

Phytoplankton trigger the production of cryptic metabolites in the marine actinobacterium *Salinispora tropica*.

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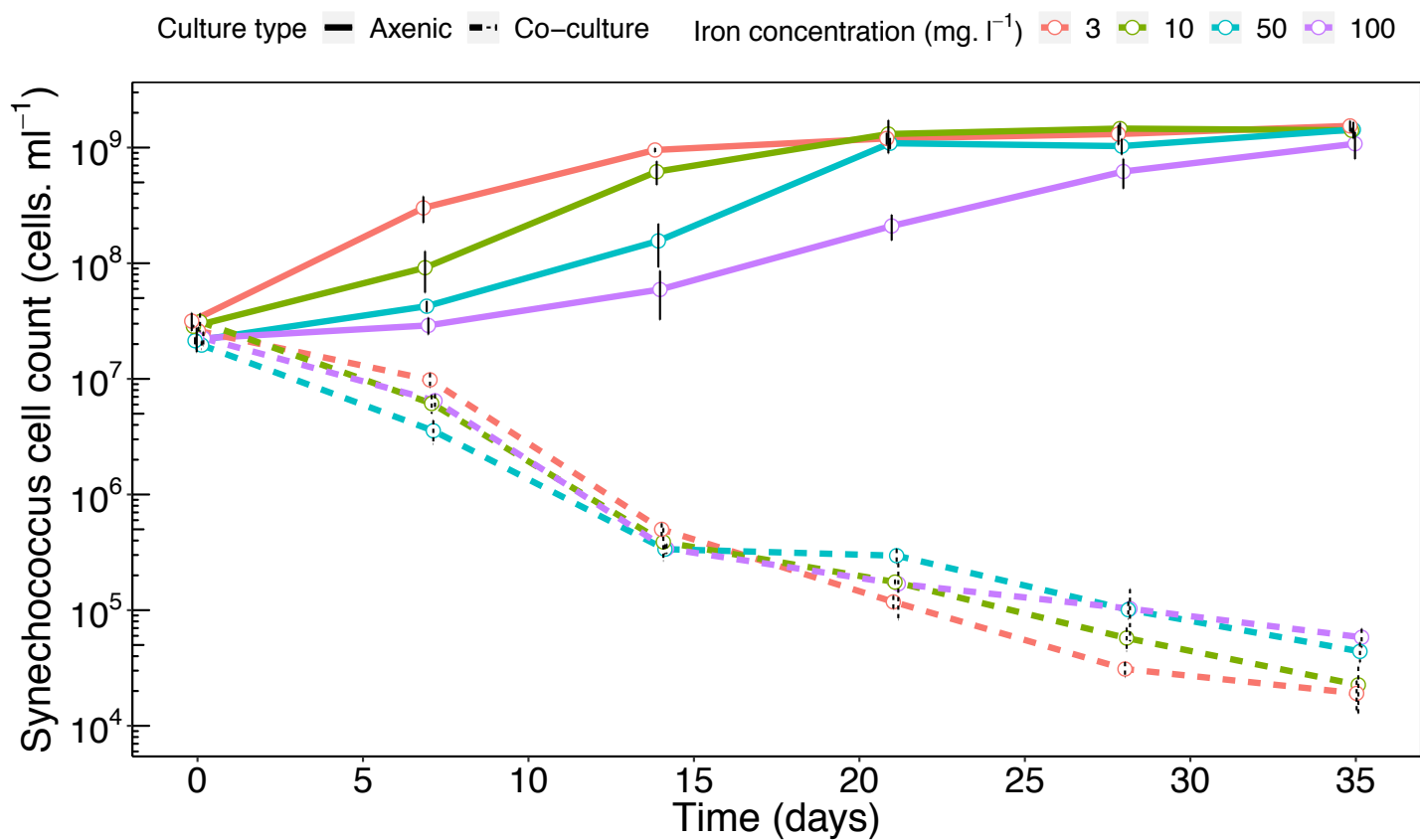
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Supplementary Figures



