Supplementary Material

Bioactivity Screening and Gene-Trait Matching Across Marine Sponge-Associated Bacteria

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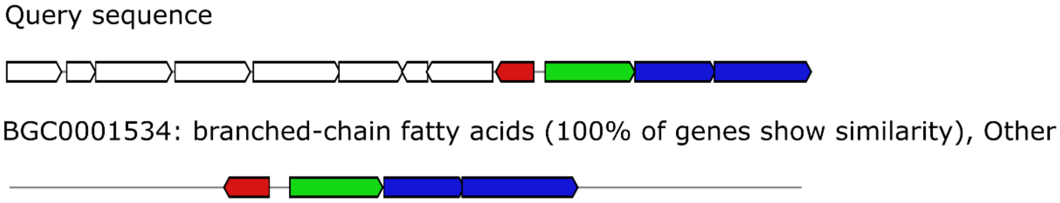
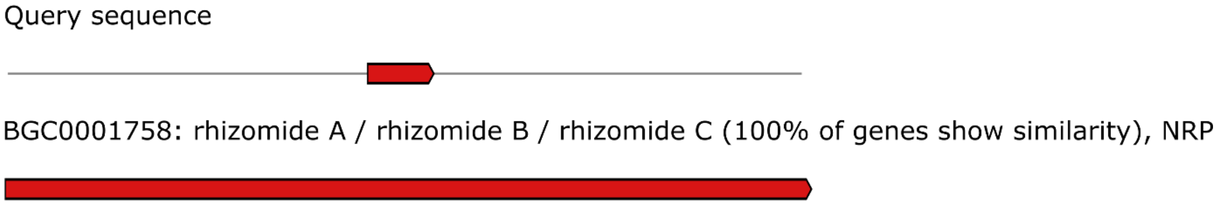
Figure S1. KnownClusterBlast output of biosynthetic gene clusters (BGCs) predicted in strain Aa3_DN216_4B10_1 showing 100% homology to reference BGCs in the Minimum Information about a Biosynthetic Gene cluster (MIBiG) database [1]. Colors indicate BLAST matches of individual genes between query and reference sequences.

a)BGC 3:Bacteriocin



b)BGC 4:NRPS-like



c)BGC 16:NRPS

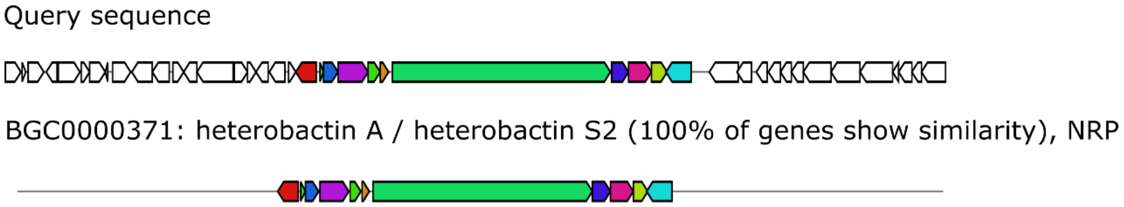


Table S1. Genome assembly metrics of strains included in the present study.

