SUPPLEMENTARY MATERIAL

Marine Bacteria from Rocas Atoll as a rich source of pharmacologically active compounds

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Table S1. Bacteria strains isolated from ascidians, sponges and sediments from the Rocas Atoll and their anticancer activity results against human colon carcinoma cell line HCT-116. Extracts were considered cytotoxic when inhibited over 75% of the cell growth at 50 µg/mL.

Strain	**Sample Source**	**Host Species**	**Bacteria Identification**[1]	**Sequence Accession number**[2]	**Cell Growth Inhibition at 5 µg/mL (%)**[3]	**Cell Growth Inhibition at 50 µg/mL (%)**[3]
BRB-256	Ascidian	*Euherdmania* sp.	*Salinispora* sp.	MK720164	47.40	94.89
BRB-283	Ascidian	*Didemnum granulatum*	-	-	25.30	64.82
BRB-284	Ascidian	*Euherdmania* sp.	*Salinispora* sp.	MK720165	64.43	91.43
BRB-285	Ascidian	Sp. nov 3	-	-	67.9	70.00
BRB-288	Ascidian	*Trididemnum rocasensis*	-	-	48.25	56.56
BRB-297	Ascidian	*Trididemnum rocasensis*	-	-	0.00	16.56
BRB-298	Ascidian	Sp. nov 3	*Streptomyces* sp.	MK720171	99.34	99.97
BRB-301	Ascidian	*Polysyncraton maurizeliae*	-	-	24.51	40.19
BRB-302	Ascidian	Sp. nov 1	*Streptomyces* sp.	MK720166	100.00	100.00
BRB-304	Ascidian	Sp. nov 3	-	-	0.00	67.43
BRB-306	Ascidian	*Polysyncraton maurizeliae*	-	-	0.00	60.76
BRB-307	Ascidian	*Euherdmania* sp.	-	-	8.19	52.08
BRB-308	Ascidian	*Ascidia viridina*	-	-	0.85	50.38
BRB-309	Ascidian	*Didemnum granulatum*	-	-	6.81	40.57
BRB-311	Ascidian	*Euherdmania* sp.	-	-	15.54	44.79
BRB-314	Ascidian	Sp. nov 2	-	-	15.75	55.10
BRB-320	Ascidian	*Euherdmania* sp.	*Salinispora* sp.	MK720162	69.12	96.37
BRB-324	Ascidian	Ascidia viridina	-	-	18.44	50.69
BRB-326	Ascidian	Sp.nov 3	-	-	0.00	0.00
BRB-346	Sediment	-	-	-	21.00	30.00
BRB-349	Sponge	*Plakinastrella microspiculifera*	*Nocardiopsis* sp.	MK720152	24.00	76.09
BRB-350	Sponge	*Plakinastrella microspiculifera*	*Streptomyces* sp.	MK720170	81.03	97.24
BRB-351	Sediment	-	-	-	22.16	69.92
BRB-352	Sediment	-	*Nocardiopsis* sp.	MK720153	77.74	77.39
BRB-353	Sponge	*Plakinastrella microspiculifera*	-	-	44.71	51.68
BRB-354	Sediment	-	-	-	23.34	71.20

BRB-355	Sponge	*Plakinastrella microspiculifera*	*Nocardiopsis* sp.	MK720167	30.80	77.98
BRB-356	Sponge	*Plakinastrella microspiculifera*	*Streptomyces* sp.	MK720154	79.47	91.72
BRB-357	Sponge	*Chondrilla cf. nucula*	-	-	20.86	73.69
BRB-358	Sponge	*Plakinastrella microspiculifera*	*Streptomyces* sp.	MK720163	74.35	83.62
BRB-359	Sediment	-	-	-	27.63	56.85
BRB-360	Sediment	-	-	-	18.97	35.74
BRB-361	Sponge	*Chondrilla cf. nucula*	-	-	16.38	49.59
BRB-362	Sponge	*Chondrilla cf. nucula*	-	-	0.00	0.85
BRB-363	Sponge	*Chondrilla cf. nucula*	-	-	28.38	59.91
BRB-364	Sponge	*Chondrilla cf. nucula*	*Nocardiopsis* sp.	MK720156	26.23	88.21
BRB-366	Sponge	*Chondrilla cf. nucula*	-	-	62.05	62.05
BRB-367	Sponge	*Chondrilla cf. nucula*	-	-	22.63	40.97
BRB-368	Sponge	*Chondrilla cf. nucula*	*Brevibacterium* sp.	MK720174	n.d	90.85
BRB-371	Sponge	*Chondrilla cf. nucula*	-	-	33.54	36.76
BRB-372	Sponge	*Chondrilla cf. nucula*	-	-	11.16	14.34
BRB-373	Ascidian	*Tridldemnum Maragogi*	-	-	19.92	43.91
BRB-374	Sponge	*Chondrilla cf. nucula*	-	-	22	49
BRB-375	Sponge	*Plakortis* sp.	-	-	22.10	51.15
BRB-383	Sponge	*Plakinastrella microspiculifera*	-	-	2.96	68.93
BRB-385	Sponge	*Chondrilla cf. nucula*	-	-	23.43	56.04
BRB-386	Sponge	*Plakinastrella microspiculifera*	-	-	51.21	65.49
BRB-388	Sediment	-	-	-	16.06	41.99
BRB-391	Ascidian	*Tridldemnum Maragogi*	*Bacillus* sp.	MK720168	85.11	100.00
BRB-392	Sediment	-	-	-	4.55	49.71
BRB-393	Sediment	-	-	-	0.00	6.31
BRB-397	Sponge	*Chondrilla cf. nucula*	*Bacillus* sp.	MK720169	35.89	92.40
BRB-398	Sediment	-	-	-	2.08	16.50
BRB-399	Sponge	*Chondrilla cf. nucula*	*Salinispora* sp.	MK720158	69.93	82.96
BRB-405	Ascidian	*Tridldemnum Maragogi*	-	-	14.36	23.00
BRB-406	Ascidian	*Tridldemnum Maragogi*	-	-	73.28	94.78
BRB-407	Ascidian	*Tridldemnum Maragogi*	-	-	80.94	92.93

