Supporting Information

Tania Keiko Shishido[1], Jouni Jokela[1], Clara-Theresia Kolehmainen[1], David P. Fewer[1], Matti Wahlsten[1], Hao Wang[1], Leo Rouhiainen[1], Ermano Rizzi[2], Gianluca Debellis[2], Perttu Permi[3], and Kaarina Sivonen[1]

[1] Department of Food and Environmental Sciences, Viikki Biocenter 1, University of Helsinki, FI-00014, Helsinki, Finland
[2] Institute of Biomedical Technologies, National Research Council**,** Via Fratelli Cervi N. 93, 20090, Segrate (Milan), Italy
[3] Program in Structural Biology and Biophysics, Institute of Biotechnology/Nuclear Magnetic Resonance Laboratory, University of Helsinki, FI-00014, Helsinki, Finland

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1. Supporting figures

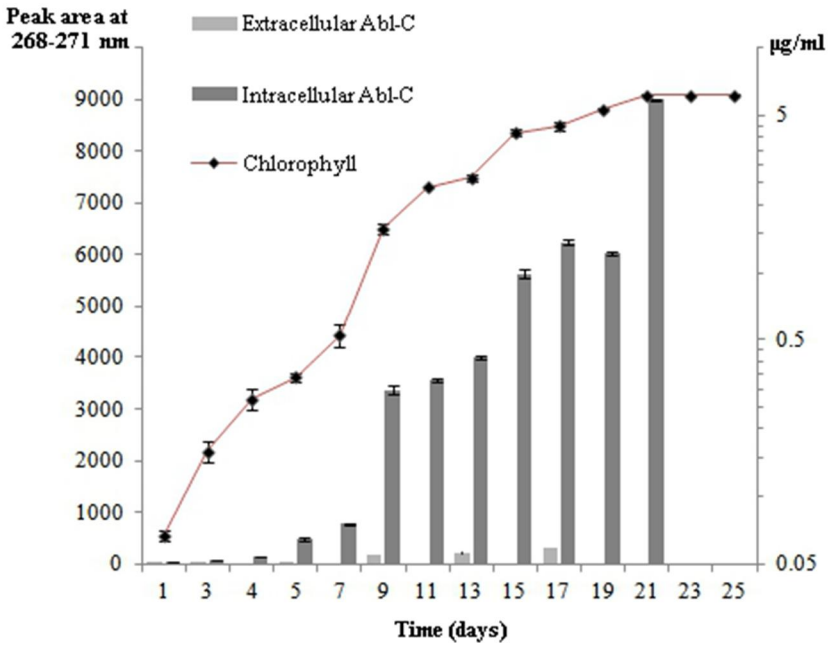
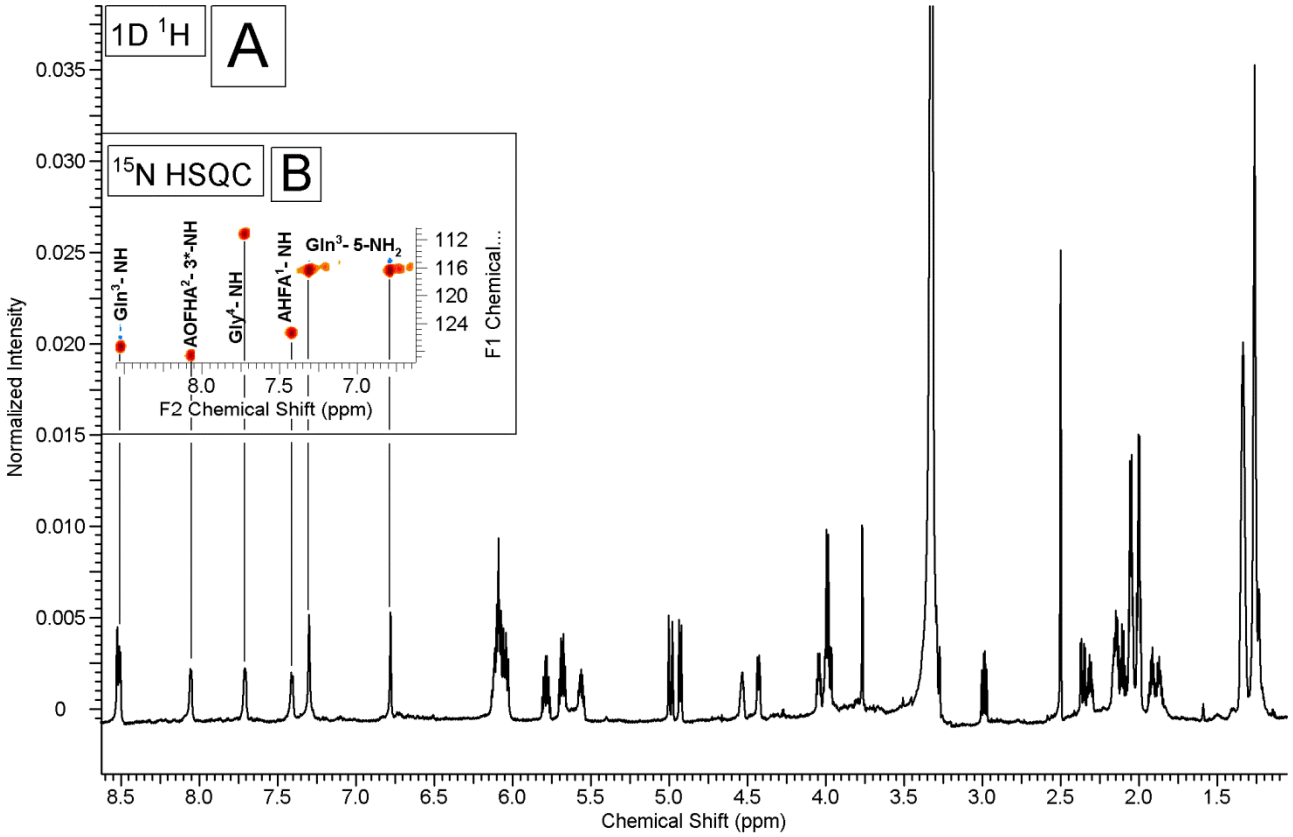
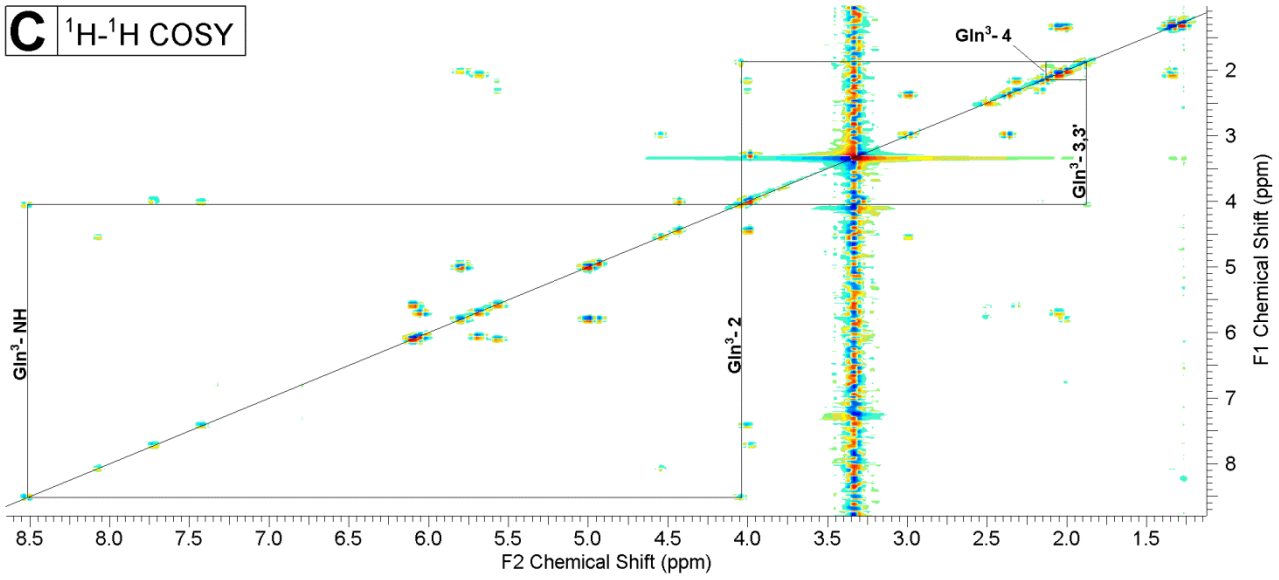
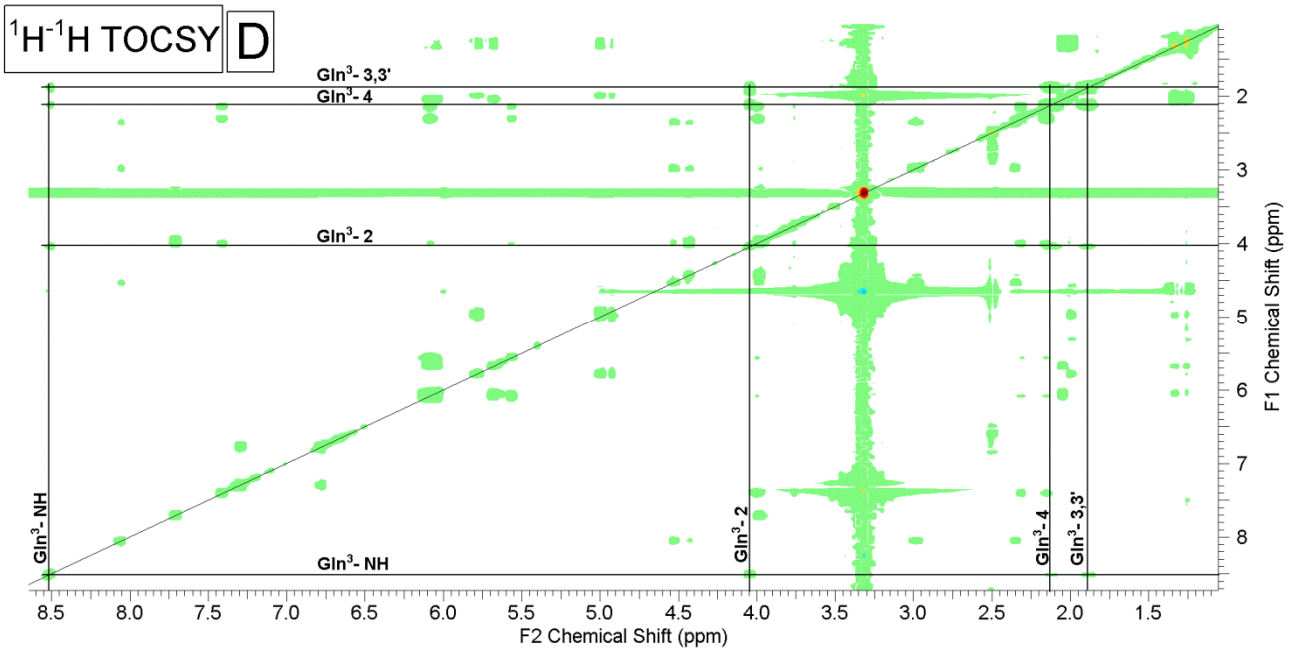
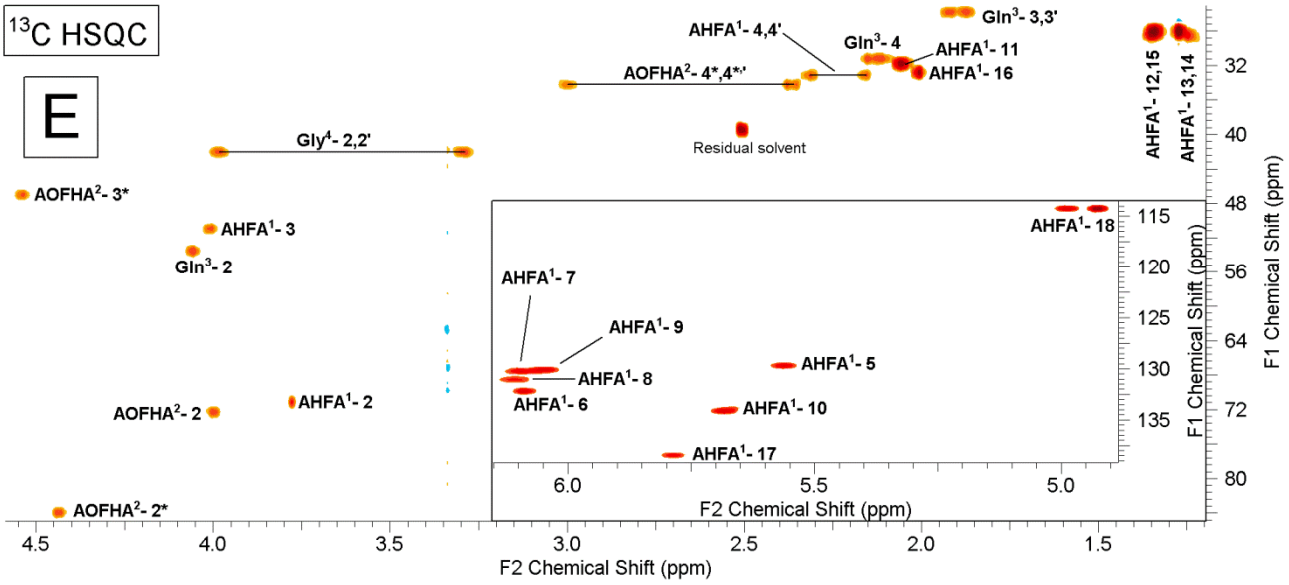