Strain ID	Isolation Source	Contigs	Genome size (Mb)	GC content (%)	N50 (Mb)	Completeness (%)	Contamination (%)	Coverage (X)	Total gene count
Aa3_DN55_6A7	*Aplysina aerophoba*	36	7.2	64.6	0.4	100.0	0.1	31	6655
Pf1_Ps_8H04_1	*Petrosia ficiformis*	18	5.7	48.2	0.5	100.0	0.2	295*	5174
Pf1_DN206_4B7	*Petrosia ficiformis*	52	5.1	52.8	0.4	100	0.8	226*	4692
Irc_Ps_AB108	*Ircinia* sp.	49	5.9	44.6	0.4	99.6	0.0	200*	5369
Pf1_DN64_8G1	*Petrosia ficiformis*	30	5.8	51.4	0.6	99.9	0.3	206	5275
Aa3_DN64_1D3	*Aplysina aerophoba*	22	5.8	50.3	0.5	99.6	0.3	711*	5239
Pf1_Ps_8H06	*Petrosia ficiformis*	40	6.1	49.7	0.4	99.6	0.6	264*	5554
Cn_Ps_AB111	*Chondrilla nucula*	36	5.9	49.8	0.4	99.9	0.0	228*	5423
Aa3_Str.68_7G12	*Aplysina aerophoba*	29	5.9	51.0	0.5	99.7	0.3	138*	5288
Acac_Ps_AB113	*Acanthella acuta*	13	5.4	51.0	0.9	99.6	0.0	191*	4886
Aa3_DN166_3E9_2	*Aplysina aerophoba*	40	4.6	56.2	0.2	99.1	0.0	237	4558
Pf1_DN81_6F7_2	*Petrosia ficiformis*	30	4.5	57.9	0.3	99.9	0.0	165	4356
Cc1_DN217_4H2	*Corticium candelabrum*	108	4.7	49.8	0.1	99.6	1.1	317	4229
Aa3_DN138_5C8	*Aplysina aerophoba*	46	3.3	41.4	0.2	100.0	0.0	310	3085
Pf1_DN14_7A9_1	*Petrosia ficiformis*	51	2.9	46.8	0.1	97.7	0.3	113	2434
Aa3_DN73_5E10_2	*Aplysina aerophoba*	41	2.6	47.0	0.1	92.2	0.3	385	2194
Aa3_DN30_1H2	*Aplysina aerophoba*	39	5.4	32.9	0.2	97.3	1.5	86	4684
Aa3_DN216_4B10_1	*Aplysina aerophoba*	79	7.1	62.5	0.3	99.9	1.1	43	6770
Aa3_DN213_3F7	*Aplysina aerophoba*	60	4.2	63.0	0.2	99.4	0.0	300	3850
Aa3_DN216_4B10_2	*Aplysina aerophoba*	19	3.4	72.9	0.3	99.8	0.0	51	3220
Aa3_DN71_7G3_2	*Aplysina aerophoba*	65	4.9	40.5	0.2	90.4	1.4	112	4807

*adapted from Versluis et al. [1]

Table S2. Taxonomic classification of studied strains based on 16S rRNA gene sequences (nr/nt database) and marker genes (GTDB database).

Strain ID	16S rRNA gene length (bp)	Best BLAST hit (accession number)	ID%	E-value	GTDB-Tk classification
Aa3_DN55_6A7	1340	*Bradyrhizobium* sp. LM6 (KX774628.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Bradyrhizobium;s__Bradyrhizobium sp003020075
Pf1_Ps_8H04_1	988	*Pseudovibrio* sp. MaPt6 (JX436420.1)	99.9	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__
Pf1_DN206_4B7	1337	*Pseudovibrio* sp. MA_AMC_33 (MN703974.1)	99.85	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__
Irc_Ps_AB108	1342	*Pseudovibrio* sp. 2011SOCNI15 (KF582860.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565
Pf1_DN64_8G1	1335	*Pseudovibrio* sp. 2011SOCNI15 (KF582860.1)	99.93	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565
Aa3_DN64_1D3	1335	*Pseudovibrio* sp. 2011SOCNI15 (KF582860.1)	99.93	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565

Pf1_Ps_8H06	1325	*Pseudovibrio* sp. 2011SOCNI42 (KF582882.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565
Cn_Ps_AB111	906	*Pseudovibrio* sp. 2011SOCNI15 (KF582860.1)	99.89	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565
Aa3_Str.68_7G12	1325	*Pseudovibrio* sp. 2011SOCNI42 (KF582882.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565
Acac_Ps_AB113	1341	*Pseudovibrio* sp. ESS-18 (MH057247.1)	99.93	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Stappiaceae;g__Pseudovibrio;s__Pseudovibrio sp900143565
Aa3_DN166_3E9_2	1316	*Ruegeria* sp. 70077 (KX833139.1)	99.93	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Ruegeria;s__
Pf1_DN81_6F7_2	991	*Ruegeria atlantica* DN83_2B6 (KP769432.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Ruegeria;s__
Cc1_DN217_4H2	1377	*Microbulbifer echini* ROA029 (MT510173.1)	99.85	0.0	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Cellvibrionaceae;g__Microbulbifer;s__
Aa3_DN138_5C8	1396	*Acinetobacter radioresistens* OsEp_Plm_15B15 (MT367790.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;s__Acinetobacter radioresistens