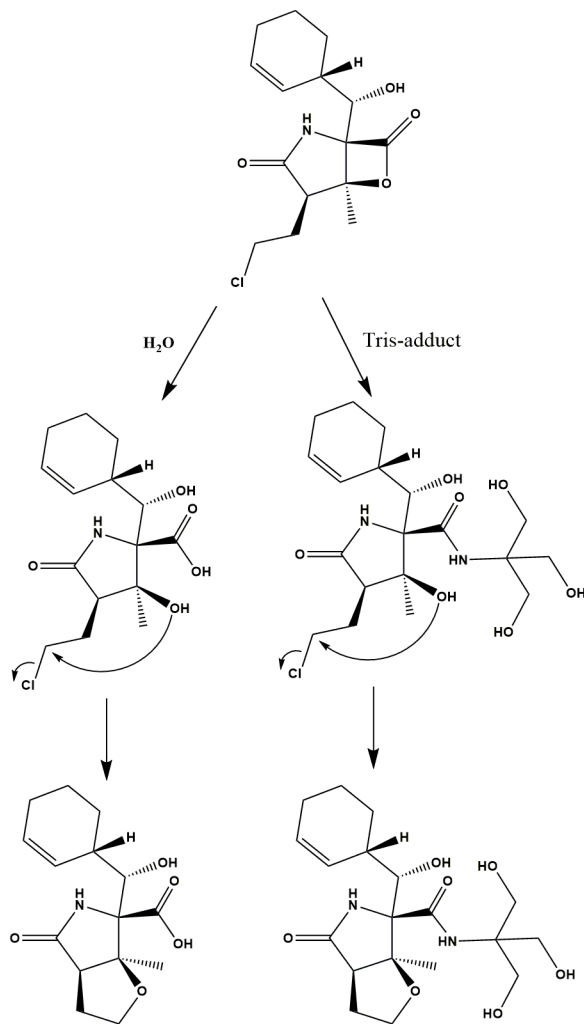
Supplementary Figure S1 | *Synechococcus* inhibition by *S. tropica* is not mediated by iron depletion. Monitoring of *Synechococcus* population grown axenically or in co-culture with *S. tropica*, in media supplemented with 3, 10, 50 or 100 mg l⁻¹ Fe(III). Graph shows mean ± standard deviation of three biological replicates.

Supplementary Table S1 | Molecular ions detected by LC-MS in *S. tropica*-*Synechococcus* co-culture only. Table shows molecular ions detected by high-resolution LC/(+)ESI-MS. Peak numbering is based on HPLC retention time and colors indicate groups of related compounds. Observed m/z values and predicted chemical formulae for $[M+H]^+$ are provided. Observed mass of main ions obtained after MS2 fragmentation are given.

MS Peak	Observed m/z	Chemical formulae for $[M+H]^+$ (calculated m/z ; err [ppm])	MS/MS
1	399.2135	$[C_{19}H_{31}N_2O_7]^+$ (399.2126; -2.4)	296.1498
			271.1295
			186.0765
			168.0659
2	401.2294	$[C_{19}H_{33}N_2O_7]^+$ (401.2292; -2.8)	298.1657
			273.1453
			255.1348
			152.0712
3	438.1701	$[C_{28}H_{24}NO_4]^+$ (438.1700; -0.3)	194.0817 177.1279
4	464.2509	$[C_{22}H_{34}N_5O_6]^+$ (464.2504; -1.2)	276.16
			171.088
			154.0615
5	296.1499	$[C_{15}H_{22}NO_5]^+$ (296.1492; -2.1)	296.1499
			318.132
			168.066
6	435.2609	$[C_{22}H_{35}N_4O_5]^+$ (435.2602; -1.7)	372.229
			276.1599
			142.0979
7	449.2764	$[C_{23}H_{37}N_4O_5]^+$ (449.2758; -1.3)	156.1135
8	298.1653	$[C_{15}H_{24}NO_5]^+$ (298.1649; -1.5)	320.1447
			298.1654
			170.0815

Salinosporamide A (Marizomib)