Fig. S1. Growth of *Anabaena* sp. XPORK13A and quantification of intra- and extracellular anabaenolysin. Cell growth measured with chlorophyll (µg/ml) content in relation to relative intra- and extracellular anabaenolysin C (Abl-C) content (peak area between 268 and 271 nm). Nodes represent mean values. Standard deviation is indicated at each node.


1D ^{1}H
A
^{15}N HSQC
B
Gln^{3}- NH
AOFHA^{2}- 3*-NH
Gly^{4}- NH
AHFA^{1}- NH
Gln^{3}- 5-NH$_2$
F1 Chemical...
F2 Chemical Shift (ppm)
Normalized Intensity
Chemical Shift (ppm)


C
^{1}H-^{1}H COSY
Gln^{3}- 4
Gln^{3}- 3,3'
Gln^{3}- NH
Gln^{3}- 2
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


^{1}H-^{1}H TOCSY
D
Gln^{3}- 3,3'
Gln^{3}- 4
Gln^{3}- 2
Gln^{3}- NH
Gln^{3}- NH
Gln^{3}- 2
Gln^{3}- 4
Gln^{3}- 3,3'
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


^{13}C HSQC
E
Gln^{3}- 3,3'
AHFA^{1}- 4,4'
Gln^{3}- 4
AHFA^{1}- 11
AHFA^{1}- 16
AOFHA^{2}- 4*,4*'
Residual solvent
Gly^{4}- 2,2'
AOFHA^{2}- 3*
AHFA^{1}- 3
Gln^{3}- 2
AOFHA^{2}- 2
AHFA^{1}- 2
AOFHA^{2}- 2*
AHFA^{1}- 12,15
AHFA^{1}- 13,14
AHFA^{1}- 18
AHFA^{1}- 7
AHFA^{1}- 9
AHFA^{1}- 8
AHFA^{1}- 6
AHFA^{1}- 5
AHFA^{1}- 10
AHFA^{1}- 17
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

