

Table S1. Taxonomic identification of the actinobacterial strains isolated from the marine sediment collected at Cepães beach in the Parque Natural do Litoral Norte, northern Portugal, and corresponding GenBank accession number.

Strain	Sample treatment	Isolation Medium	Medium used for biomass growth	Closest identification*	Similarity %	Sequence length (bp)	GenBank accession number
MS3B	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.78	1361	MN134370
MS3C	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora</i> sp.	100	1056	MN134369
MS3C1	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.91	1057	MN134368
MS3D1	No treatment - Method 1	NPS	Marine Broth	<i>Streptomyces</i> sp.	100	1374	MN134350
MS3E	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.85	1377	MN134367
MS5B	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.71	1385	MN134380
MS5C	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.71	1388	MN134379
MS5D	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.91	1057	MN134378
MS5E	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora aurantiaca</i>	100	1379	MN134392
MS8A1	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	ISP2	<i>Streptomyces</i> sp.	100	1364	MN134348
MS8B	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora</i> sp.	100	1350	MN134362
MS8C	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora chalcea</i>	99.85	1377	MN134385
MS14B	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Streptomyces</i> sp.	98.13	1385	MN134356
MS16B	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora chalcea</i>	99.85	1376	MN134391
MS18A	Water bath (60 °C for 5 min) - Method 2	NPS	ISP2	<i>Streptomyces</i> sp.	99.71	1399	MN134355
MS18B	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Streptomyces</i> sp.	99.85	1376	MN134354

MS19	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.57	1386	MN134375
MS19A	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora chalcea</i>	100	1354	MN134390
MS19B1	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora aurantiaca</i>	100	1357	MN134395
MS19C	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.93	1363	MN134374
MS19D	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora marina</i>	99.78	1373	MN134377
MS19E	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.85	1377	MN134384
MS19F	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora chalcea</i>	99.78	1383	MN134389
MS23A	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora chalcea</i>	99.83	1380	MN134388
MS23B	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.78	1381	MN134383
MS23C	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.78	1367	MN134373
MS23D	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora chalcea</i>	99.85	1377	MN134387
MS24	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.71	1379	MN134372
MS26H	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.71	1372	MN134361
MS26I	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.71	1376	MN134371
MS27A	Water bath (60 °C for 5 min) - Method 2	NPS	ISP2	<i>Streptomyces</i> sp.	100	1357	MN134353
MS29	Water bath (60 °C for 5 min) - Method 2	NPS	ISP2	<i>Streptomyces</i> sp.	100	1346	MN134352
MS32	No treatment - Method 1	NPS	ISP2	<i>Streptomyces</i> sp.	99.93	1386	MN134351
MS35	No treatment - Method 1	nps	ISP2	<i>Nocardiopsis</i> sp.	100	1141	MN134360
MS38	Water bath (60 °C for 5 min) - Method 2	SCN	ISP2	<i>Herbiconiux</i> sp	100	1367	MN134396
MS39B	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	SCN	ISP2	<i>Arthrobacter pascens</i>	100	1384	MN134398

MS39C	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	SCN	ISP2	<i>Arthrobacter</i> sp.	100	1059	MN134397
MS40A	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	SCN	ISP2	<i>Pseudarthrobacter</i> sp.	99.25	938	MN134357
MS40B1	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	scn	ISP2	<i>Pseudarthrobacter</i> sp.	99.82	1087	MN134358
MS42	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora aurantiaca</i>	100	1382	MN134394
MS46	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.64	1382	MN134382
MS48	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora</i> sp.	100	1372	MN134366
MS49	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora chalcea</i>	99.63	1358	MN134386
MS50	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora peucetia</i>	99.21	1384	MN134376
MS51	No treatment - Method 1	NPS	Marine Broth	<i>Micromonospora</i> sp.	100	1056	MN134365
MS52A	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora coxensis</i>	99.64	1391	MN134381
MS52B	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Micromonospora</i> sp.	100	1360	MN134364
MS53	Water bath (60 °C for 5 min) - Method 2	NPS	Marine Broth	<i>Polymorphospora rubra</i>	99.78	1368	MN134359
MS54	No treatment - Method 1	NPS	ISP2	<i>Streptomyces</i> sp.	99.93	1380	MN134349
MS55	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora aurantiaca</i>	100	1376	MN134393
MS56	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Micromonospora</i> sp.	99.85	1377	MN134363
MS58	Antibiotics (20ppm) + Water bath (28°C for 30 min) - Method 3	NPS	Marine Broth	<i>Actinomadura sputi</i>	99.39	814	MN134399

*According to Nucleotide collection (nr/nt) database from NCBI BLAST.