Chemical Formula: $C_{15}H_{20}ClNO_4$
Exact Mass: 313.1081



Compound 5

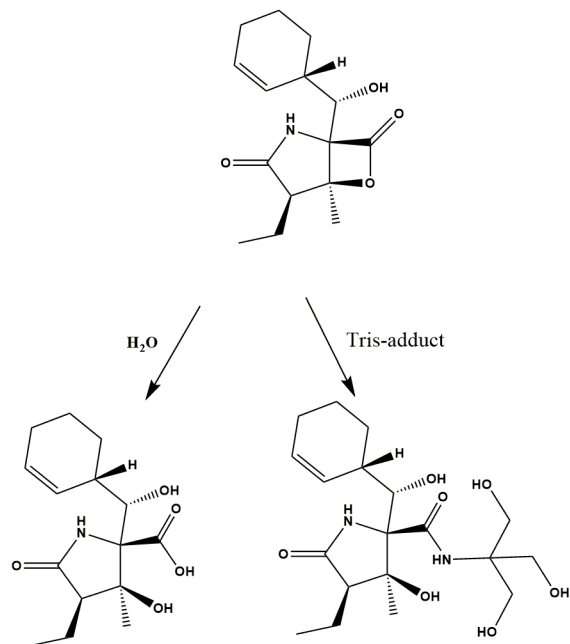
Chemical Formula: $C_{15}H_{21}NO_5$
Exact Mass: 295.1420