BRB-408	Sediment	-	*Bacillus* sp.	MK720155	88.94	100.97
BRB-412	Ascidian	*Trididemnum Maragogi*	-	-	9.45	46.35
BRB-414	Ascidian	*Trididemnum Maragogi*	*Salinispora* sp.	MK720161	86.52	98.86
BRB-415	Ascidian	*Trididemnum Maragogi*	*Salinispora* sp.	MK720172	64.51	97.97
BRB-417	Sediment	-	*Streptomyces* sp.	MK720159	17.21	95.73
BRB-418	Sediment	-	*Streptomyces* sp.	MK720157	34.44	98.88
BRB-455	Sediment	-	-	-	12.00	35.00
BRB-456	Sediment	-	-	-	21.04	71.98
BRB-457	Sediment	-	-	-	23.19	67.36
BRB-458	Sediment	-	-	-	14.63	27.64
BRB-462	Ascidian	*Trididemnum Maragogi*	*Salinispora* sp.	MK720160	75.39	92.81
BRB-463	Sponge	*Chondrilla cf. nucula*	-	-	29.00	57.00
BRB-466	Sponge	*Plakortis sp.*	-	-	21.42	49.35
BRB-468	Sponge	*Chondrilla cf. nucula*	*Salinispora* sp.	MK720173	61.19	94.04
BRB-474	Sponge	*Chondrilla cf. nucula*	-	-	14.67	25.07
BRB-475	Sediment	-	-	-	0.00	21.23
BRB-476	Sediment	-	-	-	14.28	20.82
BRB-502	Ascidian	*Trididemnum Maragogi*	-	-	16.77	29.52
BRB-504	Ascidian	*Trididemnum Maragogi*	-	-	44.10	102.30

[1] Bacterial identification based on 16S rRNA sequencing for those strains considered active

[2] Available at (http://www.ncbi.nlm.nih.gov/genbank).

[3] Cell growth inhibition was obtained by MTT assay, using HCT-116 colon cancer cells, after 72h incubation.

n.d. – not determined

Figure S1. Origin of the 80 bacterial strains recovered from the Atlantic Rocas Atoll. (A) Bacteria recovered from ascidians (40%), sponges (35%) and sediments (25%); (B) Identification of ascidians, sponge and sites where sediments were collected.