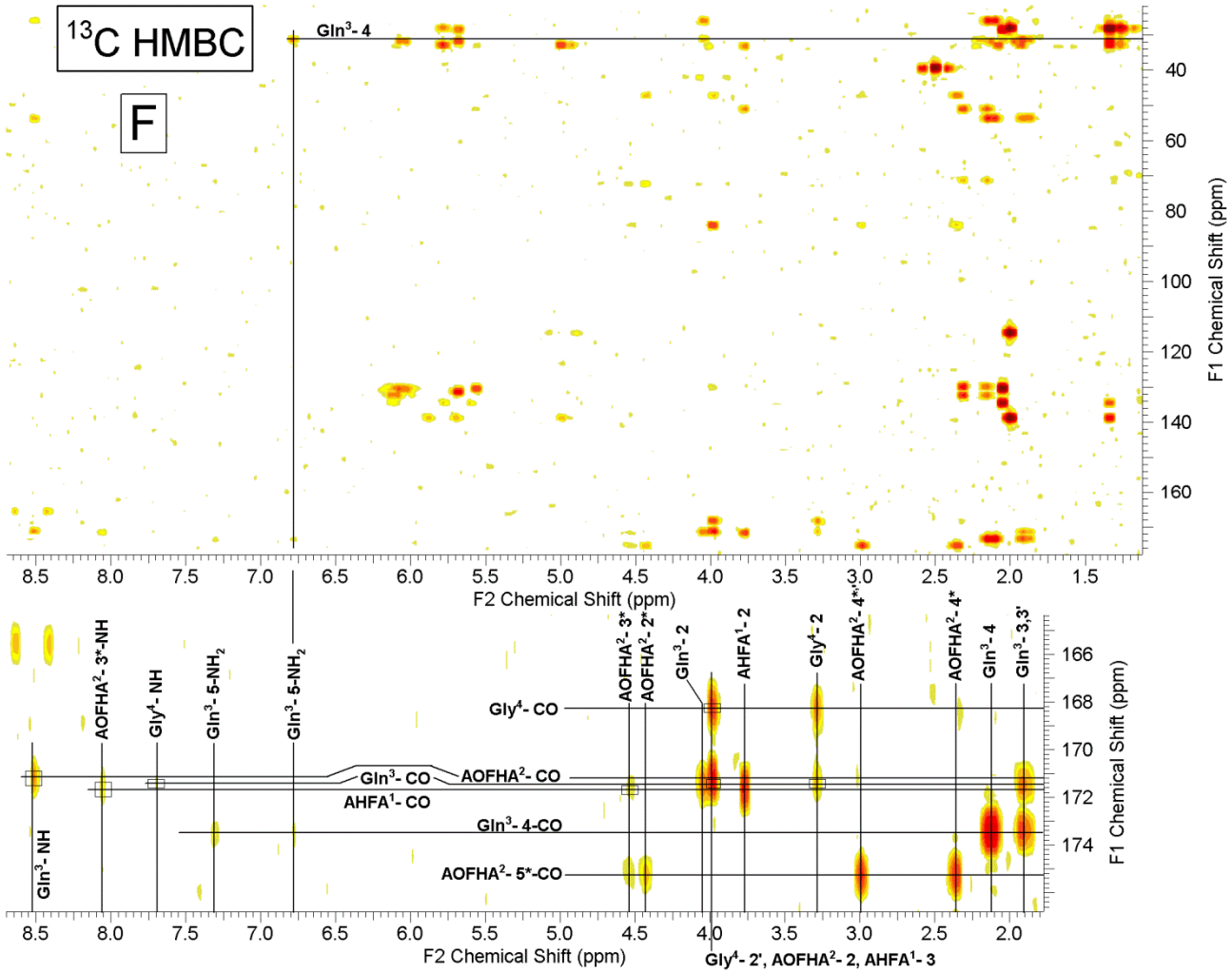


Fig. S2. NMR spectra of anabaenolysin C (1, Fig. 2). (A) ^{1}H NMR spectrum, (B) annotated ^{15}N-HSQC spectrum, (C) Gln^{3} annotated ^{1}H-^{1}H COSY spectrum, (D) ^{1}H-^{1}H TOCSY spectrum, (E) annotated ^{13}C-HSQC spectrum, (F) partially annotated ^{13}C HMBC spectrum in which boxes show the correlations between the subunits. AHFA, AOFHA; see Table S4.

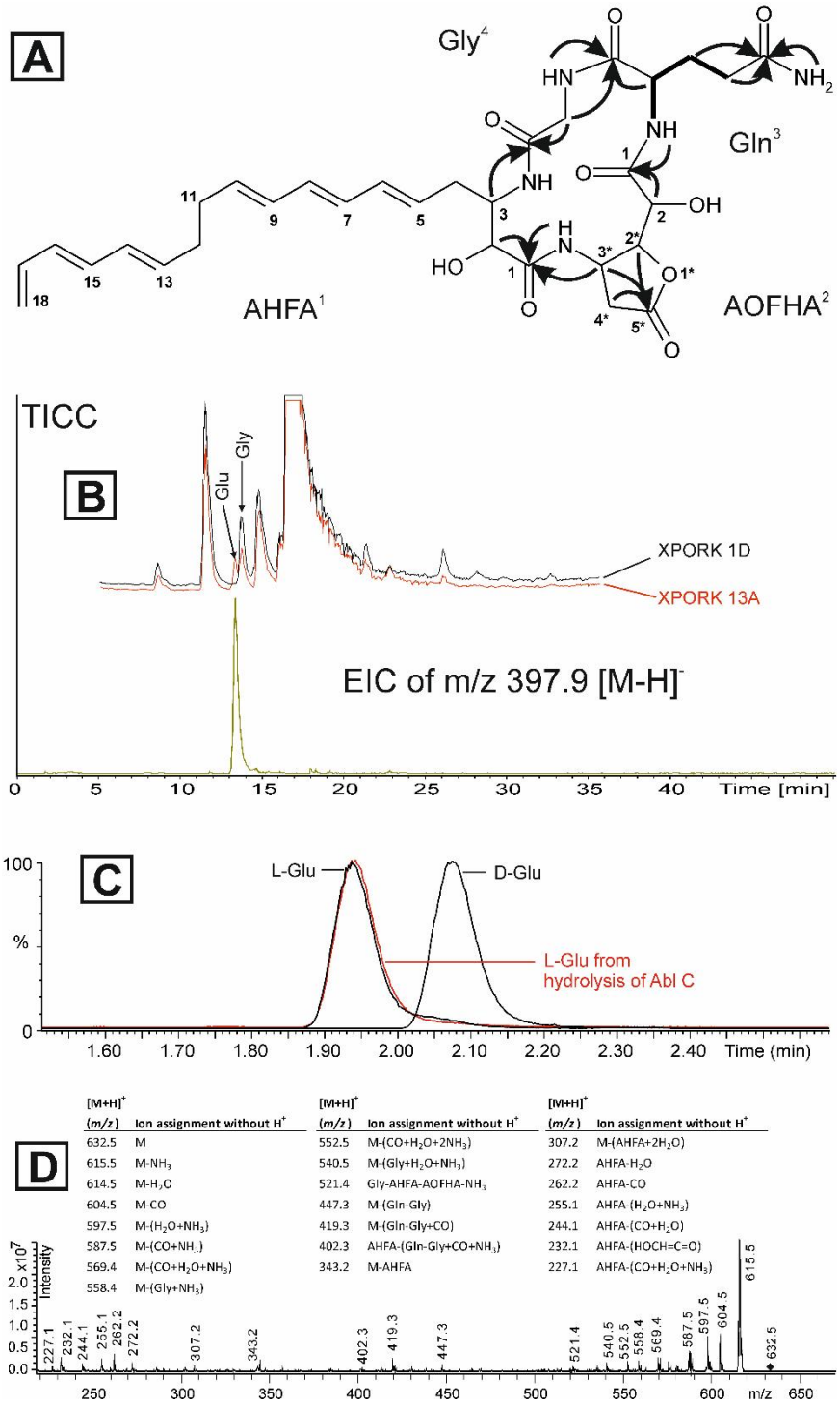


Fig. S3. Analysis to reveal the chemical structure of anabaenolysin C. (A) Structure of anabaenolysin C (**1**) with ^{1}H-^{1}H DQF-COSY for Gln^{3} (bold lines) and ^{13}C HMBC (arrows) correlations. (B) Total ion current (TICC) and extracted ion chromatogram (EIC *m/z* 397.9 corresponding to a deprotonated Marfey derivative of Glutamine) of anabaenolysin C from *Anabaena* sp. XPORK13A, and of anabaenolysin B from strain *Anabaena* sp. XPORK1D. (C) Extracted ion chromatograms (EIC *m/z* 398.0 corresponding to a deprotonated Marfey derivative of Glutamine) of L- and D-Glu standards (R_t 1.94 and 2.07 min, respectively) (Sigma) and L-Glu from acid-hydrolyzed anabaenolysin C (R_t 1.94 min) from *Anabaena* sp. XPORK13A (D) Product ion spectrum from anabaenolysin C (*m/z* 632.5) from *Anabaena* sp. XPORK13A. AHFA, AOFHA; see Table S4.

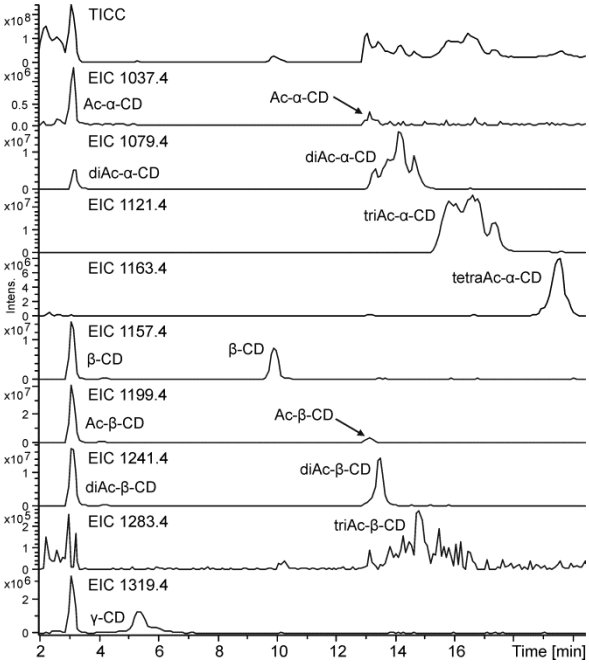


Fig. S4. Chromatograms of cyclodextrins produced by *Anabaena* sp. XSPORK2A. TICC = Total ion current chromatogram, EIC = extracted ion chromatogram of cyclodextrins (CD).