Compound 1

Chemical Formula: $C_{19}H_{30}N_2O_7$
Exact Mass: 398.2053

Salinosporamide B

Chemical Formula: $C_{15}H_{21}NO_4$
Exact Mass: 279.1471



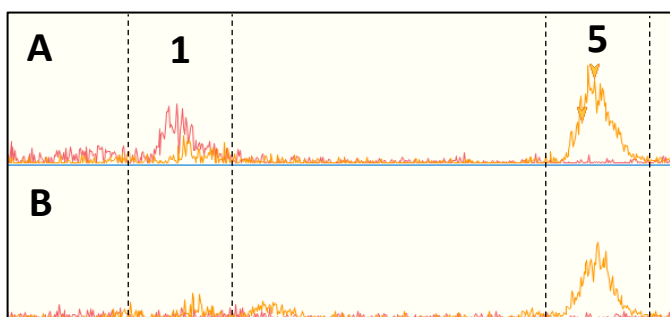
Compound 8

Chemical Formula: $C_{15}H_{23}NO_5$
Exact Mass: 297.1576

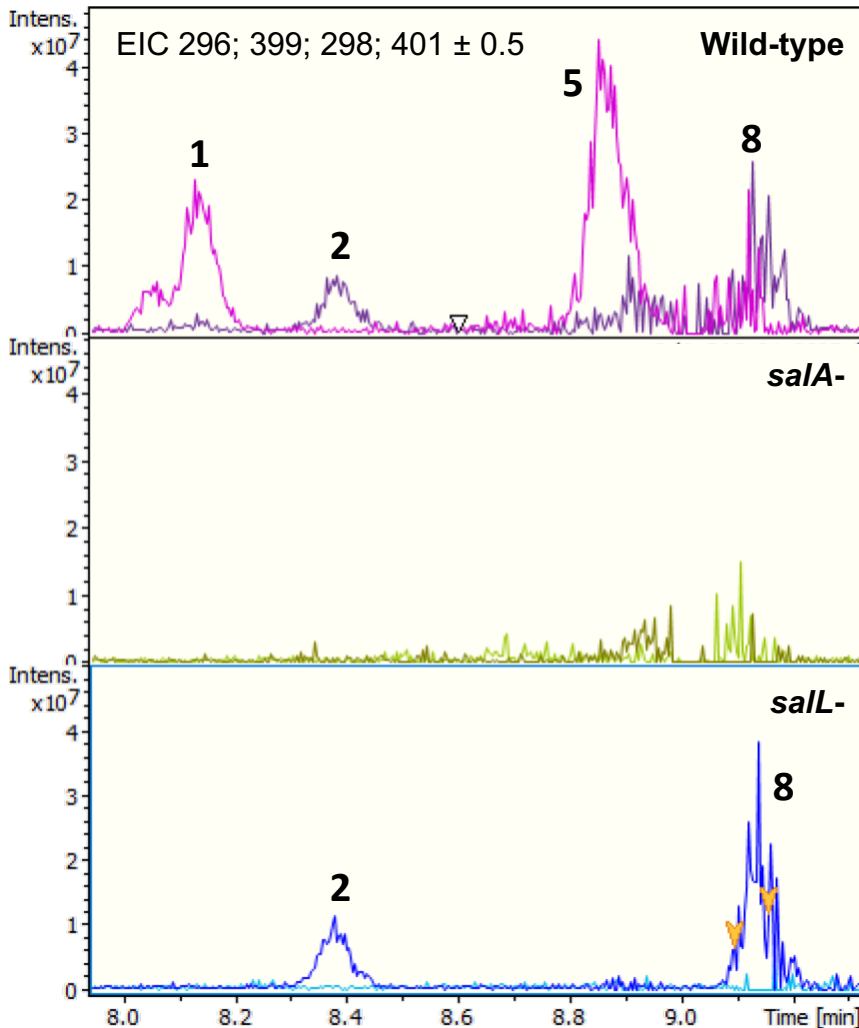
Compound 2

Chemical Formula: $C_{19}H_{32}N_2O_7$
Exact Mass: 400.2210

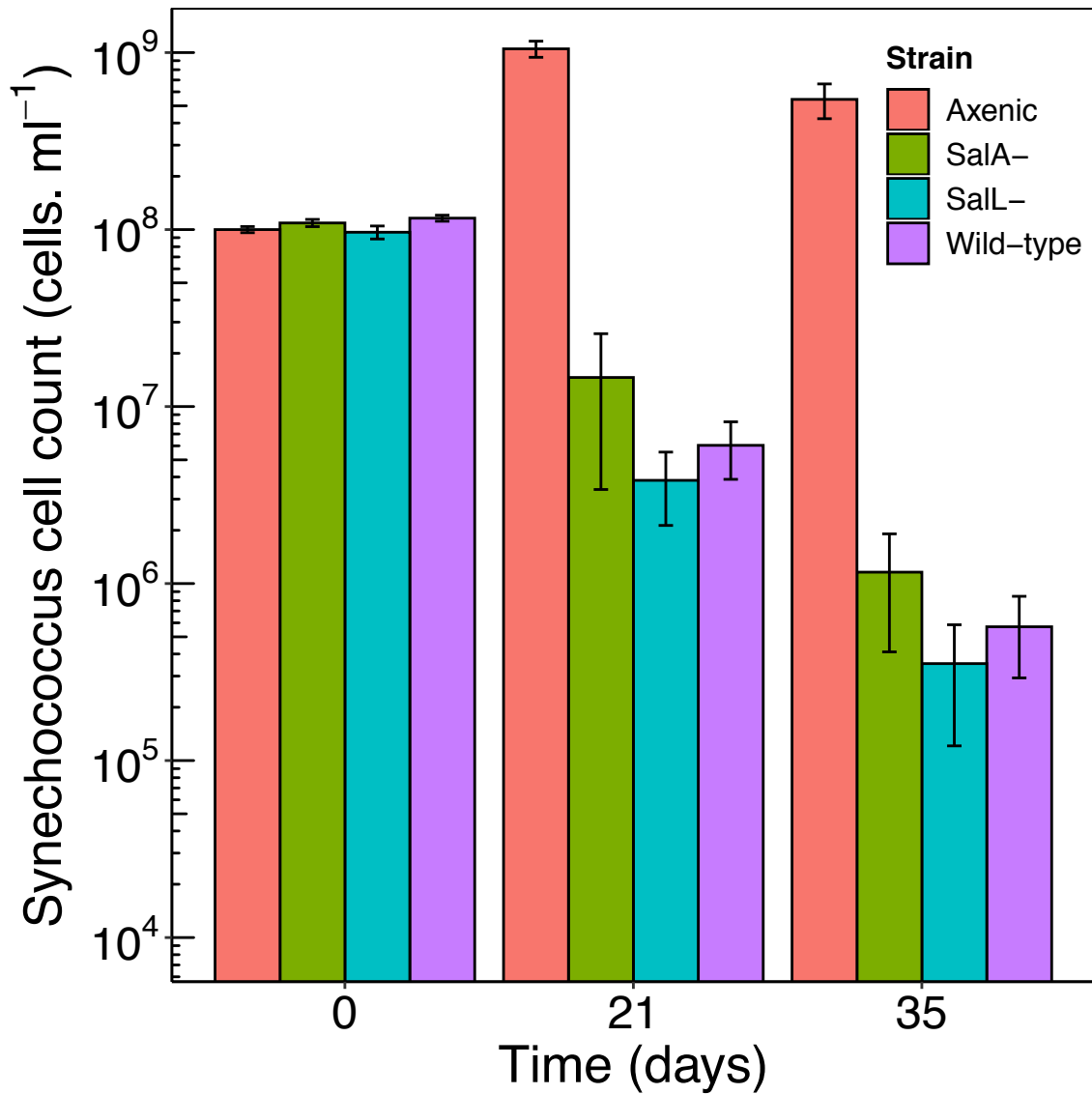
Supplementary Figure S2 | Chemical structure of salinosporamide A and B, with their respective degradation products. Salinosporamide A ($C_{15}H_{20}^{35}ClNO_4$; mass 313.11) hydrolyzes to form the molecule NPI-0065 (**5**) ($C_{15}H_{21}NO_5$; mass 295.14) or reacts with Tris to form the hypothetical molecule (**1**) ($C_{19}H_{30}N_2O_7$; mass 398.21). Salinosporamide B ($C_{15}H_{21}NO_4$; mass 279.15) hydrolyzes to form the molecule (**8**) ($C_{15}H_{23}NO_5$; mass 297.16) or reacts with Tris to form the hypothetical molecule (**2**) ($C_{19}H_{32}N_2O_7$; mass 400.22).