Table S2. GNPS dereplication results for the 26 actinobacterial crude extracts that showed antimicrobial and/or cytotoxic activities. The Table shows the compounds recorded in the database for each extract and the correspondent cosine score (indicates the similarity of two MS/MS spectra from 0 to 1, totally dissimilar and completely identical, respectively).

Strain	Closest identification	Compound	Cosine	m/z Error ppm	Lib m/z
MS3B	<i>Micromonospora</i> sp.	No match	-	-	-
MS3C	<i>Micromonospora</i> sp.	No match	-	-	-
MS3C1	<i>Micromonospora</i> sp.	No match	-	-	-
MS3D1	<i>Streptomyces</i> sp.	Antimycin A2	0.92	11	535.27
		Antimycin A1	0.91	0	557.25
MS3E	<i>Micromonospora</i> sp.	No match	-	-	-
MS5B	<i>Micromonospora coxensis</i>	No match	-	-	-
MS5E	<i>Micromonospora aurantiaca</i>	No match	-	-	-
MS8A1	<i>Streptomyces</i> sp.	Dehydroxynocardamine	0.97	1	585.36
		Deferrioxamine E	0.95	0	601.36
		Antimycin A1	0.94	0	557.25
		Antimycin A2	0.93	10	535.27
		Antimycin A3	0.92	15	521.26
		Desferrioxamine E	0.86	2	623.34
MS8B	<i>Micromonospora</i> sp.	No match	-	-	-
MS8C	<i>Micromonospora chalcea</i>	No match	-	-	-
MS14B	<i>Streptomyces</i> sp.	No match	-	-	-
MS16B	<i>Micromonospora chalcea</i>	No match	-	-	-
MS18A	<i>Streptomyces</i> sp.	Deferrioxamine E	0.96	0	601.36
		Dehydroxynocardamine	0.96	0	585.36
		Antimycin A1	0.94	0	557.25
		Antimycin A2	0.93	10	535.27
		Antimycin A3	0.92	16	521.26
		Desferrioxamine E	0.9	2	623.34
MS18B	<i>Streptomyces</i> sp.	Dehydroxynocardamine	0.97	0	585.36
		Deferrioxamine E	0.96	0	601.36
		Antimycin A1	0.95	0	557.25
		Antimycin A2	0.93	10	535.27
		Desferrioxamine E	0.91	2	623.34
		Antimycin A3	0.92	16	521.26
MS19C	<i>Micromonospora</i> sp.	No match	-	-	-
MS19E	<i>Micromonospora coxensis</i>	No match	-	-	-
MS27A	<i>Streptomyces</i> sp.	Deferrioxamine E	0.97	0	601.36
		Dehydroxynocardamine	0.95	0	585.36
		Desmethylenylnocardamine	0.95	0	587.34
		Desferrioxamine	0.95	1	561.36
		Antimycin A1	0.94	0	557.25
		Desferrioxamine E	0.93	1	623.34
		Antimycin A2	0.93	9	535.27
		Antimycin A3	0.91	15	521.26
MS29	<i>Streptomyces</i> sp.	Dehydroxynocardamine	0.97	0	585.36
		Deferrioxamine E	0.96	0	601.36
		Antimycin A1	0.94	0	557.25
		Antimycin A2	0.93	10	535.27
		Antimycin A3	0.92	16	521.26
		Desferrioxamine E	0.92	2	623.34
MS48	<i>Micromonospora</i> sp.	No match	-	-	-
MS49	<i>Micromonospora chalcea</i>	No match	-	-	-

MS50	<i>Micromonospora peucetia</i>	No match	-	-	-
MS52A	<i>Micromonospora coxensis</i>	No match	-	-	-
MS54	<i>Streptomyces</i> sp.	Antimycin A2	0.9	10	535.27
		Antimycin A1	0.9	0	557.25
MS55	<i>Micromonospora aurantiaca</i>	No match	-	-	-
MS56	<i>Micromonospora</i> sp.	No match	-	-	-
MS58	<i>Actinomadura sputi</i>	No match	-	-	-

