

Supplementary Material

Diversity and Antimicrobial Potential of Predatory Bacteria from the Peruvian Coastline

Luis Linares-Otoya,^{1,2,5} Virginia Linares-Otoya,^{3,5} Lizbeth Armas-Mantilla,³ Cyntia Blanco-Olano,³ Max Crüsemann,² Mayar L. Ganoza-Yupanqui,³ Julio Campos-Florian,³ Gabriele M. König,^{2,4} Till F. Schäberle^{1,2,4,*}

¹ Institute for Insect Biotechnology, Justus Liebig University of Giessen, Giessen, Germany

² Institute for Pharmaceutical Biology, University of Bonn, Bonn, Germany

³ Department of Pharmacology, Faculty of Pharmacy and Biochemistry, National University of Trujillo, Trujillo, Peru

⁴ German Centre for Infection Research (DZIF) Partner Site Bonn/Cologne

⁵ Research Centre for Sustainable Development Uku Pacha, Peru

* Correspondence till.f.schaeberle@agrar.uni-giessen.de; Tel.: +49-641-9937140

Supplementary Table S1. Isolated strains that showed antibiotic activity

Strain identifier	Strain name as deposited in NCBI database	Closest 16S rRNA gene sequence hit in BLAST	Identity %	Isolation bait	Isolation site
2	Tenacibaculum sp. s2	Tenacibaculum sp. QDHT-02	99	<i>E. coli</i>	Paracas
16	Tenacibaculum sp. S16	Tenacibaculum sp. sw0106-03(3)	99	<i>E. coli</i>	Paracas
17	Kocuria rosea s17	Kocuria rosea	99	<i>E. coli</i>	Paracas
21	Reichenbachiella sp. S21	Reichenbachiella agariperforans	95	<i>E. coli</i>	Paracas
23	Oceanicola marinus s23	Oceanicola marinus S15	99	<i>E. coli</i>	Paracas
37	Limibacter armeniacum s37	Limibacter armeniacum YM11-159	99	<i>E. coli</i>	Paracas
42	Fulvivirga sp. s42	Fulvivirga sp. ana H.e.	99	<i>P. inhibens</i>	Isla Foca
47	Porifericola rodea s47	Porifericola rhodea	99	<i>P. inhibens</i>	Paracas
48	Fulvivirga kasyanovii s48	Fulvivirga kasyanovii	99	<i>P. inhibens</i>	Isla Foca
68	Rapidithrix sp. s68	Rapidithrix sp. TISTR 1768	99	<i>P. inhibens</i>	Paracas
80	Rapidithrix sp s80	Rapidithrix sp. TISTR 1768	99	<i>P. inhibens</i>	Paracas
82	Labrenzia sp. strain s82	Labrenzia sp. strain 1334-392	98	<i>E. coli</i>	Paracas
83	Microbulbifer hydrolyticus s83	Microbulbifer hydrolyticus strain DSM 11525	99	<i>E. coli</i>	Manglares
90	Bacillus sp. s90	Bacillus thuringiensis serovar finitimus YBT-020	99	<i>P. inhibens</i>	Paracas
93	Labrenzia sp. s93	Labrenzia sp. R-66638	99	<i>E. coli</i>	Isla Foca
100	Nitratireductor sp. s100	Nitratireductor sp.	99	<i>E. coli</i>	Manglares
106	Staphylococcus succinus s106	Staphylococcus succinus	99	<i>P. inhibens</i>	Paracas
107	Microbulbifer sp. s107	Microbulbifer sp. CMC	99	<i>P. inhibens</i>	Manglares
108	Euzebyella sp. s108	Euzebyella sp. CY01	97	<i>P. inhibens</i>	Manglares
120	Streptomyces sp. s120	Streptomyces sp. NPA1	99	<i>E. coli</i>	Manglares
122	Nocardiopsis sp. s122	Nocardiopsis sp. 13-112	99	<i>E. coli</i>	Manglares
123	Nitratireductor sp. s123	Nitratireductor sp. OM-1	99	<i>E. coli</i>	Isla Foca
102 ^a	Microbacterium sp. s102a	Microbacterium sp. CC4P	99	<i>P. inhibens</i>	Paracas
102b	Paenibacillus sp. s102b	Paenibacillus glucanolyticus strain 5162	99	<i>P. inhibens</i>	Paracas
118b	Echinicola shivajiensis s118b	Echinicola shivajiensis strain AK12	99	<i>E. coli</i>	Manglares
17_1c	Planomicrobium sp. s171c	Planomicrobium sp. PK32_S1	99	<i>E. coli</i>	Paracas
17_2c	Paracoccus sp. s172c	Paracoccus sp. MAR824	98	<i>E. coli</i>	Paracas
30b1	Ponticoccus sp. s30b1	Ponticoccus sp. strain 7002-260	100	<i>E. coli</i>	Paracas
49b1	Bacillus aquimaris s49b1	Bacillus aquimaris strain M12	99	<i>E. coli</i>	Paracas
49b2	Euzebyella sp. s49b2	Euzebyella sp. B39	99	<i>E. coli</i>	Paracas