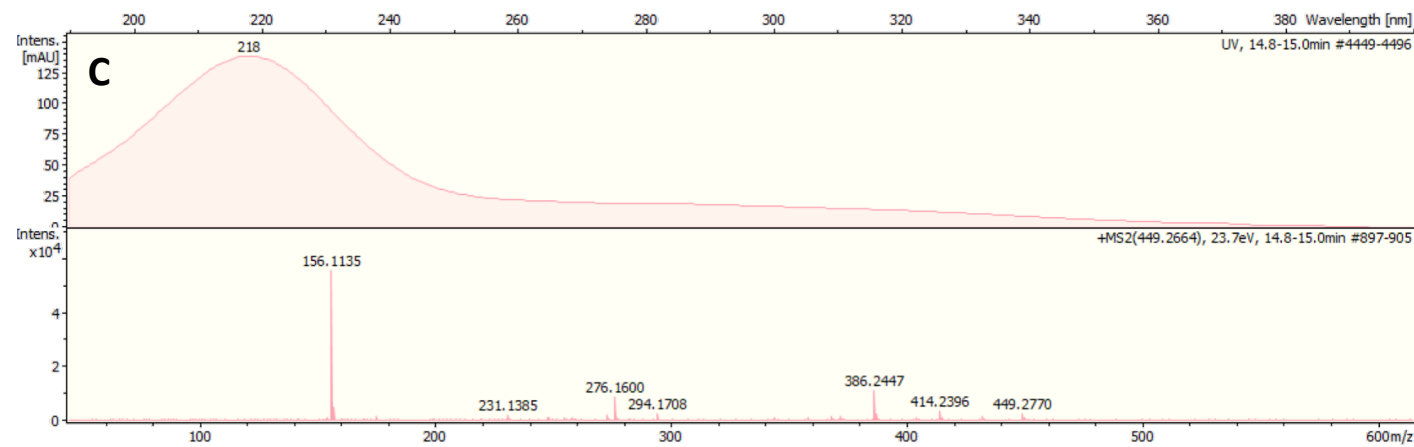
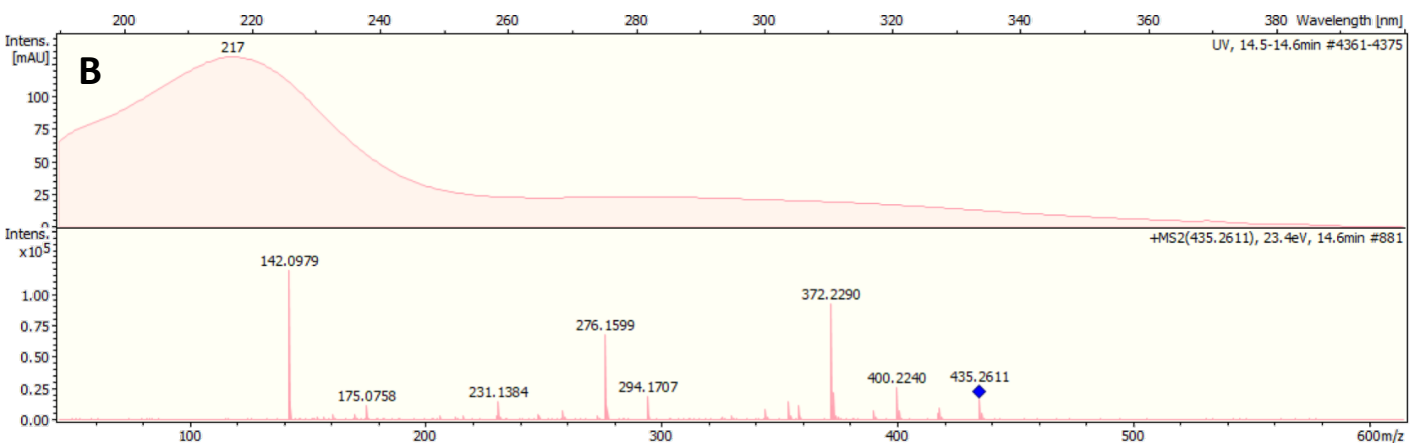
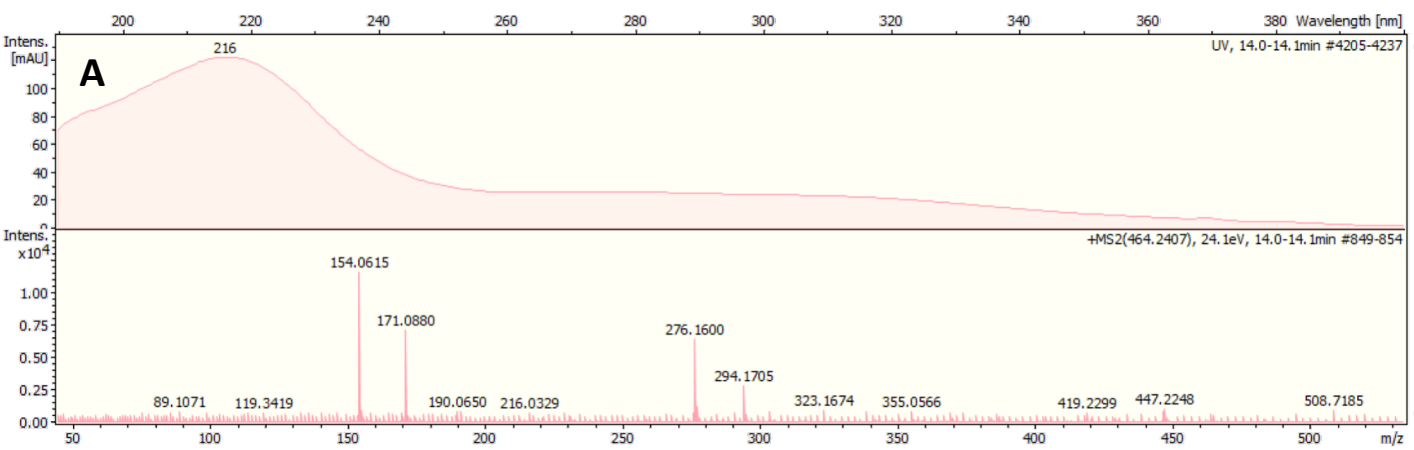
Supplementary Figure S3 | Extracted Ion chromatograms of molecules 1 and 5 in the supernatant of *S. tropica* cultures in marine broth. A. Culture supernatant of *S. tropica* grown in marine broth supplemented with trizma base. **B.** Culture supernatant of *S. tropica* grown in marine broth. Graphs show molecules detected with a retention time between 8.8 and 11.1 minutes. In red is shown the extracted ion chromatogram for m/z 399 (\pm 0.5). In orange is shown the extracted ion chromatogram for m/z 296 (\pm 0.5).