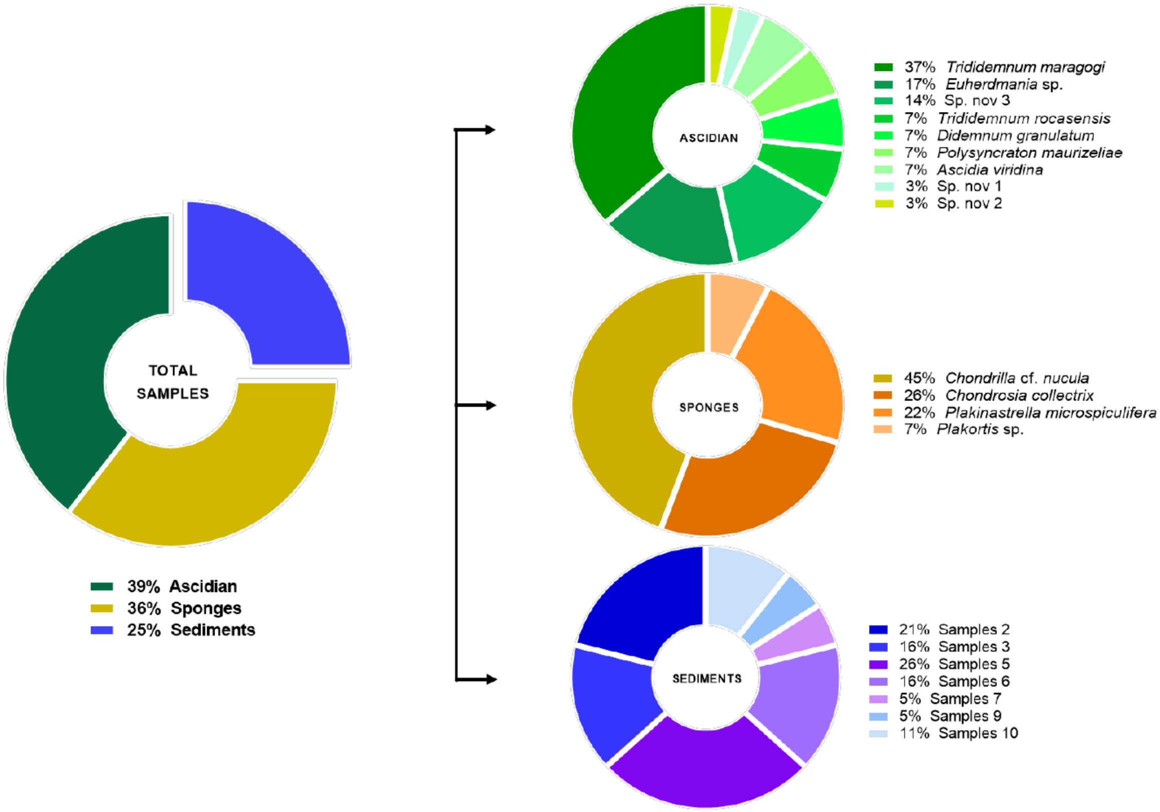

Figure S2. Molecular Network of crude extracts produced by bacteria recovered from Rocas Atol, using positive ionization mode (ESI$^+$) data. Node colors represent the strains extracts accordingly to the legend. Nodes highlighted in black boxes represent parent ions that are identified by GNPS library, and blue box represent cluster of ions that are present only in active crude extracts.

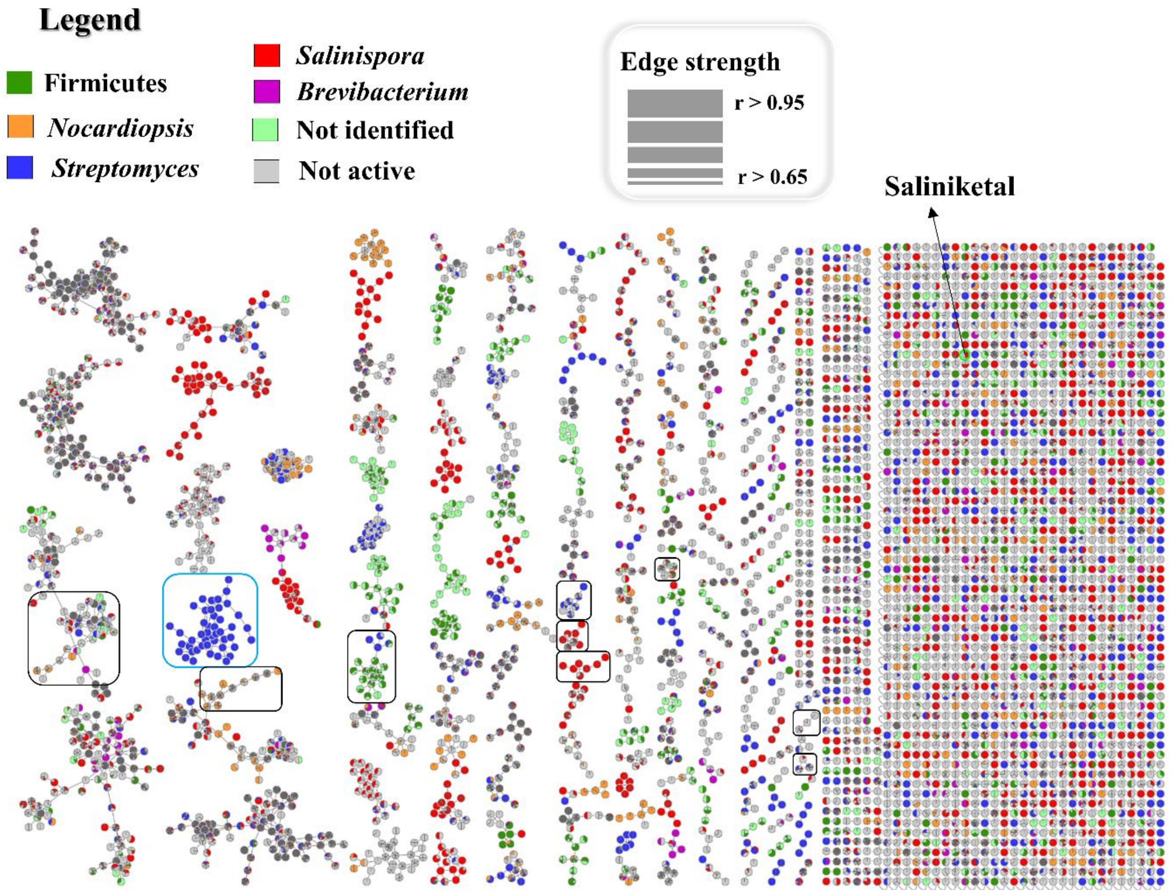

Figure S3. Cluster of the diketopiperazine family, observed as protonated adducts ($[M+H]^+$), produced by all investigated strain. The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated nodes are circled in green.