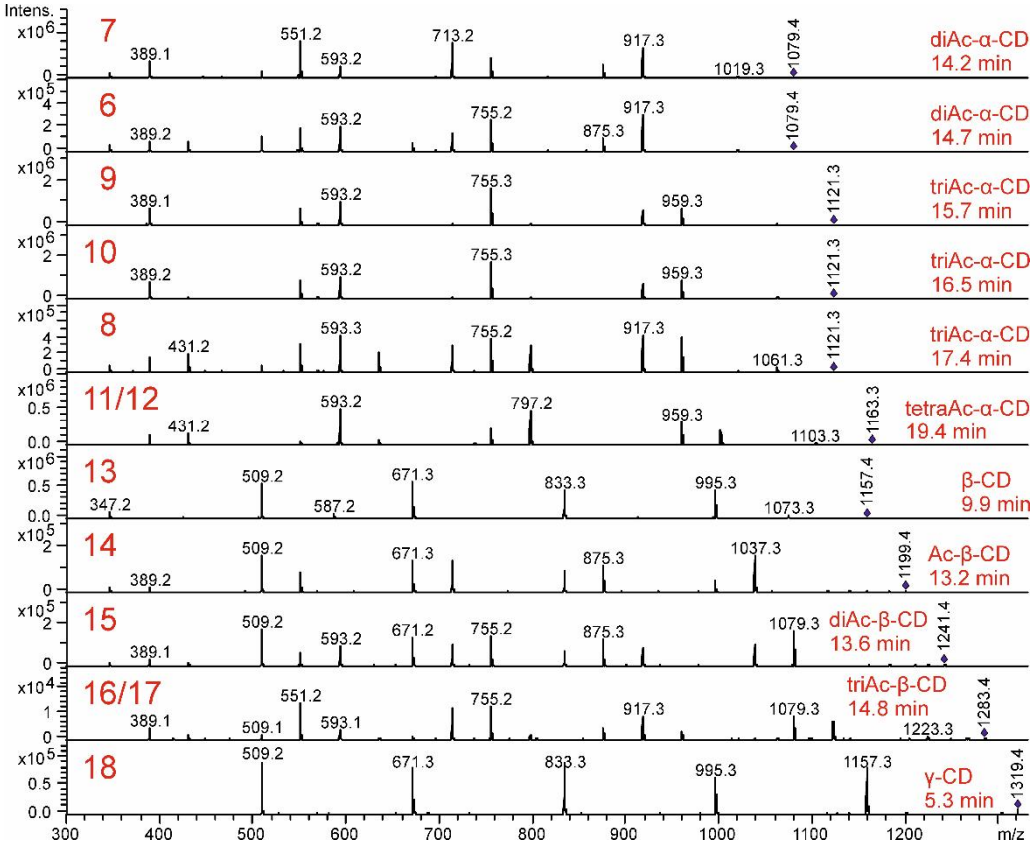
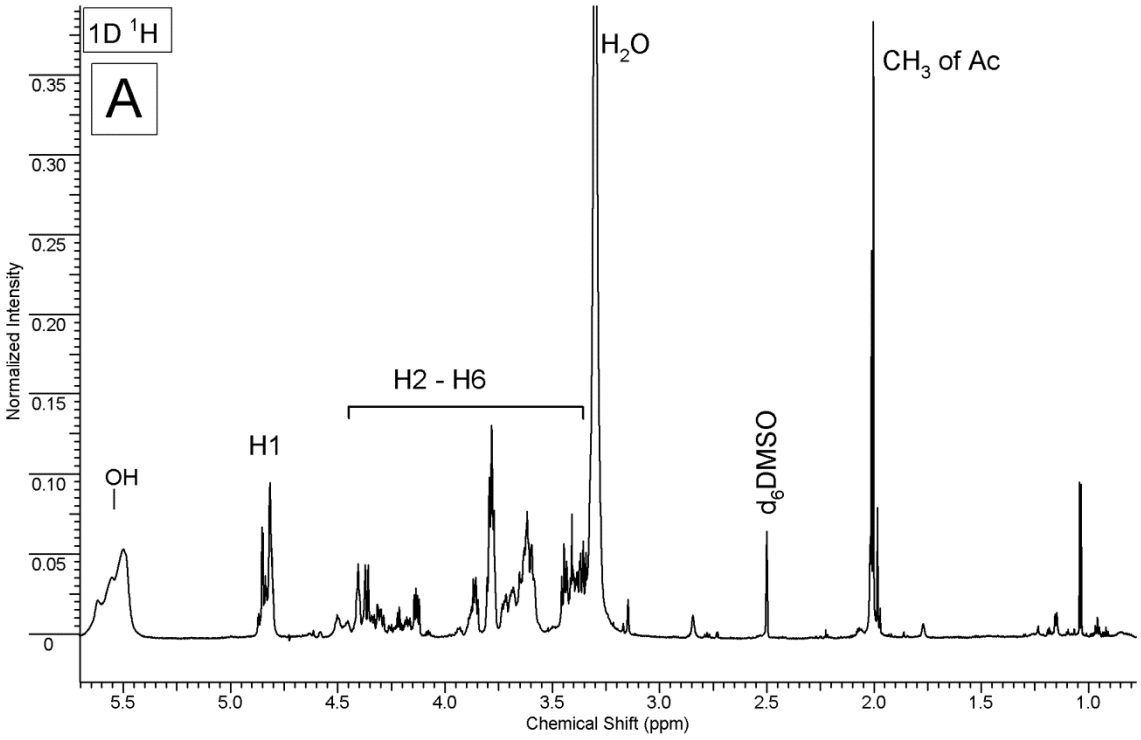
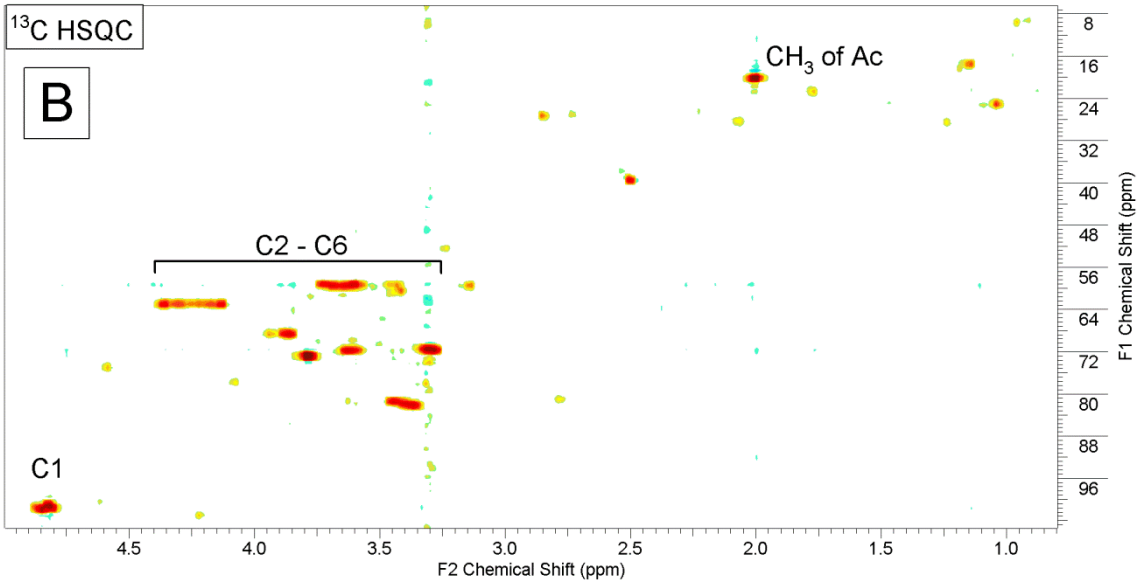


Fig. S5. Product ion spectra of protonated cyclodextrins produced by *Anabaena* sp. XSPORK2A. Retention times (min) come from the chromatograms presented in Fig. S4. See compound numbers 6 – 18 from Fig.2.