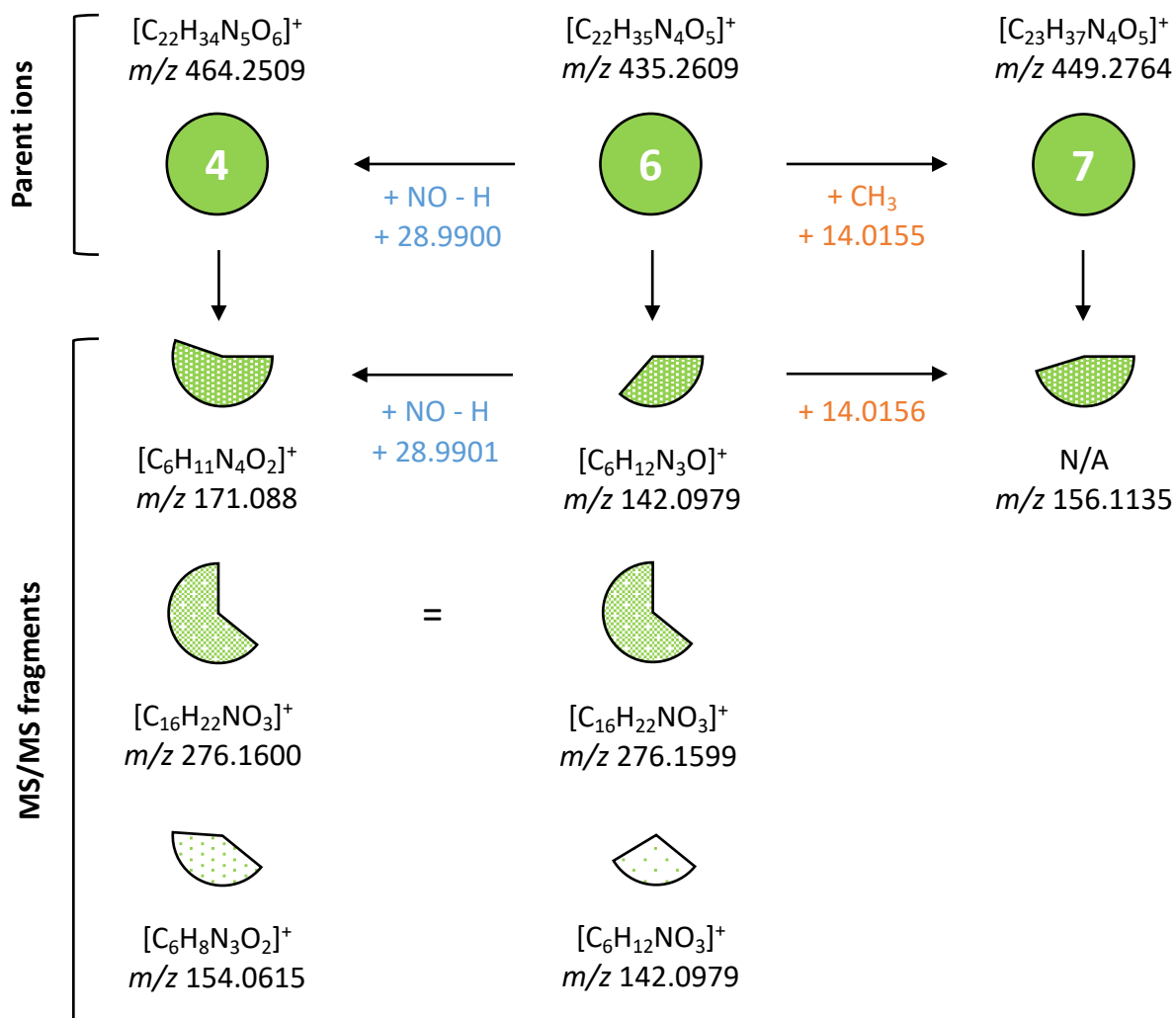
Supplementary Figure S4 | Extracted ion chromatograms of molecules 1, 2, 5 and 8 in the culture supernatant of *S. tropica* wild-type (top panel), and the salinosporamide mutants *saIA*⁻ (middle panel) and *saII*⁻ (bottom panel). The *saIA*⁻ strain does not produce salinosporamide A or any derivatives, while the *saII*⁻ strain still produces salinosporamide B. Graphs show molecules detected with a retention time between 8.0 and 9.4 minutes.