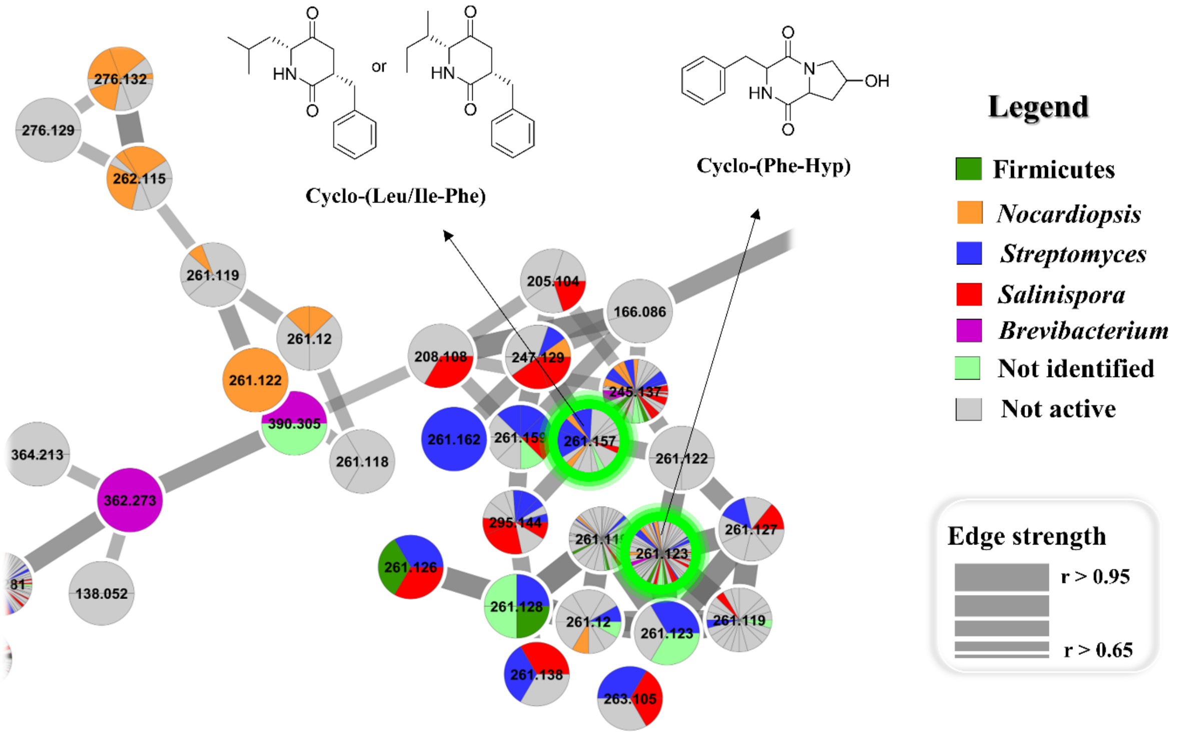

Figure S4. Cluster of the rifamycin family, observed as protonated adducts ([M+H]$^+$), produced mainly by *Salinispora* (BRB-256, BRB-399, BRB-414, BRB-415 and BRB-468), but also by BRB-283, a not active strain. The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated node is circled in green.