Supplementary Table S2. Growth behavior at different NaCl concentrations

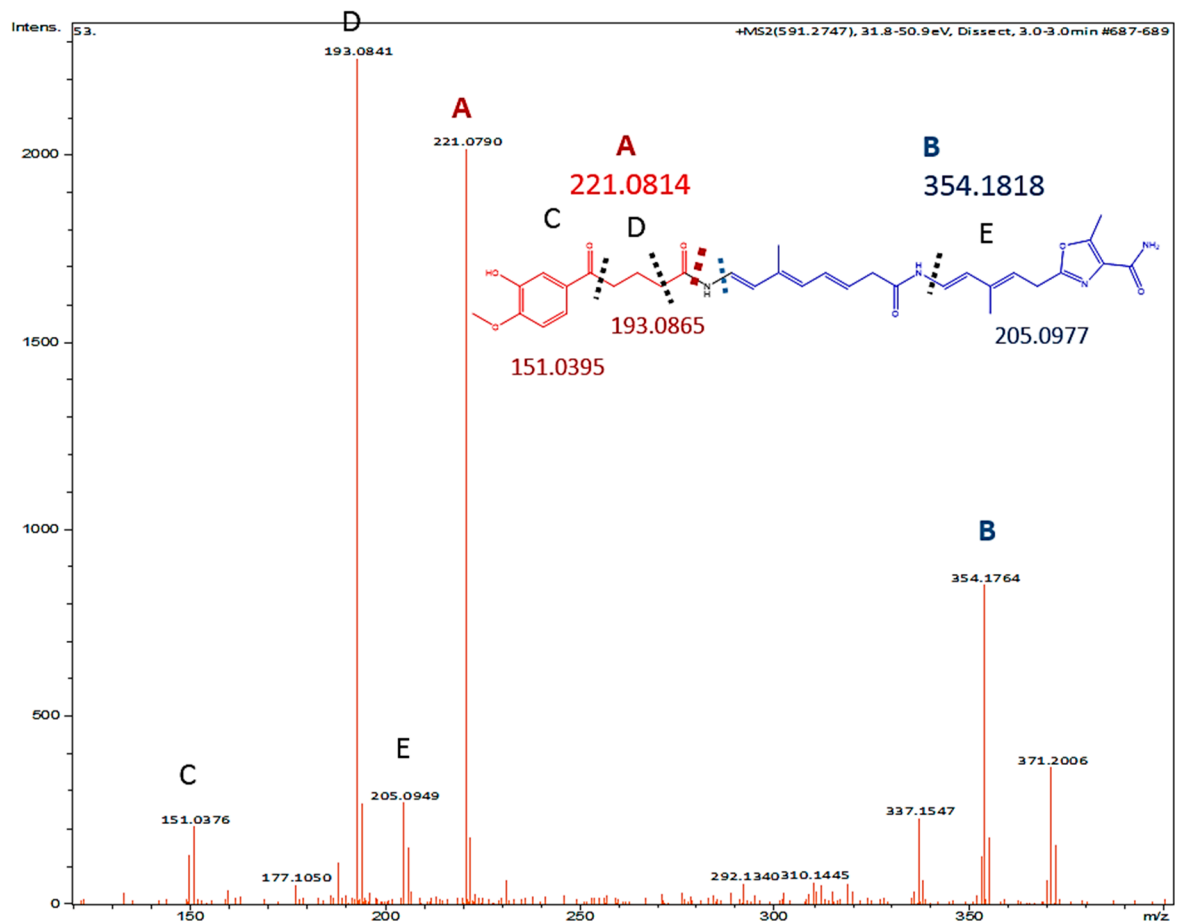
Strain identifier	% NaCl							
	0.5%	1%	1.95%	2.14%	2.23%	3.89%	5%	10%
2	--	++	+++	+++	++	++	--	--
16	--	+	++	+++	++	+	+	--
17	++	++	++	++	++	++	+	--
17_1c	++	++	++	++	++	++	+	--
17_2c	++	++	++	++	++	++	+	--
21	+	+	++	+++	++	++	+	+
23	--	--	--	+	++	+++	++	--
30b1	++	+++	+++	++++	+++	+++	+++	++
37	++	++	++	++	++	++	--	--
42	+	++	+++	+++	++	+	+	+
47	+	++	+++	++	++	++	--	--
48	+	++	++	++	+	--	--	--
49b1	+++	+++	+++	+++	+++	++	+	--
49b2	+	+	++	++	++	++	+	--
68	+	++	+++	+++	++	+	--	--
80	+	++	+++	+++	++	+	--	--
82	++	++	+++	+++	++	++	++	--
83	+	++	+++	+++	++	++	++	+
90	+++	+++	+++	+++	+++	+++	++	--
93	+	++	++	++	+	+	--	--
100	++	++	++	++	++	++	++	-
102a	+	++	++	++	++	+	+	+
102b	+	++	+++	+++	++	+	+	+
106	++	+++	++++	+++	+++	+++	++	++
107	+	++	+++	+++	++	+	+	+
108	+	+	++	+++	++	+	+	--
118b	--	+	++	++	+	+	+	--
120	++	++	++	++	++	++	--	--
122	+	++	++	++	++	+	+	+
123	++	+++	+++	++	++	++	+	+