Supplementary Figure S5 | Monitoring of *Synechococcus* grown in axenic culture and in co-culture with the wild-type, *salA*- or *sall*- *S. tropica* strains. Graph shows mean of triplicates ± standard deviation.



Supplementary Figure S6 | MS/MS fragmentation spectra of the cryptic molecules. High-resolution LC/(+)ESI-MS/MS spectra obtained for molecule 4 (A), 6 (B), and 7 (C).



Supplementary Figure S7 | The cryptic molecules 4, 6 and 7 are related. Schematic of the cryptic compounds and their corresponding daughter ions generated by MS/MS. Observed m/z values detected by high-resolution LC/(+)-ESI-MS and predicted chemical formulae for $[M+H]^+$ are provided. N/A indicate chemical formulae that could not be generated by the DataAnalysis software.

Supplementary Table S2 | Summary of the proteomics dataset.

Strain studied (No. of proteins)	Growth medium	Detected proteins (% of total proteins)	Detected proteins related to BGCs (% of total detected)	Relative abundance ^a (%)
<i>S. tropica</i> CNB-440 (4,522)	Marine Broth (MB)	1,869 (41.3)	179 (9.6)	9.6 ± 0.9
	Artificial Seawater (ASW)	1,797 (39.7)	172 (9.6)	11.4 ± 0.4
	Phototroph supernatant (SUPSYN)	1,831 (40.5)	181 (9.9)	15.0 ± 1.2

**

^a Table shows the cumulated relative abundance of the detected proteins linked to biosynthetic gene clusters, indicated as mean ± standard deviation of three biological replicates. Tukey HSD test, ** significant at q -value < 0.01; *** significant at q -value < 0.001.

Supplementary Table S3 | Detected proteins from the *pks3* orphan BGC in *S. tropica* CNB-440. Table shows protein identifiers, annotation and relative abundance (expressed as the abundance of the protein over the abundance of the total proteome normalized to 1).

Protein ID	Gene name	Locus tag	Annotation	Relative abundance
A4X7T4	Strop_2488	STROP_RS12520	DUF3050 domain-containing protein	0.000750547
A4X7T5	Strop_2489	STROP_RS12525	phytanoyl-CoA dioxygenase	0.130375917
A4X7T6	Strop_2490	STROP_RS12530	4-hydroxyphenylpyruvate dioxygenase	0.002402502
A4X7T7	Strop_2491	STROP_RS12535	beta-ketoacyl	0.001818263
A4X7T8	Strop_2492	STROP_RS12540	acyl--CoA ligase	0.031677582
A4X7U0	Strop_2494	STROP_RS12550	3-ketoacyl-ACP synthase	0.014597734
A4X7U3	Strop_2497	STROP_RS12570	long-chain fatty acid--CoA ligase	0.034956859
A4X7U4	Strop_2498	STROP_RS12575	antibiotic biosynthesis monooxygenase	0.129801561
A4X7U5	Strop_2499	STROP_RS12580	actinorhodin polyketide beta-ketoacyl synthase	0.006074535
A4X7U6	Strop_2500	STROP_RS12585	beta-ketoacyl	0.00502919
A4X7U7	Strop_2501	STROP_RS12590	cupin domain-containing protein	0.146326909
A4X7U8	Strop_2502	STROP_RS12595	cyclase	0.495965212
A4X7U9	Strop_2503	STROP_RS12600	acetyl-CoA carboxylase biotin carboxylase subunit	0.006262552
A4X7V0	Strop_2504	STROP_RS12605	acetyl-CoA carboxylase biotin carboxyl carrier protein	0.10444248
A4X7V1	Strop_2505	STROP_RS12610	acetyl-CoA carboxylase carboxyltransferase subunit alpha	0.000773389
A4X7V2	Strop_2506	STROP_RS12615	Tcml family type II polyketide cyclase	0.117717019
A4X7V4	Strop_2508	STROP_RS12625	Tcml family type II polyketide cyclase	0.229200995