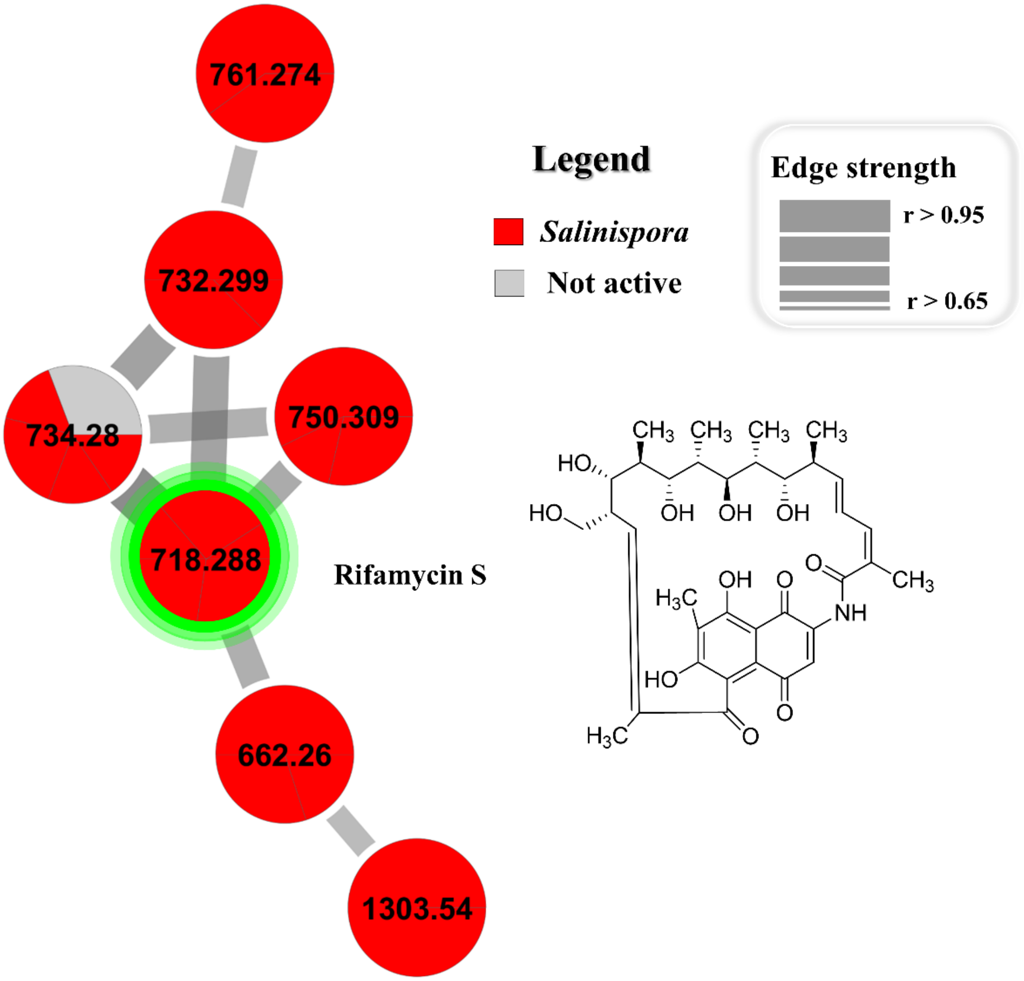

Figure S5. Cluster of lipopeptide family, including surfactins and esperin, observed as protonated adducts ($[M+H]^+$), produced by *Bacillus* (Firmicutes) (BRB-391, BRB-397 and BRB-408), *Nocardiopsis* (BRB-364), *Streptomyces* (BRB-298, BRB-302, BRB-350, BRB-356, BRB-358, BRB-417 and BRB-418), *Salinispora* (BRB-320 and BRB-415) not identified (BRB-406, BRB-407 and BRB-504) and not active (BRB-346 and BRB-359) strains. The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated nodes are circled in pink.

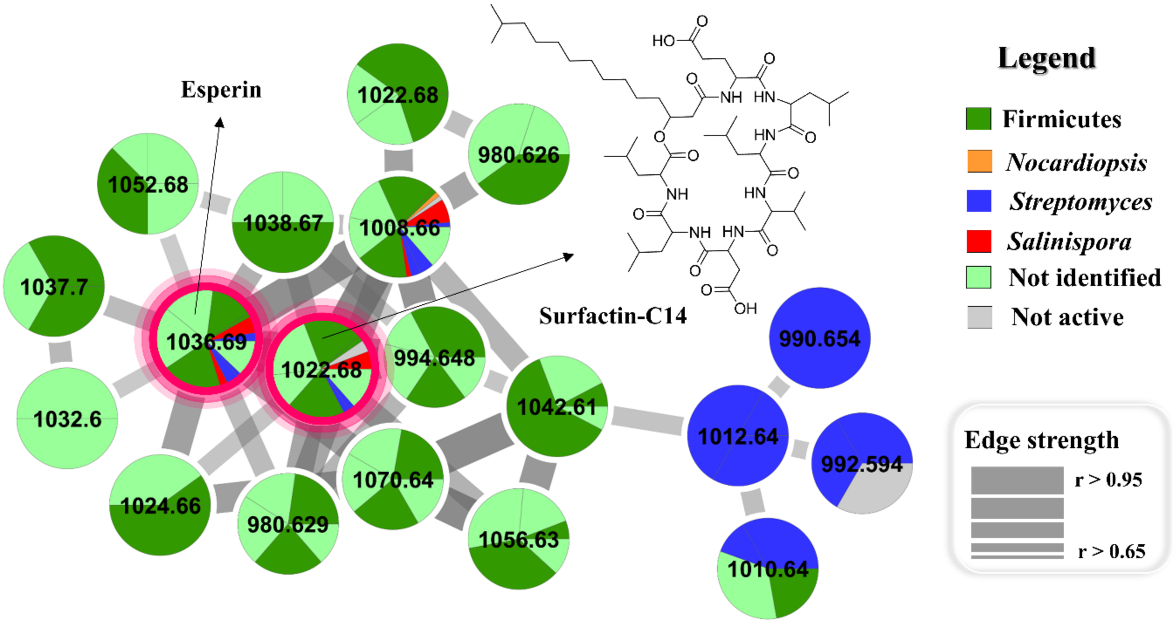

Figure S6. Cluster of the erythromycin family, observed as protonated adducts ($[M+H]^+$), produced mainly by not identified (BRB-346, BRB-374, BRB-375, BRB-405, BRB-455 and BRB-463) strains, but also by one *Salinispora* (BRA-415) strain. The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated node is circled in green.