Supplementary Table S3. Growth behavior at different temperatures

Strain identifier	10°C		27 °C		30°C		45°C	
	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H
2	--	--	++	+++	++	+++	--	--
16	--	--	++	+++	++	+++	--	--
17	--	--	++	++	++	++	--	--
17_1c	--	--	++	++	++	++	+	+
17_2c	--	--	++	++	++	++	--	--
21	--	--	++	+++	++	+++	--	--
23	--	--	++	+++	++	+++	--	--
30b1	--	--	+	++	++	+++	--	--
37	--	--	++	++	++	+++	--	--
42	--	--	--	+	+	++	--	--
47	--	--	--	+	++	+++	+	+
48	--	--	--	+	+	++	--	--
49b1	--	--	+	++	+	++	+	+
49b2	--	--	--	++	++	+++	--	--
68	--	--	--	++	+	++	--	--
80	--	--	+++	+++	+++	+++	--	--
80	--	--	--	++	+	++	--	--
82	--	--	++	+++	++	+++	+	+
83	--	--	+	++	++	+++	--	+
90	--	--	++	+++	++	+++	++	++
93	--	--	+	++	+	++	+	+
100	--	--	++	++	++	++	--	--
102a	--	--	+	++	+	++	+	+
102b	--	--	+	++	+	++	+	+
106	--	--	++	++	++	+++	+++	+++
107	--	--	+	++	++	+++	+	+
108	--	--	++	+++	+++	+++	--	--
118b	--	--	--	+	+	++	+	+
120	--	--	++	+++	+++	+++	++	+++
122	--	--	+	++	+	++	+	+
123	--	--	+	++	+	++	+	+

Supplementary Table S4. Selected predatory bacteria reported in the literature

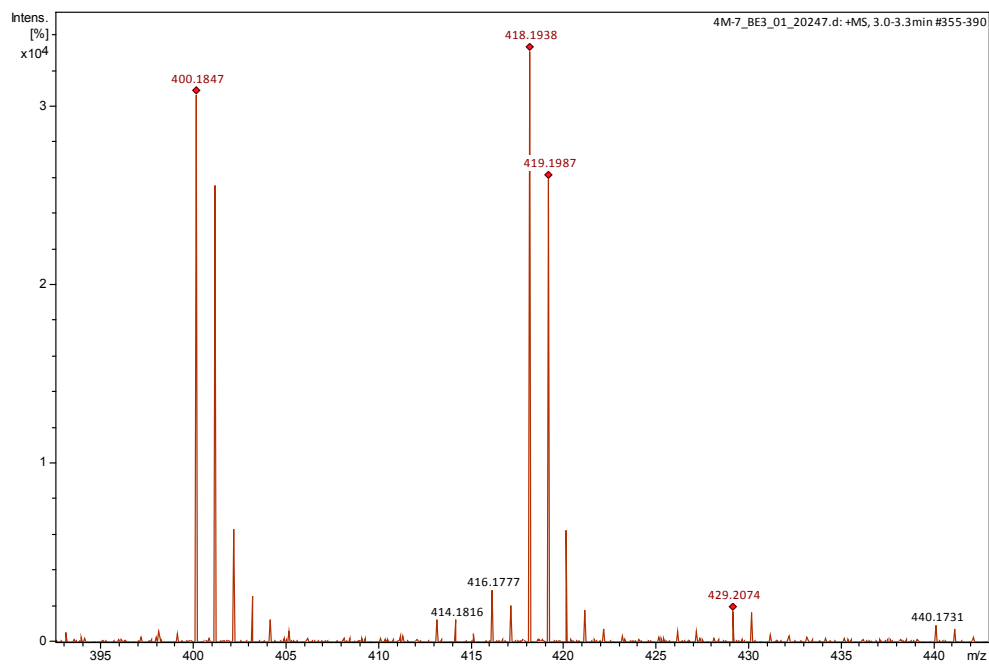
Genus and or species	Reference
<i>Agromyces ramosus</i>	[1]
<i>Herpetosiphon</i>	[2]
<i>Bdellovibrio bacteriovorus</i>	[3]
<i>Halobacteriovorax</i>	[4]
<i>Myxococcus xanthus</i>	[2]
<i>Vampirococcus</i>	[5]
<i>Ensifer</i>	[6]
<i>Cupriavidus necator</i>	[7]
<i>Lysobacter</i>	[8]
<i>Saprospira grandis</i>	[9]
<i>Tenacibaculum</i>	[10]
<i>Olleya</i>	[10]
<i>Cytophaga</i>	[11]
<i>Rapidithrix</i>	[12]
<i>Porifericola rhodea</i>	[13]
<i>Vampirovibrio chlorellavorus</i>	[2]
<i>Micavibrio</i>	[14]