Pf1_DN14_7A9_1	1373	*Psychrobacter celer* 7A3 (KU525106.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Psychrobacter;s__Psychrobacter sp002810365
Aa3_DN73_5E10_2	1385	*Psychrobacter celer* G205M1 (MH256047.1)	100.0	0.0	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Psychrobacter;s__Psychrobacter sp002810365
Aa3_DN30_1H2	1044	*Aquimarina macrocephali* XH119 (KC178950.1)	100.0	0.0	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Aquimarina;s__Aquimarina megaterium
Aa3_DN216_4B10_1	1068	*Rhodococcus erythropolis* KB1 (CP050124.1)	100.0	0.0	d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Mycobacteriales;f__Mycobacteriaceae;g__Rhodococcus;s__Rhodococcus erythropolis_D
Aa3_DN213_3F7	1368	*Brevibacterium aurantiacum* SMQ-1419 (CP025333.1)	100.0	0.0	d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Brevibacteriaceae;g__Brevibacterium;s__Brevibacterium aurantiacum
Aa3_DN216_4B10_2	1360	*Janibacter melonis* M0604 (KF924217.1)	99.93	0.0	d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Dermatophilaceae;g__Janibacter;s__Janibacter melonis
Aa3_DN71_7G3_2	1397	*Bacillus frigoritolerans* ZB201705 (CP030063.1)	100.0	0.0	d__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae_A;g__Bacillus_X;s__Bacillus_X frigoritolerans

Table S3. Relative abundance of biosynthetic gene cluster (BGC) types identified by antiSMASH [2]. Numbers were normalized according to the genome size. NRPS; non-ribosomal peptide synthetase.

Strain ID	BGC types						Total BGCs
	Bacteriocin	NRPS	Terpene	Betalactone	Siderophore	Others	
Aa3_DN55_6A7	0.3	0.3	0.0	0.3	0.0	0.0	0.7
Pf1_Ps_8H04_1	0.2	0.0	0.2	0.2	0.0	0.4	0.9
Pf1_DN206_4B7	0.2	0.2	0.2	0.2	0.0	0.4	1.2
Irc_Ps_AB108	0.3	0.2	0.2	0.2	0.2	0.5	1.5
Pf1_DN64_8G1	0.3	0.2	0.2	0.3	0.2	0.7	1.9
Aa3_DN64_1D3	0.3	0.2	0.2	0.3	0.2	0.5	1.7
Pf1_Ps_8H06	0.2	0.2	0.2	0.2	0.0	0.7	1.3
Cn_Ps_AB111	0.3	0.0	0.2	0.2	0.0	0.3	1.0
Aa3_Str.68_7G12	0.2	0.2	0.2	0.2	0.2	0.7	1.5
Acac_Ps_AB113	0.4	0.0	0.2	0.2	0.2	0.4	1.3
Aa3_DN166_3E9_2	0.2	0.0	0.0	0.2	0.0	1.1	1.5
Pf1_DN81_6F7_2	0.2	0.0	0.0	0.2	0.0	0.9	1.3
Cc1_DN217_4H2	0.2	0.0	0.0	0.0	0.2	0.9	1.3
Aa3_DN138_5C8	0.0	0.0	0.0	0.6	0.3	0.3	1.2
Pf1_DN14_7A9_1	0.0	0.0	0.0	0.3	0.0	0.0	0.3
Aa3_DN73_5E10_2	0.0	0.0	0.0	0.4	0.0	0.0	0.4
Aa3_DN30_1H2	0.6	0.4	0.4	0.0	0.0	0.4	1.7
Aa3_DN216_4B10_1	0.1	1.0	0.1	0.0	0.0	1.5	2.8
Aa3_DN213_3F7	0.2	0.7	0.2	0.0	0.2	0.7	2.1
Aa3_DN216_4B10_2	0.0	0.0	0.3	0.0	0.3	0.6	1.2
Aa3_DN71_7G3_2	0.0	0.2	0.4	0.2	0.2	0.4	1.4

Table S4. Information on BGCs detected by antiSMASH [2] of strains selected for gene-trait matching and comparison to the Minimum Information about a Biosynthetic Gene cluster (MIBiG) database [3].