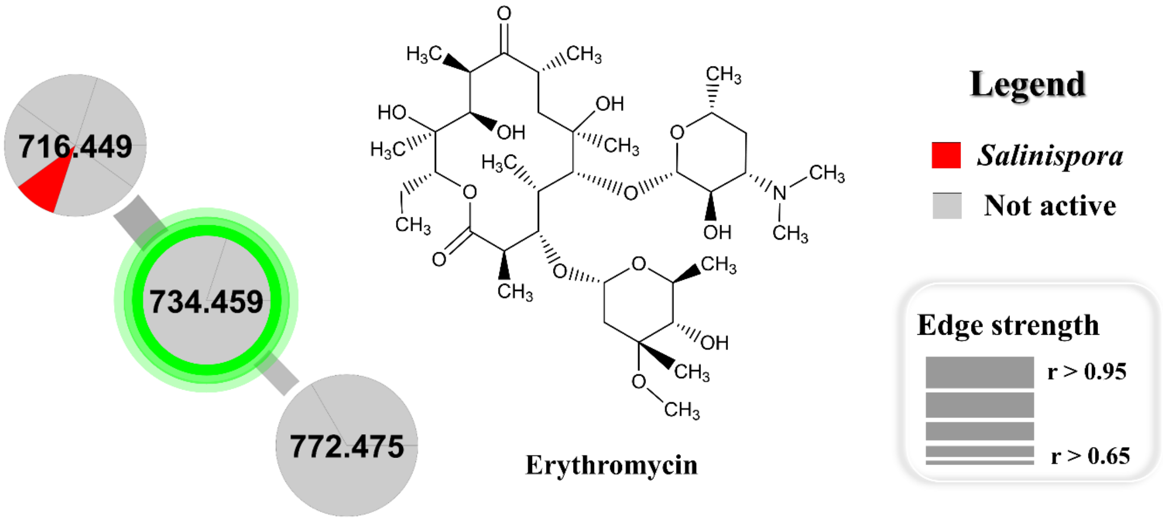

Figure S7. Cluster of the antibiotic TAN family, observed as protonated adducts ($[M+H]^+$), produced mainly by *Nocardiopsis* (BRB-349, BRB-352, BRB-355 and BRB-364), but also Firmicutes (BRB-397), *Streptomyces* (BRB-302 and BRB-358), *Salinispora* (BRB-414 and BRB-468), not identified (BRB-407) and not active (BRB-351, BRB-354, BRB-360, BRB-361, BRB-371, BRB-372, BRB-373, BRB-383, BRB-385, BRB-388, BRB-393 and BRB-455) strains. The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated node is circled in green.

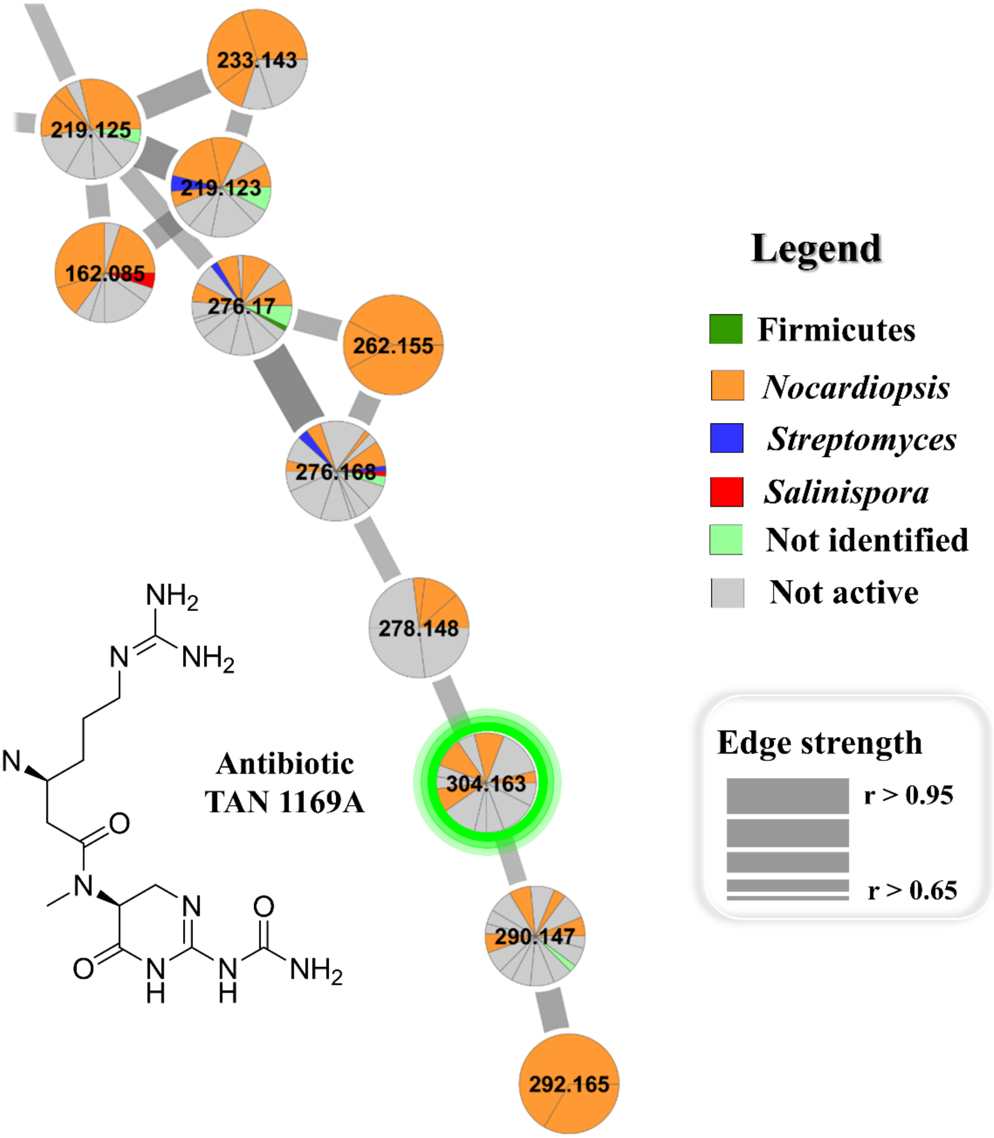

Figure S8. Cluster of the surugamide family, observed as protonated adducts ($[M+H]^+$), produced mainly by *Streptomyces* strains (BRB-350, BRB356 and BRB-358), *Nocardiopsis* (BRB-352) and not active (BRB-353, BRB-366, BRB-375 and BRB-386). The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated nodes are circled in green.

