibrioferrin.

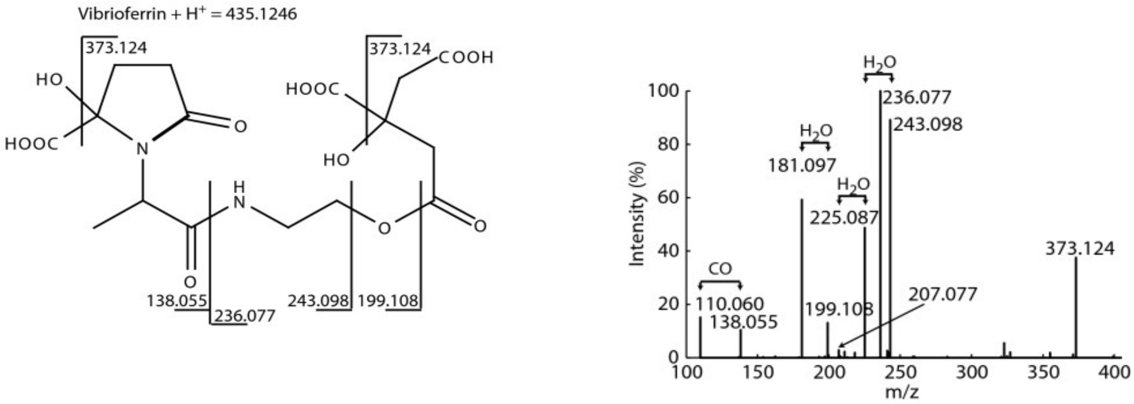
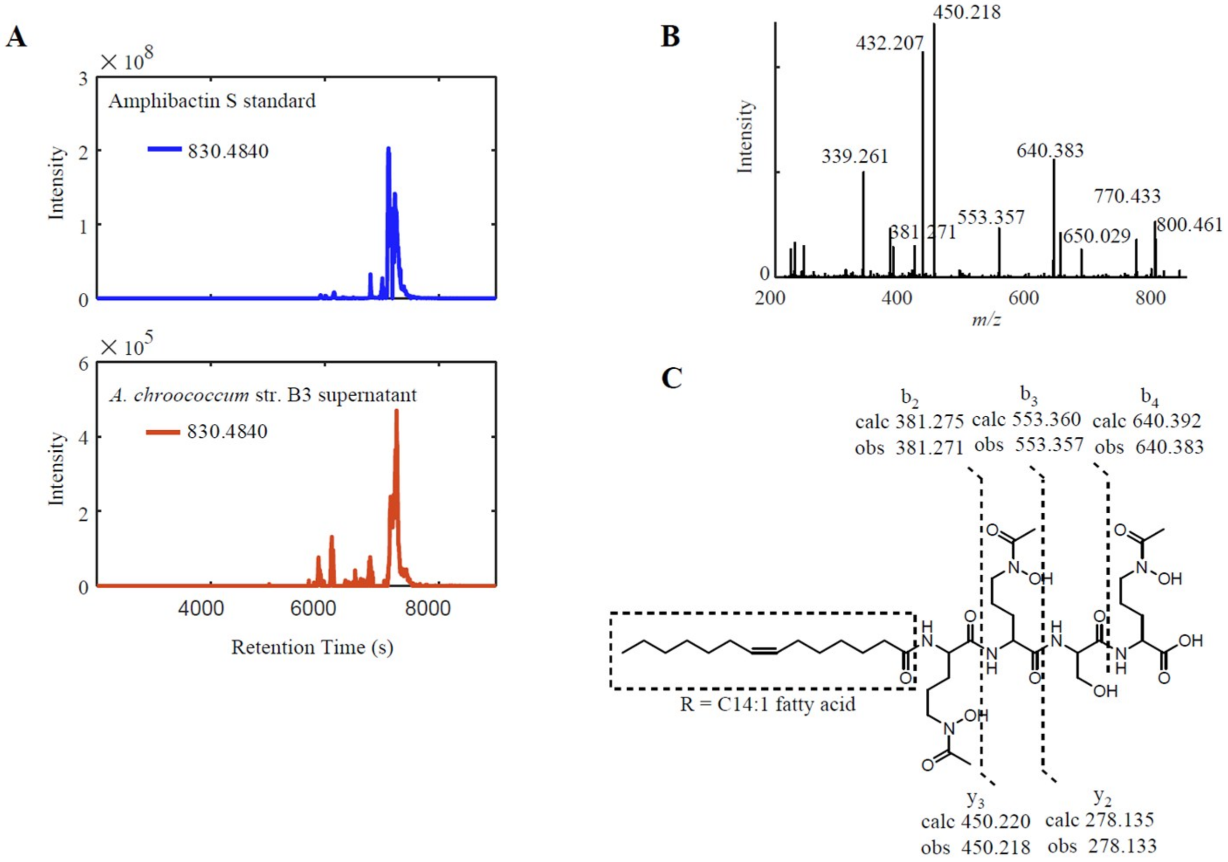
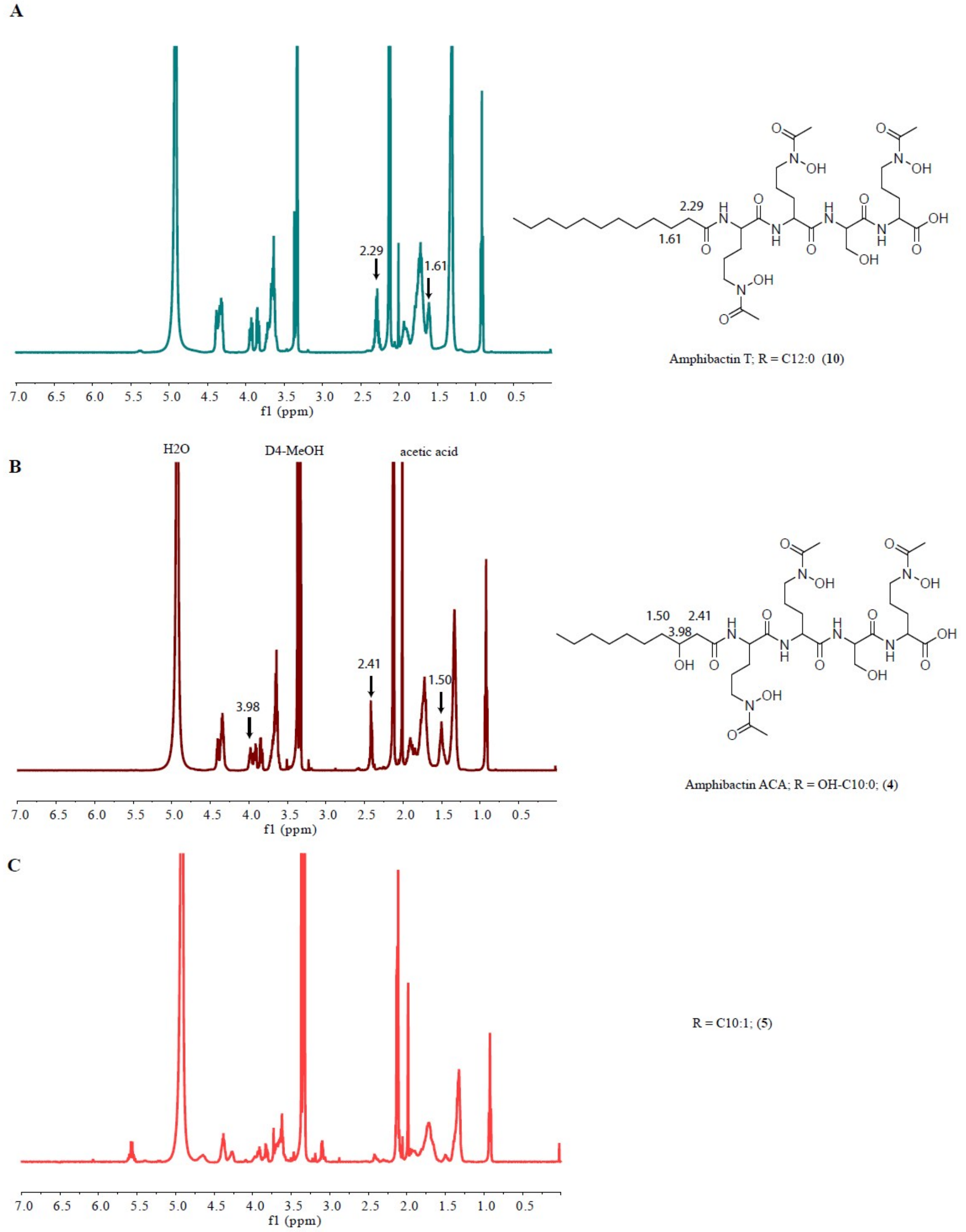