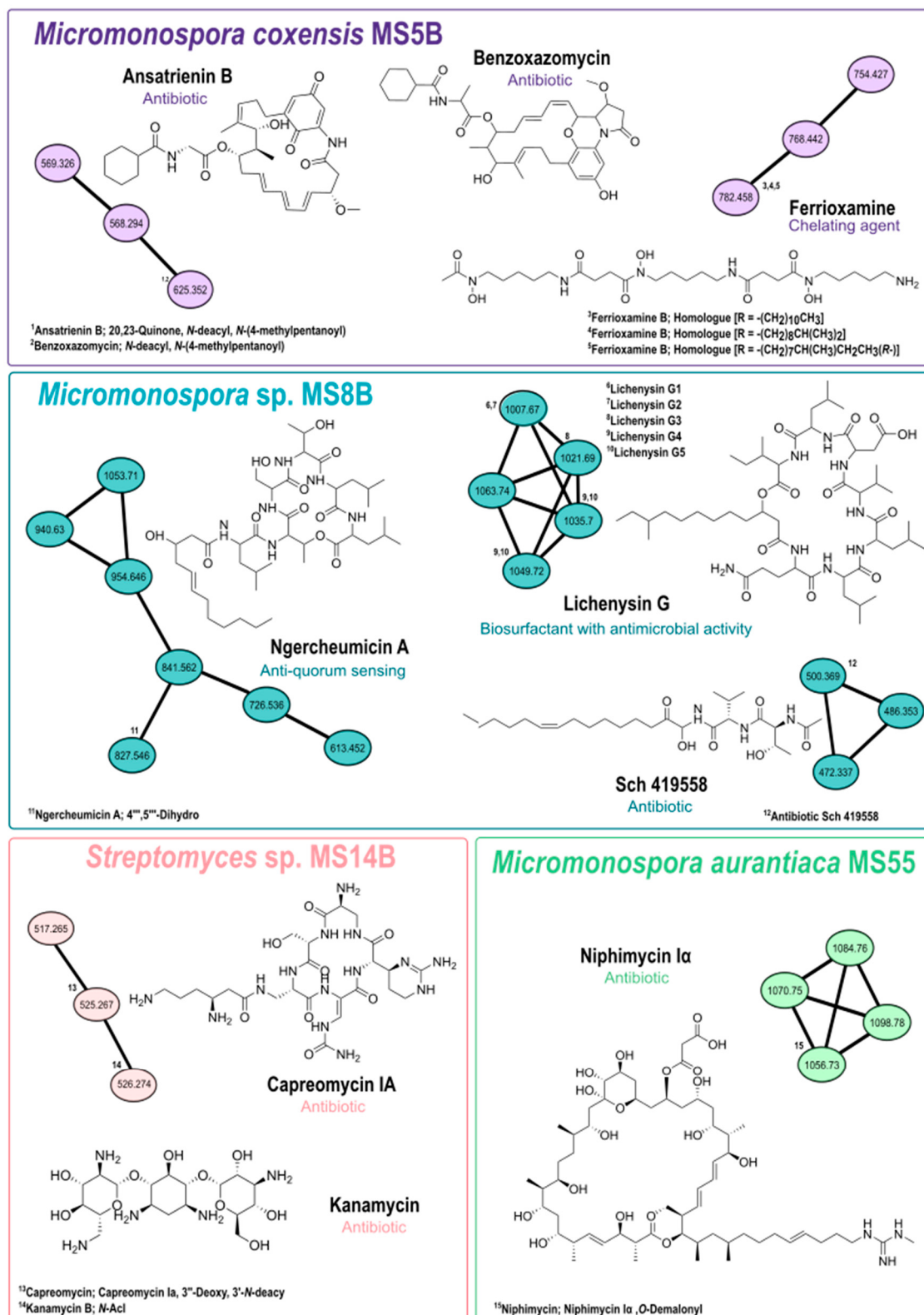


Figure 1. Dereplication of GNPS molecular network data for bioactive actinobacterial extracts. The extracts for the strains MS5B, MS8B, MS14B and MS55 showed clusters with accurate masses matching known natural products, according to the dereplication results from Insilico Peptidic Natural Product Dereplicator, Dictionary of NP and NP atlas database.