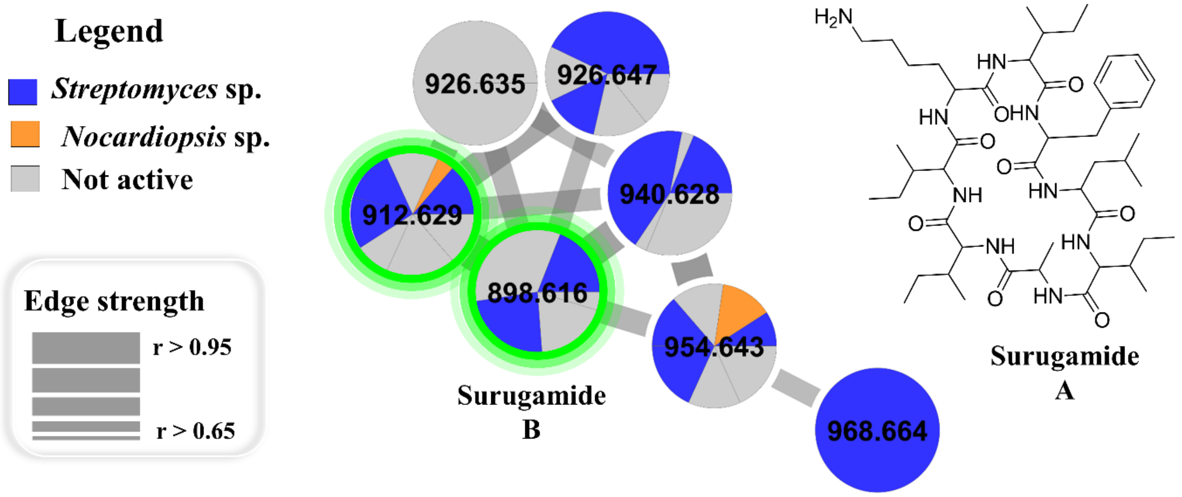

Figure S9. Cluster of the staurosporine family, observed as protonated adducts ($[M+H]^+$), produced mainly by *Salinispora* (BRB-256, BRB-320, BRB-399, BRB-414, BRB-415, BRB-462 and BRB-468), *Streptomyces* (BRB-302) not active (BRB-285, BRB-304, BRB-306, BRB-307, BRB-308, BRB-309, BRB-324 and BRB-405) strains. The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated nodes are circled in green.

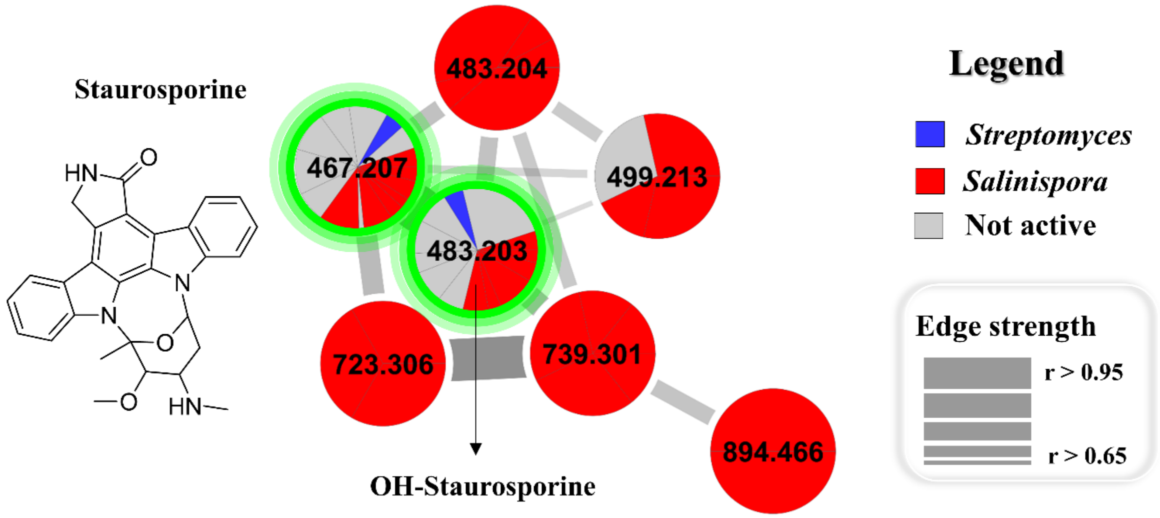

Figure S10. Cluster of the desferrioxamine family, observed as protonated adducts ($[M+H]^+$), produced mainly by not active (BRB-367, BRB-375, BRB-386, BRB-412, BRB-463 and BRB-476) strains, but also by *Streptomyces* (BRB-350 and BRB-358) and *Salinispora* (BRB-414). The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated nodes are circled in green.