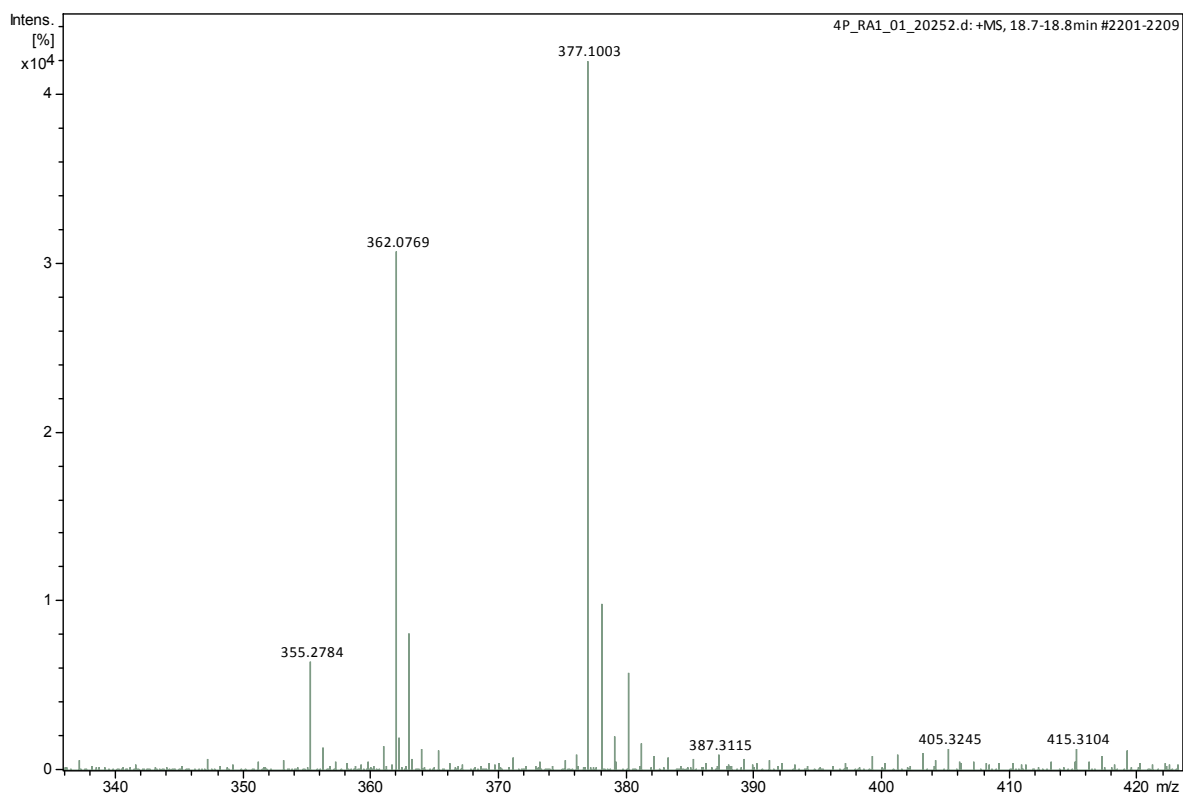
Supplementary Figure S1. MS/MS spectrum of *Rapidithrix thailandica* s80 crude extract showing the fragmentation pattern expected for ariakemicins isolated before from *Rapidithrix* sp [15].

Supplementary Table S5. Antismash bioinformatic of secondary metabolites BGC present in the genome of *Streptomyces sp. s120* showing the presence of the naphthyridinomycin and resistomycin BGC [16].

Type	From	To	Most similar known cluster	MIBiG BGC-ID
Otherks- T1pks- Nrps	1	58334	Naphthyridinomycin_biosynthetic_gene_cluster (60% of genes show similarity)	BGC0000394_c1
Other	1	16023	Naphthyridinomycin_biosynthetic_gene_cluster (39% of genes show similarity)	BGC0000394_c1
T2pks	422	42943	Resistomycin_biosynthetic_gene_cluster (88% of genes show similarity)	BGC0000264_c1