1D ^{1}H
A
H_2O
CH_3 of Ac
H2 - H6
H1
OH
d_6DMSO
Normalized Intensity
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)


^{13}C HSQC
B
CH_3 of Ac
C2 - C6
C1
8
16
24
32
40
48
56
64
72
80
88
96
F1 Chemical Shift (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F2 Chemical Shift (ppm)

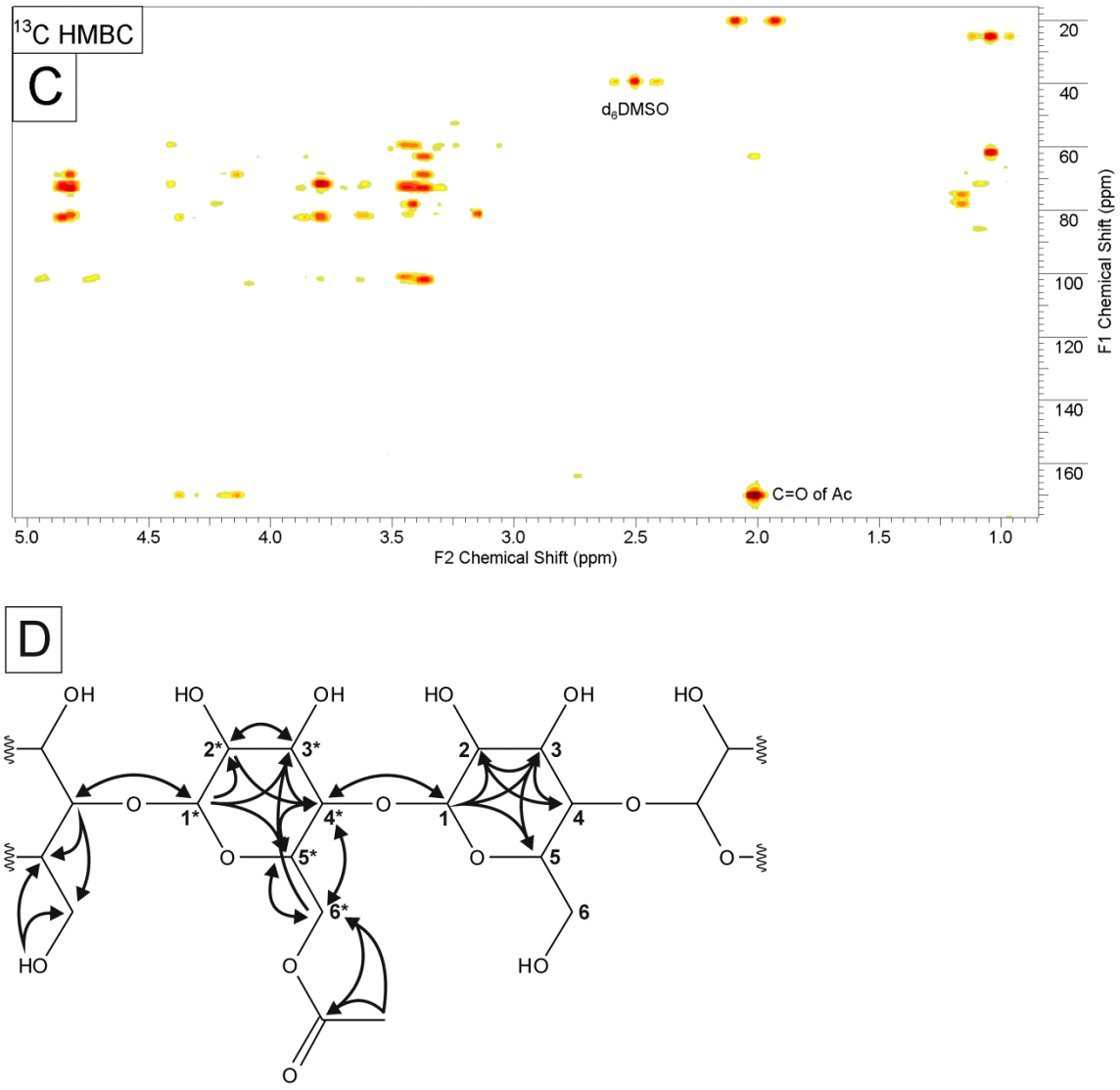


Fig. S6. NMR spectra of the cyclodextrin mixture from *Anabaena* sp. XSPORK2A and the structure with NMR correlations (See Fig. S4). ^{1}H NMR spectrum (A), ^{13}C-HSQC spectrum (B), ^{13}C HMBC (C) and partial cyclodextrin structure with ^{13}C HMBC correlations (arrows).

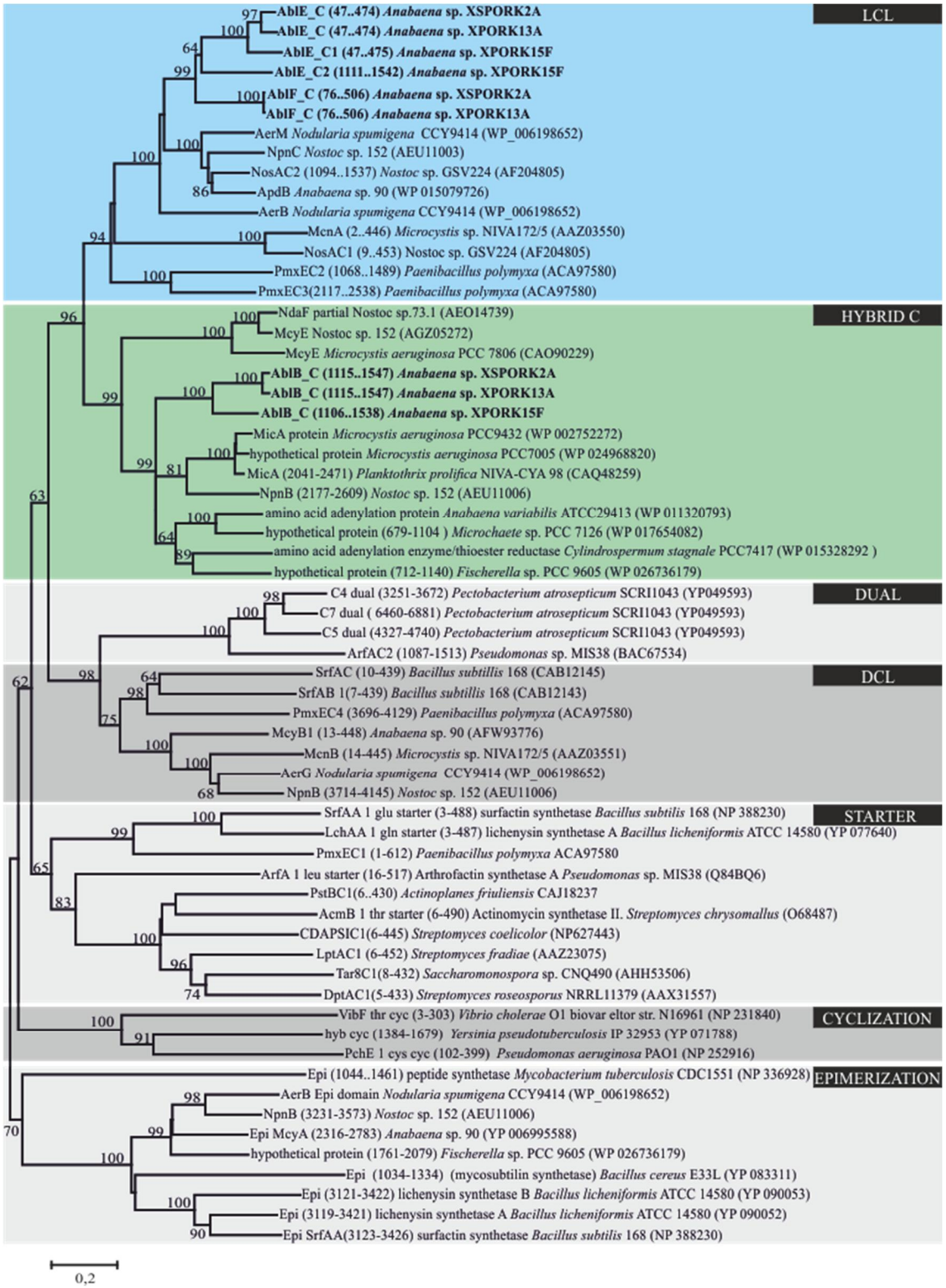


Fig. S7. Phylogenetic tree based on sequences of the condensation domains of the anabaenolysin gene cluster. Phylogenetic tree constructed using the Neighbor-joining method with 1000 bootstrap (values over 60% are indicated at the node).