Figure S4. The retention time (panel **A**), *m/z* value, and MS/MS pattern (panel **B**) of amphibactin S matched one of the amphibactin analogs present in the supernatant and cell pellet of *A. chroococcum* str. B3. Different amphibactins have common characteristic fragment ions (y ions in panel **C**) but differ in the mass of the acyl fatty acid residues. Differences in the high-resolution MS and MS/MS measurements thus revealed the different fatty acid residues of all major amphibactin analogs (see also Table **S10**).



Amphibactin S (MH^+ = 830.4840)

Figure S5. ^{1}H-NMR spectra for three isolated amphibactin analogs for use as quantification standards. Comparison of the spectra between the previously described amphibactin T (panel **A**) and the new amphibactin ACA (panel **B**) also confirmed the position of the hydroxyl group in the fatty acyl tail of amphibactin ACA (panel **B**). Another isolated analog (panel **C**) contained a singly unsaturated decanoyl-group (compound **5**) based on HR-LC-MS and HR-MS/MS analysis (Tables **S8**, **S10**).



Amphibactin T; R = C12:0 (**10**)

Amphibactin ACA; R = OH-C10:0; (**4**)

R = C10:1; (**5**)

Figure S6. Growth curves for the two *A. chroococcum* strains and *A. vinelandii* in cultures with three different levels of Fe availability. The conditions correspond to the siderophore concentration results presented in **Fig. 6** in the main manuscript.