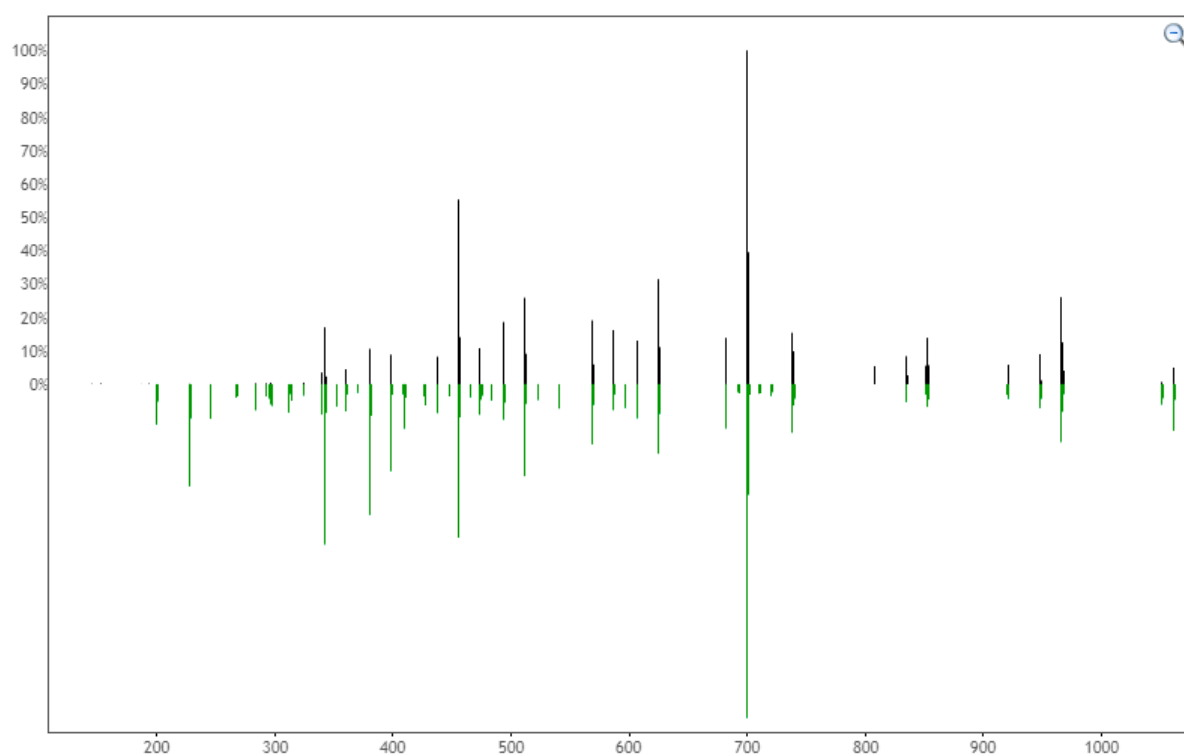
Supplementary Figure S2. MS spectrum of *Streptomyces sp.* s120 extract showing the presence of naphthyridinomycin (observed m/z 418.1938, expected 418.1978) [17].



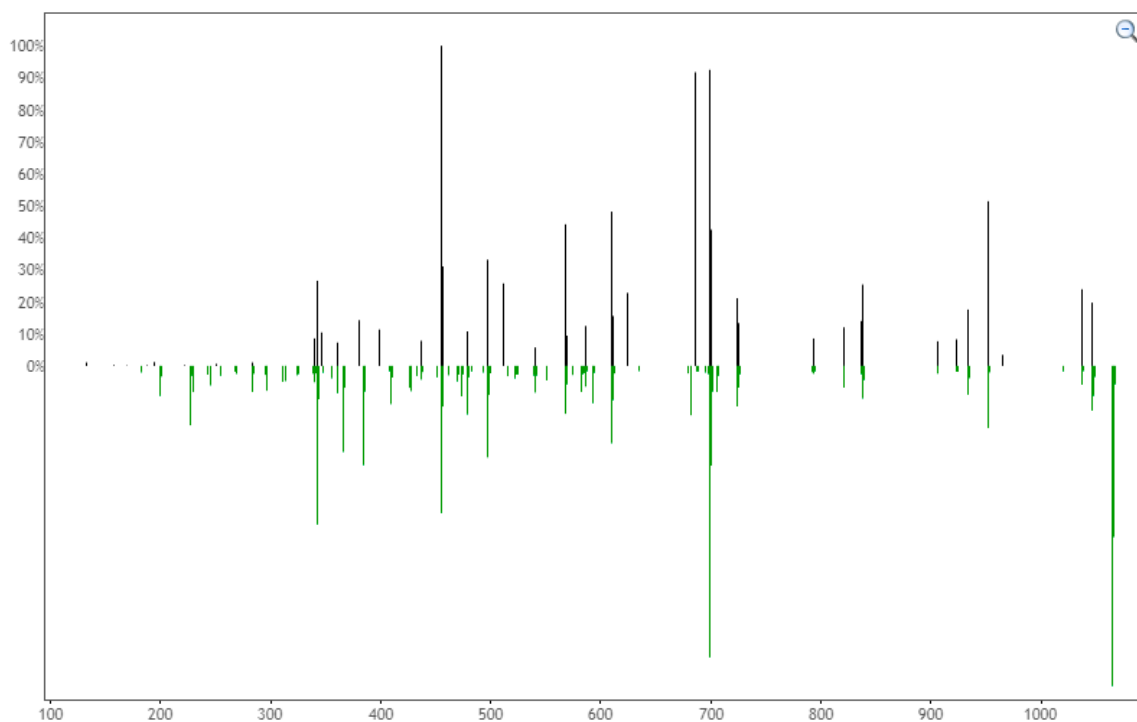
Supplementary Figure S3. MS spectrum of *Streptomyces sp.* s120 extract showing the presence of resistomycin (observed m/z 377.1003, expected 377.1025) [18].