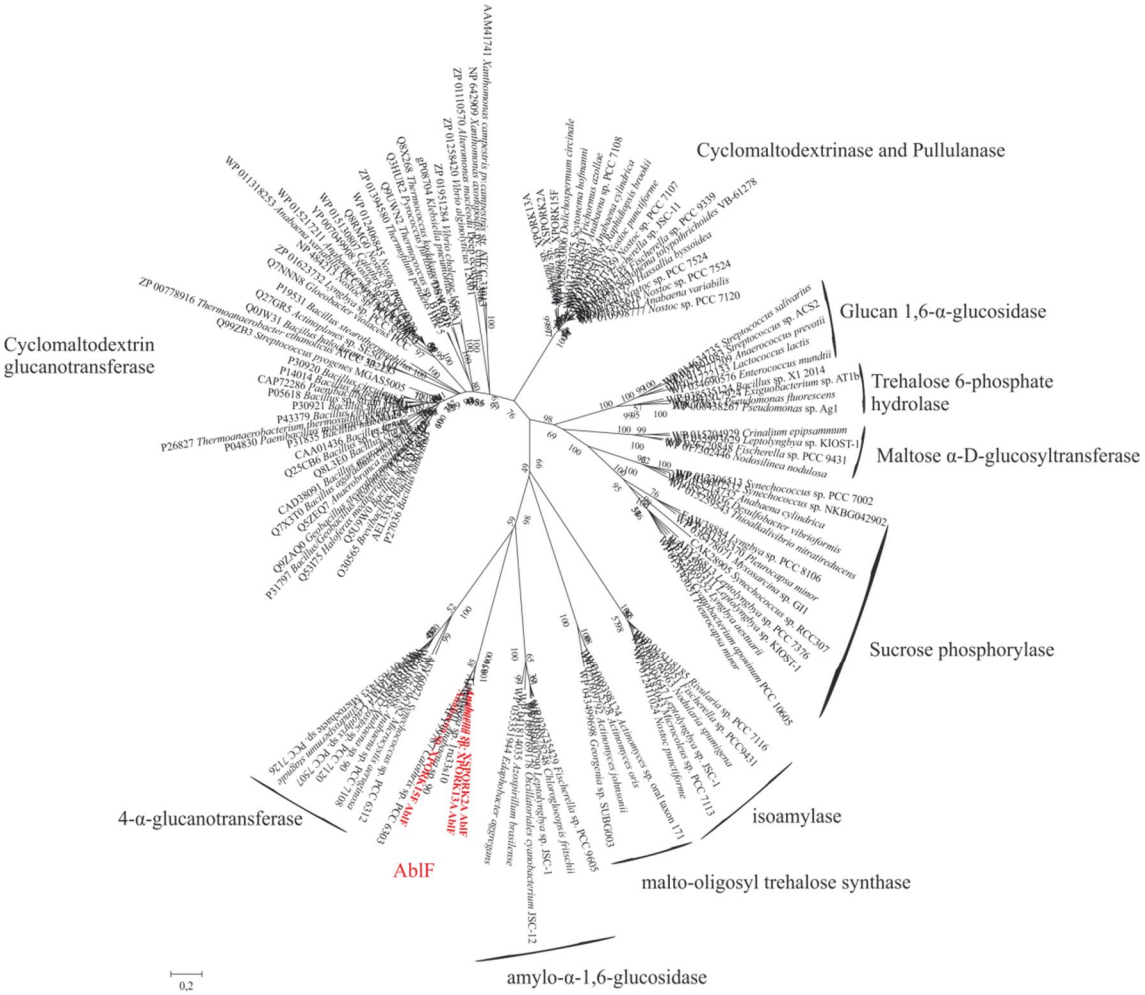


Fig. S8. Phylogenetic tree based on amino acid sequences from alpha-amylases. The phylogenetic tree was constructed using the Neighbor-joining method with 1000 bootstrap replicates, indicated at the node.

2. Supporting tables

Table S1. Strains analyzed with HPLC for the detection of anabaenolysins.

Taxon	Strain	Year	Location	Habitat
Anabaena				
Anabaena sp.	37	1985	Lake Sääksjärvi, Finland	
Anabaena sp.	318	1998	Helsinki coast, "39A", Baltic Sea	planktonic
Anabaena sp.	1TU35s12	2001	Lake Tuusulanjärvi, Finland	planktonic
Anabaena sp.	BECID19	2001	Helsinki, Vuosaari, Gulf of Finland	benthic
Anabaena sp.	BECID20	2001	Helsinki, Gulf of Finland	benthic
Anabaena sp.	BECID22	2001	Helsinki, Gulf of Finland	benthic
Anabaena sp.	BECID23	2001	Helsinki, Gulf of Finland	benthic
Anabaena sp.	BECID30	2001	Helsinki, Gulf of Finland	benthic
Anabaena sp.	BECID32	2001	Helsinki, Gulf of Finland	benthic
Anabaena sp.	BIR3	2003	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR25	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR32			
Anabaena sp.	BIR42			
Anabaena sp.	BIR 49	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR52	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR78	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR84	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR94	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR162	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR208	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR241	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR246	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR247	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR256	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR258	2004	Baltic Sea, Gulf of Finland	planktonic

Anabaena sp.	BIR260	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR348	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	BIR441	2004	Baltic Sea, Gulf of Finland	planktonic
Anabaena sp.	SYKE 658	1999	Enäjärvi. Finland	planktonic
Anabaena sp.	SYKE 748A	1999	Tuusulajärvi, Finland	
Anabaena sp.	SYKE 844A	1999	Kisakallio, Finland	
Anabaena sp.	XPORK1C	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XPORK1D	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XPORK4C	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XPORK4D	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XPORK5C	1999	Baltic Sea, Cape Porkkala, Finland	epithytic
Anabaena sp.	XPORK6A	1999	Baltic Sea, Cape Porkkala, Finland	benthic sediment
Anabaena sp.	XPORK6C	1999	Baltic Sea, Cape Porkkala, Finland	benthic sediment
Anabaena sp.	XPORK13A	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XPORK15D	1999	Baltic Sea, Cape Porkkala, Finland	benthic, epilithic
Anabaena sp.	XPORK15F	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XPORK36C	1999	Baltic Sea, Cape Porkkala, Finland	periphytic
Anabaena sp.	XPORK36D	1999	Baltic Sea, Cape Porkkala, Finland	benthic sediment
Anabaena sp.	XSPORK2A	1999	Baltic Sea, Cape Porkkala, Finland	benthic, gastropod
Anabaena sp.	XSPORK7B	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Anabaena sp.	XSPORK14D	1999	Baltic Sea, Cape Porkkala, Finland	benthic sediment
Anabaena sp.	XSPORK27C	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Aphanizomenon				
Aphanizomenon sp.	3	1985	Lake Långajön, Ahvenanmaa, Finland	
Aphanizomenon sp.	37	1985	Lake Sääskjörvi, Finland	
Aphanizomenon sp.	201	1987	Lake Bodomjärvi, Finland	
Aphanizomenon sp.	313	1997	Tuusulajarvi, Finland	
Aphanizomenon sp.	326	1998	Lake Lohjanjärvi, Finland	
Aphanizomenon sp.	0TU37S7	2000	Lake Tuusulanjärvi, Finland	

Aphanizomenon sp.	1TU26S2	2001	Lake Tuusulanjärvi, Finland	
Aphanizomenon sp.	1TU29S13	2001	Lake Tuusulanjärvi, Finland	
Aphanizomenon sp.	SYKE 741		Maarian allas, Finland	
Aphanizomenon sp.	SYKE 761		Raisio-Naantali, Finland	
Calothrix				
Calothrix sp.	336/1		Vihti, Enäjärvi Laukilanlahti, Finland	
Calothrix sp.	336/3		Vihti, Enäjärvi Laukilanlahti, Finland	benthic
Calothrix sp.	441/2		Lake Säyhteenjärvi,Finland	
Calothrix sp.	BECID1	2001	Helsinki, Matosaari, Finland	periphytic
Calothrix sp.	BECID4	2001	Baltic Sea, Gulf of Finland	Brackish water
Calothrix sp.	BECID9	2001	Baltic Sea, Gulf of Finland	benthic, epilithic
Calothrix sp.	BECID18	2001	Helsinki, Vuosaari, Gulf of Finland	benthic, sediment
Calothrix sp.	BECID26	2001	Baltic Sea, Vuosaari, Finland	benthic, epilithic
Calothrix sp.	BECID33	2001	Helsinki, Vuosaari, Gulf of Finland	benthic, epilithic
Calothrix sp.	PCC 7507	1972	near Kastanienbaum, Vierwaldstättersee, Switzerland	sphagnum bog
Calothrix sp.	PCC 7714	1969	Small pool, Aldabra atoll, India	planktonic
Calothrix sp.	PCC 7715	1964	Thermal spring, Dax, FRA	planktonic
Calothrix sp.	XPORK2B	1999	Baltic Sea, Cape Porkkala, Finland	benthic, periphytic
Calothrix sp.	XPORK11C	1999	Baltic Sea, Cape Porkkala, Finland	benthic sediment
Calothrix sp.	XSPORK3	1999	Baltic Sea, Cape Porkkala, Finland	-
Calothrix sp.	XSPORK4A	1999	Baltic Sea, Cape Porkkala, Finland	epiphytic
Calothrix sp.	XSPORK10A	1999	Baltic Sea, Cape Porkkala, Finland	benthic, epilithic
Calothrix sp.	XSPORK36C	1999	Baltic Sea, Cape Porkkala, Finland	periphytic
Cylindrospermum				
Cylindrospermum sp.	PCC 7417	1972	Greenhouse, Stockholm, Sweden	soil
Fischerella				
Fischerella sp.	PCC 7414	1979	Hot spring, New Zealand	planktonic