Strain ID	BGC	BGC type	Most similar known (MIBiG)	Similarity	MIBiG accession [3]
Aa3_DN216_4B10_1	1	NRPS, terpene	SF2575	6%	BGC0000269
	2	NRPS	coelichelin	27%	BGC0000325
	3	bacteriocin	branched-chain fatty acids	100%	BGC0001534
	4	NRPS-like	rhizomide A/rhizomide B/ rhizomide C	100%	BGC0001758
	5	NRPS	bacillomycin D	20%	BGC0001090
	6	NRPS	-	-	-
	7	NRPS	rifamorpholine A/rifamorpholine B/rifamorpholine C/rifamorpholine D/rifamorpholine E	4%	BGC0001759
	8	ectoine	ectoine	75%	BGC0000853
	9	NRPS	erythrochelin	57%	BGC0000349
	10	lanthipeptide	-	-	-
	11	PKS-like, amglyccycl	acarbose	7%	BGC0000691
	12	butyrolactone	-	-	-
	13	NRPS-like	-	-	-
	14	NRPS	chloramphenicol	17%	BGC0000893
	15	NRPS-like	thiotulin	8%	BGC0001193
	16	NRPS	heterobactin A/heterobactin S2	100%	BGC0000371
	17	LAP	diisonitrile antibiotic SF2768	11%	BGC0001574
	18	T1PKS	-	-	-
	19	T1PKS	kirromycin	8%	BGC0001070
	20	terpene	carotenoid	18%	BGC0000633
Pf1_DN64_8G1	1	acyl_amino_acids	-	-	-
	2	NRPS	rimosamide	14%	BGC0001760
	3	betalactone	pseudaminic acid	22%	BGC0001747
	4	T1PKS, T3PKS	-	-	-
	5	bacteriocin	-	-	-
	6	terpene	-	-	-
	7	betalactone	fengycin	13%	BGC0001095
	8	arylpolyene, ladderane	-	-	-

	9	siderophore	-	-	-
	10	bacteriocin	-	-	-
Aa3_DN64_1D3	1	betalactone	pseudaminic acid	22%	BGC0001747
	2	NRPS	rimosamide	14%	BGC0001760
	3	acyl_amino_acids	-	-	-
	4	arylpolyene, ladderane	-	-	-
	5	terpene	-	-	-
	6	bacteriocin	-	-	-
	7	siderophore	-	-	-
	8	bacteriocin	-	-	-
	9	betalactone	fengycin	13%	BGC0001095
	10	T3PKS, T1PKS	-	-	-
Aa3_DN213_3F7	1	NRPS	coelibactin	27%	BGC0000324
	2	NRPS	streptobactin	11%	BGC0000368
	3	NRPS-like	-	-	-
	4	siderophore	desferrioxamine E	50%	BGC0001478
	5	terpene	carotenoid	85%	BGC0000636
	6	LAP	corynazolicin	42%	BGC0001174
	7	ectoine	ectoine	75%	BGC0000853
	8	NRPS	caboxamycin	20%	BGC0001444
	9	bacteriocin	-	-	-

Table S5. Growth media and isolation source of the studied bacterial strains. Details on the media preparation can be found in the References mentioned below. MA; Marine Agar, MA/10; Marine Agar (10x diluted), MHA; Mueller-Hinton Agar, MHA/10; Mueller-Hinton Agar (10x diluted).

Strain ID	**Isolation source**	**Medium**	**Reference**
Aa3_DN55_6A7	*Aplysina aerophoba*	MA/10	[4]
Pf1_Ps_8H04_1	*Petrosia ficiformis*	MA	[1]
Pf1_DN206_4B7	*Petrosia ficiformis*	MA	[4]
Irc_Ps_AB108	*Ircinia* sp.	MA	[1]
Pf1_DN64_8G1	*Petrosia ficiformis*	MA	[4]
Aa3_DN64_1D3	*Aplysina aerophoba*	MA	[4]
Pf1_Ps_8H06	*Petrosia ficiformis*	MA	[1]
Cn_Ps_AB111	*Chondrilla nucula*	MA	[1]
Aa3_Str.68_7G12	*Aplysina aerophoba*	MA	[1]
Acac_Ps_AB113	*Acanthella acuta*	MA	[1]
Aa3_DN166_3E9_2	*Aplysina aerophoba*	MA	[4]
Pf1_DN81_6F7_2	*Petrosia ficiformis*	MHA	[4]
Cc1_DN217_4H2	*Corticium candelabrum*	MA	[4]
Aa3_DN138_5C8	*Aplysina aerophoba*	MA	[4]
Pf1_DN14_7A9_1	*Petrosia ficiformis*	MHA/10	[4]
Aa3_DN73_5E10_2	*Aplysina aerophoba*	MHA	[4]
Aa3_DN30_1H2	*Aplysina aerophoba*	MHA	[4]
Aa3_DN216_4B10_1	*Aplysina aerophoba*	MHA	[4]
Aa3_DN213_3F7	*Aplysina aerophoba*	MA	[4]
Aa3_DN216_4B10_2	*Aplysina aerophoba*	MHA	[4]
Aa3_DN71_7G3_2	*Aplysina aerophoba*	MHA	[4]

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