Supplementary Table S6. GNPS dereplication analysis of crude extract of *Paenibacillus sp. s102b* showing the presence of pumilacidin C and E as well as surfactin [19].

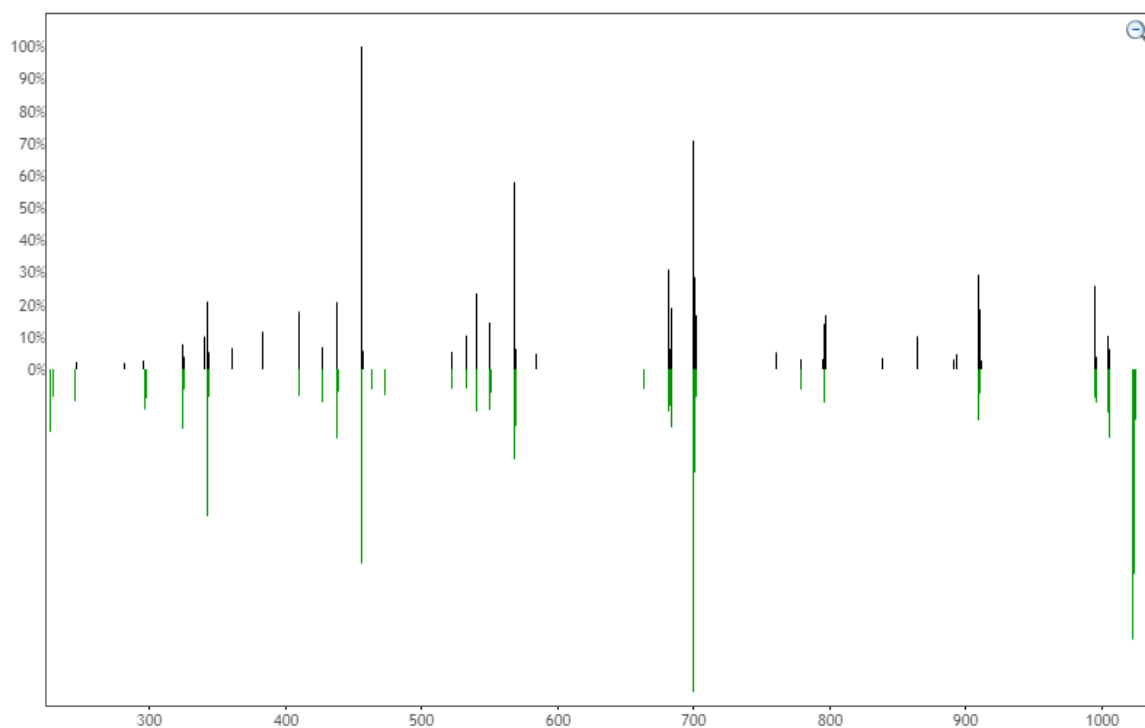
Compound_Name	Library Class	Cosine	Shared Peaks	TIC	RT	Mass Diff	SpecMZ	LibMZ	PI
pumilacidin_C_14344_der eplictor_pv_2.93242e-26	Bronze	0.82	36	6691. 19	419. 89	0.01	1078.75	1078.74	Pevzner
pumilacidin_E_14342_der eplictor_pv_1.13518e-25	Bronze	0.65	25	4486. 22	402. 57	0.02	1064.73	1064.72	Pevzner
[Ile2_4_7]Surfactin_43293_ dereplictor_pv_2.3158e-16	Bronze	0.73	20	1797. 96	1152 .71	0.01	1022.68	1022.67	Pevzner



Supplementary Figure S4. MS/MS mirror plot of crude extract of *Paenibacillus sp. s102b* against GNPS database showing the presence of pumilacidin C [19]. Source: Green: Pumilacidin C MS/MS spectrum from GNPS database, black: Crude extract of *Paenibacillus sp. s102b*.



Supplementary Figure S5. MS/MS mirror plot of crude extract of *Paenibacillus sp.* s102b against GNPS database showing the presence of pumilacidin E [19]. Source: Green: Pumilacidin E MS/MS spectrum from GNPS database Black: Crude extract of *Paenibacillus sp.* s102b.



Supplementary Figure S6. MS/MS mirror plot of crude extract of *Paenibacillus sp.* s102b against GNPS database showing the presence of surfactin [19]. Source: Green: Surfactin MS/MS spectrum from GNPS database, black: Crude extract of *Paenibacillus sp.* s102b.