Fischerella sp.	SAG 1427-1	1951	Rice field, Allahabad, India	soil
Hapalosiphon				
Hapalosiphon hibernicus	BZ23-1	1984		
Nodularia				
Nodularia sp.	7804	1966	Thermal spring, Dax, France	benthic
Nodularia harveyana	B053	1992	Baltic Sea	Shallow coastal water
Nodularia sp.	BECID27	2001	Helsinki, Vuosaari, Gulf of Finland	benthic, epiphytic
Nodularia sp.	BECID29	2001	Baltic Sea, Vuosaari, Finland	benthic, epilithic
Nodularia sp.	BECID36	2002	Sipoo, Gulf of Finland	epilithic
Nodularia sp.	BY1	1986	Brakish water, Baltic Sea, bloom	planktonic
Nodularia sp.	HEM	1986	Brackish water, Baltic Sea	planktonic
Nodularia sp.	PCC 73104/1	1979	Spotted lake, Brit Columbia, Canada	soil
Nostoc				
Nostoc sp.	342/7		Kemiö, Pederså	
Nostoc sp.	ATCC 53789		Arron Island, Scotland	Lichen
Nostoc sp.	BECID2	2001	Suomenlahti, Karpinlahti, Finland	
Nostoc sp.	IO-102-I	2000	Sysmä, Finland (Pannaria pezizoides)	lichen
Nostoc sp.	UK 18 BV		Autti, Finland	lichen
Nostoc ellipsosporum	V	1990	Nezamyslice, Czech Republic	field
Nostoc calcicola	VI	1998	Dobre Pole, Czech Republic	field
Nostoc sp.	XHIID A6		Hiidenvesi, Kirkkojärvi, Finland	benthic
Nostoc sp.	XHIID C12		Hiidenvesi, Nummelanselkä, Finland	
Nostoc sp.	XPORK4A	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Nostoc sp.	XPORK5A	1999	Baltic Sea, Cape Porkkala, Finland	epithytic
Nostoc sp.	XPORK14A	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Nostoc sp.	XPORK15C	1999	Baltic Sea, Cape Porkkala, Finland	benthic
Nostoc sp.	XPORK24A	1999	Baltic Sea, Cape Porkkala, Finland	-

Nostoc sp.	XPORK24B	1999	Baltic Sea, Cape Porkkala, Finland	-
Oscillatoria				
Oscillatoria sp.	UK3		Itä-Pakila, Helsinki, Finland	lichen
Planktothrix				
Planktothrix sp.	126/8	1984	Lake Vesijärvi, Finland	
Planktothrix sp.	CYA 18	1971	Lake Gjersjøen, Norway	planktonic
Rivularia				
Rivularia sp.	BECID10	2001	Helsinki, Herttoniemi, Baltic Sea, Finland	benthic, epilithic
Rivularia sp.	BECID14	2001	Helsinki Hettoniemi, Gulf of Finland	benthic, epilithic
Rivularia sp.	XPORK3A	1999	Baltic Sea, Cape Porkkala, Finland	periphytic, benthic
Rivularia sp.	XPORK16B	1999	Baltic Sea, Cape Porkkala, Finland	benthic, epilithic
Scytonema				
Scytonema sp.	PCC 7110	1971	Limestone, Bermuda	limestone
Snowella				
Snowella sp.	0TU37S4	2000	Lake Tuusulanjärvi, Finland	
Tolypothrix				
Tolypothrix sp.	TOL328	1999	Kuopio, FI, Greenhouse	soil
Trichormus				
Trichormus doliolum	1		unknown	
Trichormus sp.	HIID B6.A	1999	Hiidenvesi, Mustionselkä, Finland	
Trichormus azollae	Kom BAI/1983	1983	Unknown	
Trichormus variabilis	Tric Von Greifswald	1992	Unknown	

unknown				
unknown	Has 1458			
unknown	HIID A18.A	1999	Hiidenvesi, Kirkkojärvi, Finland	
unknown	HIID A25.A	1999	Hiidenvesi, Kirkkojärvi, Finland	
unknown	HIID A5.A	1999	Hiidenvesi, Kirkkojärvi, Finland	
unknown	HIID A7	1999	Hiidenvesi, Kirkkojärvi Finland	
unknown	HIID B11.B	1999	Hiidenvesi, Mustionselkä, Finland	
unknown	HIID B22.A	1999	Hiidenvesi, Mustionselkä, Finland	
unknown	HIID B4.B	1999	Hiidenvesi, Mustionselkä, Finland	
unknown	335/4		Juupajoki, Kukkolahti, Finland	
unknown	P10			
unknown	SMIX5			
unknown	UHCC0003		Tuusulanjarvi, Finland	
unknown	UHCC0006		Pernajanlahti, Finland	
unknown	UHCC0007		Pernajanlahti, Finland	
unknown	UHCC0008		Vaskijärvi, Finland	
unknown	UHCC0013		Vuotiainen, Finland	
unknown	UHCC0020			
unknown	UHCC0021			
unknown	UHCC0023			
unknown	UHCC019			
unknown	XHIID B16.A			
unknown	XHIID C1		Hiidenvesi, Nummelanselkä, Finland	benthic
unknown	XHIID D14		Hiidenvesi, Kiihtelyksenselkä, Finland	benthic
unknown	XHIID D4		Hiidenvesi, Kiihtelyksenselkä, Finland	
unknown	XHIID D8		Hiidenvesi, Kiihtelyksenselkä, Finland	
unknown	XPORK2C	1999	Baltic Sea, Cape Porkkala, Finland	gastropod
unknown	XPORK14F	1999	Baltic Sea, Cape Porkkala, Finland	benthic
unknown	XSPORK14A	1999	Baltic Sea, Cape Porkkala, Finland	benthic sediment
unknown	XSPORK15B	1999	Baltic Sea, Cape Porkkala, Finland	benthic

unknown	XSPORK15C	1999	Baltic Sea, Cape Porkkala, Finland	benthic
unknown	XSPORK20A	1999	Baltic Sea, Cape Porkkala, Finland	benthic
unknown	XSPORK22A	1999	Baltic Sea, Cape Porkkala, Finland	benthic
unknown	XSPORK24A	1999	Baltic Sea, Cape Porkkala, Finland	benthic
unknown	XSPORK24B	1999	Baltic Sea, Cape Porkkala, Finland	-
unknown	XSPORK34A	1999	Baltic Sea, Cape Porkkala, Finland	periphytic

Table S2. Growth inhibition (mm) of selected mold and yeast species by crude extracts of anabaenolysin-producing *Anabaena* spp. assessed with standard disk diffusion assays. No inhibition is marked with a minus (-).

Strain	Compound	Amount (µg)	*Candida albicans* HAMBI 261	*Aspergillus flavus* HAMBI 829	*Aspergillus parasiticus* HAMBI 827
XPORK1D	Abl-B	37.5	18	12	10
XPORK1C	Abl-B	37.5	12	18	20
XSPORK2A	Abl-B	37.5	20	-	-
XPORK6C	Abl-B	37.5	16	14	14
XPORK13A	Abl-C	Nd	6	10	10
XPORK15F	Abl-A	25.0	10	10	8
XSPORK27C	Abl-B	37.5	20	14	16
XPORK36D	Abl-B	37.5	12	14	14

Nd: not determined.

Table S3. Calculated and experimental accurate masses of anabaenolysin variants A, B and C from *Anabaena* strains, mass error in ppm and calculated formulas.

		$[M+H]^+$ (*m/z*)		Error	
Strain	**Abl**	**Calc**	**Exp**	**(ppm)**	**Formula (M)**
XPORK15F	A	559.2762	559.2761	-0.34	$C_{28}H_{38}N_4O_8$
BECID 22	A	559.2762	559.2757	-1.05	$C_{28}H_{38}N_4O_8$
XPORK1C	B	561.2919	561.2923	0.64	$C_{28}H_{40}N_4O_8$
XPORK1D	B	561.2919	561.2917	-0.43	$C_{28}H_{40}N_4O_8$
XPORK4C	B	561.2919	561.2916	-0.60	$C_{28}H_{40}N_4O_8$
XPORK6C	B	561.2919	561.2916	-0.60	$C_{28}H_{40}N_4O_8$
XPORK36D	B	561.2919	561.2921	0.29	$C_{28}H_{40}N_4O_8$
XSPORK2A	B	561.2919	561.2919	-0.07	$C_{28}H_{40}N_4O_8$
XSPORK27C	B	561.2919	561.2917	-0.43	$C_{28}H_{40}N_4O_8$
BECID30	B	561.2919	561.2915	-0.78	$C_{28}H_{40}N_4O_8$
XPORK13A	C	632.3290	632.3289	-0.24	$C_{31}H_{45}N_5O_9$
XPORK13A	D	634,3446	634.3434	-2.05	$C_{31}H_{47}N_5O_9$

Table S4. ^{1}H and ^{13}C NMR data for anabaenolysin C (1) from *Anabaena* sp. XPORK13A in d_6-DMSO.