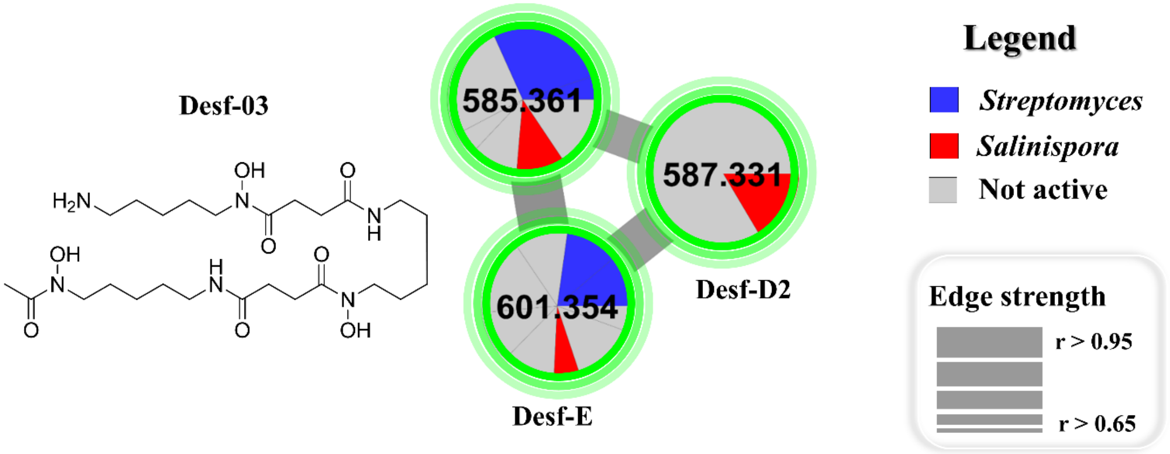

Figure S11. Cluster of the sphinganine family, observed as protonated adducts ($[M+H]^+$), produced by many different strains, including *Bacillus* (Firmicutes) (BRB-408), *Brevibacterium* (BRB-368), *Nocardiopsis* (BRB-364), *Salinispora* (BRB-256, BRB-414 and BRB-415), *Streptomyces* (BRB-298), not identified (BRB-406 and BRB-504) and not active (BRB-297, BRB-304, BRB-306, BRB-307, BRB-308, BRB-309, BRB-346, BRB-374, BRB-398, BRB-405 and BRB-463). The node color represents the genus of the bacteria, and edge size is according to the cosine score. The annotated node is circled in green.

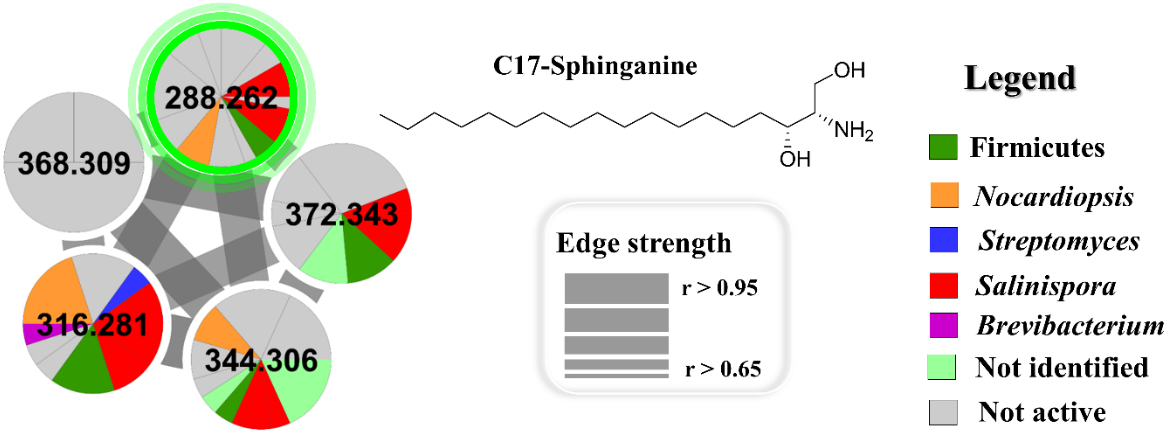

Figure S12. (A) Venn diagram and (B) distributions of samples according to the number of ions detected in cytotoxic and non-cytotoxic extracts; (C) Shannon-Wiener index. For these analyses, it was used the .csv table obtained from the molecular network containing the features information.

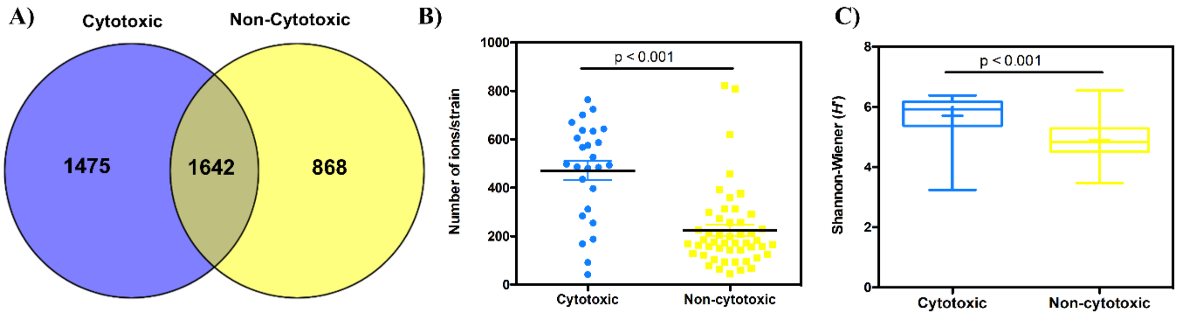

Figure S13. (a) Chromatographic profile of the extract produced by Streptomyces BRB-302 in A1 culture medium. Dashed line set the fractions collected over time. (b) Growth inhibition response in cytotoxic assay using the human colon adenocarcinoma HCT-116 cell line. In red are highlighted the fractions that contain the novonestmycin derivatives.