References

1. Casida, L.E. Interaction of *Agromyces ramosus* with Other Bacteria in Soil. *Applied And Environmental Microbiology* 1983, 46, 881–888.
2. Korp, J.; Vela Gurovic, M.S.; Nett, M. Antibiotics from predatory bacteria. *Beilstein journal of organic chemistry* 2016, 12, 594–607.
3. Jurkevitch, E.; Jacquet, S. *Bdellovibrio* and related organisms Unusual bacterial predators! *M S-Medecine Sciences* 2017, 33, 519–527.
4. Kandel, P.P.; Pasternak, Z.; van Rijn, J.; Nahum, O.; Jurkevitch, E. Abundance, diversity and seasonal dynamics of predatory bacteria in aquaculture zero discharge systems. *FEMS microbiology ecology* 2014, 89, 149–161.
5. Guerrero, R.; Pedrosalio, C.; Esteve, I.; Mas, J.; Chase, D.; Margulis, L. Predatory prokaryotes - predation and primary consumption evolved in bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 1986, 83, 2138–2142.
6. Willems, A.; Fernández-López, M.; Muñoz-Adelantado, E.; Goris, J.; Vos, P. de; Martínez-Romero, E.; Toro, N.; Gillis, M. Description of new *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens* Casida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. Request for an opinion. *International Journal Of Systematic And Evolutionary Microbiology* 2003, 53, 1207–1217.
7. Seccareccia, I.; Kovacs, A.T.; Gallegos-Monterrosa, R.; Nett, M. Unraveling the predator-prey relationship of *Cupriavidus necator* and *Bacillus subtilis*. *Microbiological Research* 2016, 192, 231–238.
8. Seccareccia, I.; Kost, C.; Nett, M. Quantitative Analysis of *Lysobacter* Predation. *Applied And Environmental Microbiology* 2015, 81, 7098–7105.
9. Lewin, R.A. *Saprospira grandis*: A flexibacterium that can catch bacterial prey by "ixotrophy". *Microbial Ecology* 1997, 34, 232–236.
10. Banning, E.C.; Casciotti, K.L.; Kujawinski, E.B. Novel strains isolated from a coastal aquifer suggest a predatory role for flavobacteria. *FEMS microbiology ecology* 2010, 73, 254–270.
11. Imai, I.; Ishida, Y.; Hata, Y. Killing of marine-phytoplankton by a gliding bacterium *Cytophaga* sp, isolated from the coastal sea of Japan. *Marine Biology* 1993, 116, 527–532.
12. Srisukchayakul, P.; Suwanachart, C.; Sangnoi, Y.; Kanjana-Opas, A.; Hosoya, S.; Yokota, A.; Arunpairojana, V. *Rapidithrix thailandica* gen. nov., sp nov., a marine gliding bacterium isolated from samples collected from the Andaman sea, along the southern coastline of Thailand. *International Journal Of Systematic And Evolutionary Microbiology* 2007, 57, 2275–2279.
13. Yoon, J.; Oku, N.; Park, S.; Kasai, H.; Yokota, A. *Porifericola rhodea* gen. nov., sp nov., a new member of the phylum Bacteroidetes isolated by the bait-streaked agar technique. *Antonie Van Leeuwenhoek International Journal Of General And Molecular Microbiology* 2011, 100, 145–153.
14. Dashiff, A.; Junka, R.A.; Libera, M.; Kadouri, D.E. Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. *Journal of applied microbiology* 2011, 110, 431–444.
15. Oku, N.; Adachi, K.; Matsuda, S.; Kasai, H.; Takatsuki, A.; Shizuri, Y. Ariakemicins a and b, novel polyketide-peptide antibiotics from a marine gliding bacterium of the genus *Rapidithrix*. *Organic Letters* 2008, 10, 2481–2484.

16. Blin, K.; Wolf, T.; Chevrette, M.G.; Lu, X.; Schwalen, C.J.; Kautsar, S.A.; Suarez Duran, H.G.; Los Santos, E.L.C. de; Kim, H.U.; Nave, M.; et al. antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. *Nucleic acids research* 2017.
17. Zmijewski, M.J.; Millerhatch, K.; Goebel, M. Naphthyridinomycin, a DNA-reactive antibiotic. *Antimicrobial agents and chemotherapy* 1982, 21, 787–793.
18. Rosenbrook, W. The structure of resistomycin. *The Journal of organic chemistry* 1967, 32, 2924–2925.
19. Wang, M.; Carver, J.J.; Phelan, V.V.; Sanchez, L.M.; Garg, N.; Peng, Y.; Nguyen, D.D.; Watrous, J.; Kapon, C.A.; Luzzatto-Knaan, T.; et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology* 2016, 34, 828–837.