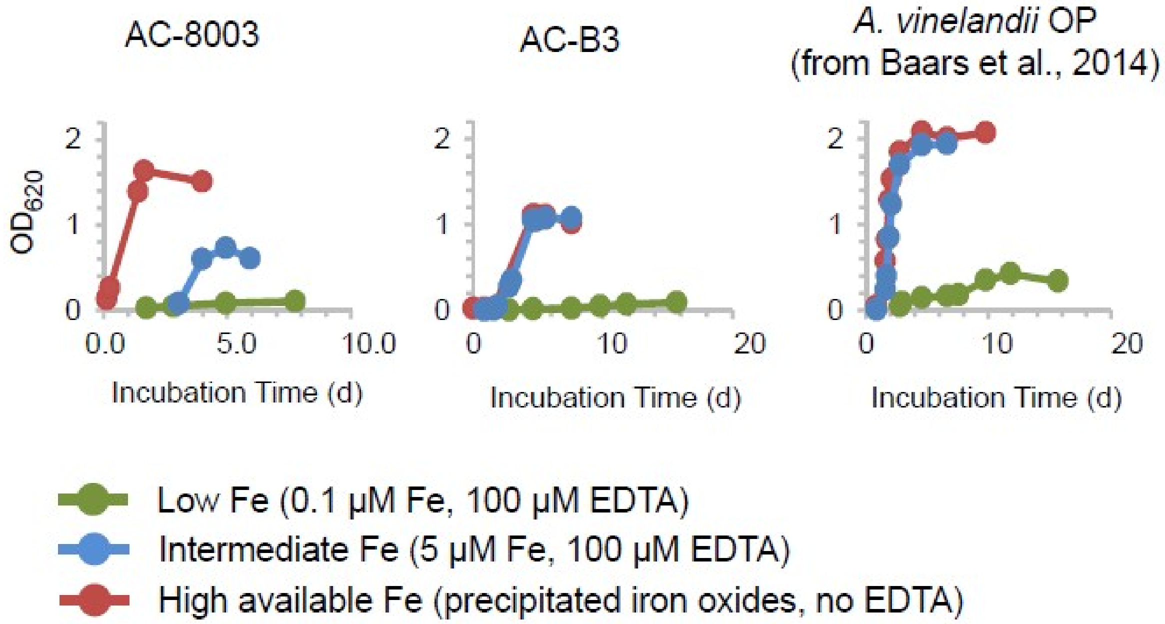


Figure S7. Dissolved Fe concentrations in filtered supernatants during growth of *A. chroococcum* str. 8003 cultured with high available Fe (precipitated amorphous Fe oxides, no EDTA), intermediate Fe (5 µM Fe, 100 µM EDTA), and low available Fe (0.1 µM Fe, 100 µM EDTA). The conditions correspond to the siderophore concentration results presented in **Fig. 6** in the main manuscript.

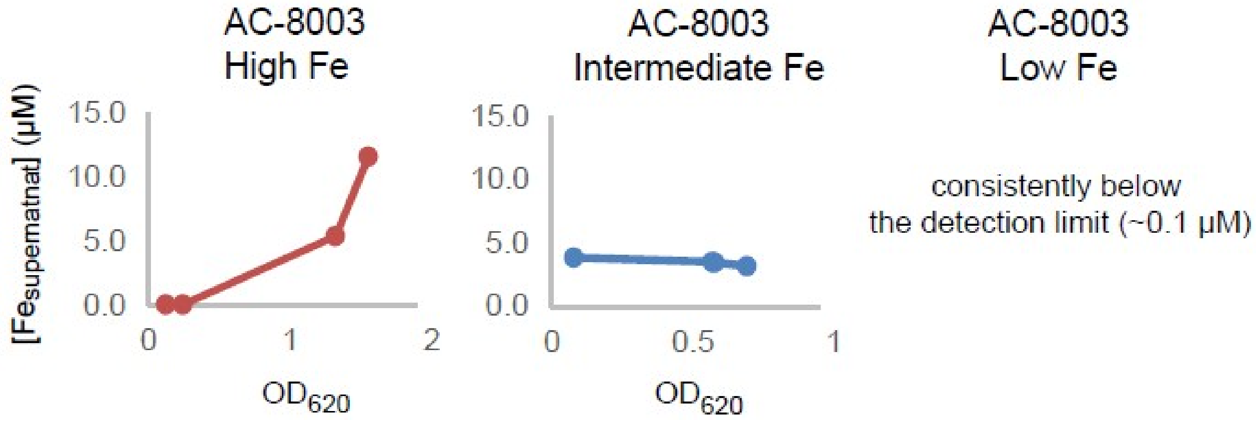


Figure S8. Siderphore production in incubations of AC-8003 and AC-B3 in media with the chelator NTA (100 µM) instead of EDTA under non-nitrogen fixing conditions (2 mM ammonia) and with lower concentrations of the glucose and mannitol carbon source (1 g/L each instead of 10 g/L).