References

1. Casida, L.E. Interaction of *Agromyces ramosus* with Other Bacteria in Soil. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* **1983**, 46, 881–888.
2. Korp, J.; Vela Gurovic, M.S.; Nett, M. Antibiotics from predatory bacteria. *Beilstein journal of organic chemistry* **2016**, 12, 594–607.
3. Jurkevitch, E.; Jacquet, S. *Bdellovibrio* and related organisms Unusual bacterial predators! *M S-MEDICINE SCIENCES* **2017**, 33, 519–527.
4. Kandel, P.P.; Pasternak, Z.; van Rijn, J.; Nahum, O.; Jurkevitch, E. Abundance, diversity and seasonal dynamics of predatory bacteria in aquaculture zero discharge systems. *FEMS microbiology ecology* **2014**, 89, 149–161.
5. GUERRERO, R.; PEDROSALIO, C.; ESTEVE, I.; MAS, J.; CHASE, D.; MARGULIS, L. PREDATORY PROKARYOTES - PREDATION AND PRIMARY CONSUMPTION EVOLVED IN BACTERIA. *Proceedings of the National Academy of Sciences of the United States of America* **1986**, 83, 2138–2142.
6. Willems, A.; Fernández-López, M.; Muñoz-Adelantado, E.; Goris, J.; Vos, P. de; Martínez-Romero, E.; Toro, N.; Gillis, M. Description of new *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens* Casida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. Request for an opinion. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY* **2003**, 53, 1207–1217.
7. Seccareccia, I.; Kovacs, A.T.; Gallegos-Monterrosa, R.; Nett, M. Unraveling the predator-prey relationship of *Cupriavidus necator* and *Bacillus subtilis*. *MICROBIOLOGICAL RESEARCH* **2016**, 192, 231–238.
8. Seccareccia, I.; Kost, C.; Nett, M. Quantitative Analysis of *Lysobacter* Predation. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* **2015**, 81, 7098–7105.
9. Lewin, R.A. *Saprosira grandis*: A flexibacterium that can catch bacterial prey by "ixotrophy". *MICROBIAL ECOLOGY* **1997**, 34, 232–236.
10. Banning, E.C.; Casciotti, K.L.; Kujawinski, E.B. Novel strains isolated from a coastal aquifer suggest a predatory role for flavobacteria. *FEMS microbiology ecology* **2010**, 73, 254–270.
11. IMAI, I.; ISHIDA, Y.; HATA, Y. KILLING OF MARINE-PHYTOPLANKTON BY A GLIDING BACTERIUM CYTOPHAGA SP, ISOLATED FROM THE COASTAL SEA OF JAPAN. *MARINE BIOLOGY* **1993**, 116, 527–532.

12. Srisukchayakul, P.; Suwanachart, C.; Sangnoi, Y.; Kanjana-Opas, A.; Hosoya, S.; Yokota, A.; Arunpairojana, V. *Rapidithrix thailandica* gen. nov., sp nov., a marine gliding bacterium isolated from samples collected from the Andaman sea, along the southern coastline of Thailand. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY* **2007**, *57*, 2275–2279.
13. Yoon, J.; Oku, N.; Park, S.; Kasai, H.; Yokota, A. *Porifericola rhodea* gen. nov., sp nov., a new member of the phylum Bacteroidetes isolated by the bait-streaked agar technique. *ANTONIE VAN LEEUWENHOEK INTERNATIONAL JOURNAL OF GENERAL AND MOLECULAR MICROBIOLOGY* **2011**, *100*, 145–153.
14. Dashiff, A.; Junka, R.A.; Libera, M.; Kadouri, D.E. Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. *Journal of applied microbiology* **2011**, *110*, 431–444.
15. Oku, N.; Adachi, K.; Matsuda, S.; Kasai, H.; Takatsuki, A.; Shizuri, Y. Ariakemicins a and b, novel polyketide-peptide antibiotics from a marine gliding bacterium of the genus *Rapidithrix*. *ORGANIC LETTERS* **2008**, *10*, 2481–2484.
16. Blin, K.; Wolf, T.; Chevrette, M.G.; Lu, X.; Schwalen, C.J.; Kautsar, S.A.; Suarez Duran, H.G.; Los Santos, E.L.C. de; Kim, H.U.; Nave, M.; *et al.* antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. *Nucleic acids research* **2017**.
17. ZMIJEWSKI, M.J.; MILLERHATCH, K.; GOEBEL, M. NAPHTHYRIDINOMYCIN, A DNA-REACTIVE ANTIBIOTIC. *Antimicrobial agents and chemotherapy* **1982**, *21*, 787–793.
18. Rosenbrook, W. The structure of resistomycin. *The Journal of organic chemistry* **1967**, *32*, 2924–2925.
19. Wang, M.; Carver, J.J.; Phelan, V.V.; Sanchez, L.M.; Garg, N.; Peng, Y.; Nguyen, D.D.; Watrous, J.; Kapono, C.A.; Luzzatto-Knaan, T.; *et al.* Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *NATURE BIOTECHNOLOGY* **2016**, *34*, 828–837.