Substructure	C/H no	δC/δN	δH	multip.; *J*(Hz)
AHFA[1]	CO	171.8	-	
	2	71.2	3.77	
	2-OH	-	-	
	3	51.0	4.00	
	3-NH	125.4	7.41	d (8.5)
	4	33.2	2.16	
	4'	-	2.31	ddd (7.2, 7.2, 14.6)
	5	129.7	5.56	(7.2)
	6	132.3	6.09	
	7	130.3	6.09	
	8	131.2	6.11	
	9	130.2	6.05	dd (9.9, 14.7)
	10	134.2	5.68	dt (7.3, 14.7)
	11	31.8	2.05	dt (6.7, 7.3)
	12	28.3	1.34	
	13	28.0	1.26	
	14	28.0	1.26	
	15	28.1	1.33	
	16	32.9	2.00	dt (7.0, 7.3)
	17	138.5	5.79	ddt (6.6, 10.3, 17.1)
	18	114.4	4.93	dd (1.8, 10.3)
			4.99	dd (1.8, 17.1)
AOFHA[2]	CO	171.2	-	
	2-OH	-	-	
	2	72.3	3.99	
	2*	84.0	4.43	dd (9.7, 3.9)
	3*	47.1	4.53	m
	3*-NH	128.6	8.05	d (5.5)
	4*	34.4	2.36	q(4.4, 18.0)
	4*, '		2.99	q(9.9, 17.6)
	5*-CO	175.2	-	
Gln[3]	CO	171.6	-	
	2	53.6	4.04	dt (7.3, 7.3)
	3	25.9	1.87	m
	3'		1.91	
	4	31,3	2.10, 2.14	
	4-CO	173.5	-	
	5-NH_2	116.5	6.78, 7.30	s, s
	NH	127.4	8.51	d (7.0)
Gly[4]	CO	168.3	-	
	2	42.1	3.28	dd (3.3, 15.4)
	2'	-	3.98	

	NH	111.3	7.71	br

AOFHA = (3-amino-5-oxotetrahydrofuran-2-yl)(hydroxy)acetic acid

AHFA = (5*E*,7*E*,9*E*)-3-amino-2-hydroxyoctadeca-5,7,9,17-tetraenoic acid

Table S5. Calculated and measured product ion intensities (%) of sodiated diAc-α-CD regioisomers AB, AC and AD; triAc-α-CD regioisomers ABC, ACD and ACE; tetraAc-α-CD regioisomers ABCD, ABCE and ABDE; diAc-β-CD regioisomers AB, AC and AD and triAc-β-CD regioisomers ABC, ABD, ABE and ACE. Glucose units A–G presented in Fig. 2. The most important characteristic intensities are highlighted with grey. CD = cyclodextrin, AC = acetyl.

			Calculated and measured (compounds 6-17) product ion intensities of sodiated α- and β-cyclodextrin (CD) isomers																					
			diAc-α-CD					triAc-α-CD						tetraAc-α-CD				diAc-β-CD				triAc-β-CD				
Compound No:						7	6				9	10	8				11/12				15					16/17
No of		[M+Na]$^+$	Calculated					Calculated						Calculated				Calculated				Calculated				
Glc	Ac	(*m/z*)	AB	AC	AD	AC	AB	ABC	ACD	ACE	ACE	ACE	ABC	ABCD	ABCE	ABDE	ABCE/ABDE	AB	AC	AD	AB	ABC	ABD	ABE	ACE	ABD/ABE
6	0	995																0	0	0	0	0	0	0	0	0
	1	1037																29	29	29	37	0	0	0	0	3
	2	1079																71	71	71	62	43	43	43	43	54
	3	1121																				57	57	57	57	43
5	0	833	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	25					
	1	875	33	33	33	31	27	0	0	0	0	0	0	0	0	0	0	29	57	57	44	0	0	0	0	0
	2	917	67	67	67	69	73	50	50	50	49	45	51	0	0	0	0	57	43	43	30	29	14	14	0	27
	3	959						50	50	50	51	55	49	67	67	67	62					29	57	57	86	54
	4													33	33	33	38					43	29	29	14	19
4	0	671	17	0	0	1	14	0	0	0	0	0	0	0	0	0	0	29	14	0	36					
	1	713	33	67	67	63	30	33	17	0	2	5	31	0	0	0	0	29	57	86	28	14	0	0	0	4
	2	755	50	33	33	36	56	33	67	100	97	91	39	50	33	33	31	43	29	14	36	29	43	29	29	43
	3	797						33	17	0	1	4	30	33	67	67	68					29	43	71	71	47
	4													17	0	0	1					29	14	0	0	7
3	0	509	33	17	0	12	23	17	0	0	0	0	7	0	0	0	0	43	29	14	53					
	1	551	33	67	100	66	38	33	50	50	41	46	32	33	17	0	6	29	57	86	18	29	14	0	0	9
	2	593	33	17	0	21	40	33	50	50	58	53	40	33	67	100	82	29	14	0	29	29	43	71	71	69
	3	635						17	0	0	0	0	21	33	17	0	11					29	43	29	29	18

	4													0	0	0	0					14	0	0	0	4
2	0	347	50	33	33	22	22	33	17	0	0	2	17	17	0	0	1	57	43	43	23					
	1	389	33	67	67	77	40	33	67	100	98	93	38	33	67	67	49	29	57	57	52	43	29	29	14	0[a]
	2	431	17	0	0	1	37	33	17	0	2	5	44	50	33	33	50	14	0	0	26	29	57	57	86	71
	3	473						0	0	0	0	0	0	0	0	0	0					29	14	14	0	29
	4													0	0	0	0					0	0	0	0	0

[a] = Poduct ions ≈ 27% smaller than the parent ion are unstable in the ion trap.

Table S6. ^{1}H and ^{13}C NMR data for the cyclodextrin mixture from *Anabaena* sp. XSPORK2A in d_6-DMSO.

No	δC	δH	mult., J (Hz)	HMBC (H → C)
1	101.8	4.85	d, 4.8	1, 2, 3, 4*, 5
2, 2*	71.6	3.31		3
3, 3*	72.9	3.79		1, 1*, 2, 2*, 4, 4*, 5, 5*
4	81.6	3.45		1*, 2, 3, 5, 6
5	71.8	3.62		1, 3, 4
6	59.5	3.62		4, 5
6'		3.69		3
OH-2, 2*, 3, 3*	-	5.50 5.55 5.62		-
OH-6	-	4.40		5, 6
1*	101.3	4.82	d, 3.3	1*, 4, 2*, 3*, 5*
4*	82.2	3.37		1, 3*, 5*, 6*
5*	68.7	3.87		4*, 6*
6*	63.0	4.16		4*, 5*, 7*
6*,'		4.34		4*, 5*, 7*
7*	171.1	-		-
8*	20.2	2.00 2.01 2.02	s	7*, 8*

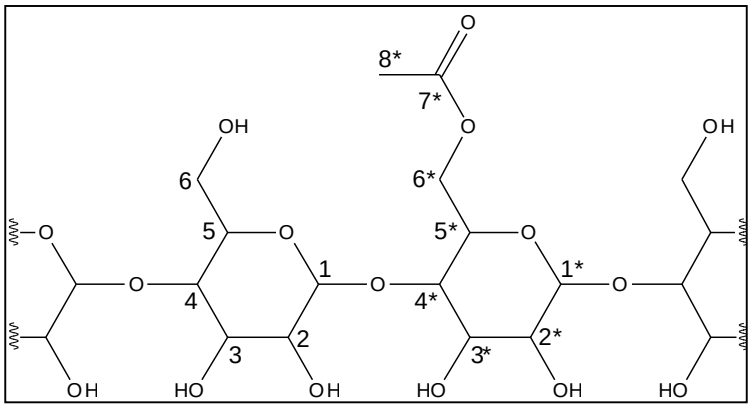


Table S7. Prediction of amino acids selected and activated by the adenylation domains in the anabaenolysin gene cluster.

Strain	NRPS module	Substrate specificity code[a]										Predicted substrate	Similarity (%)
		235	236	239	278	299	301	322	330	331	517		
XSPORK2A	AblA	D	L	T	K	I	G	H	V	G	K	Asx[b]	90
	AblD	D	I	L	Q	L	G	V	I	W	K	Gly	100
	AblE	D	I	L	Q	L	G	V	I	W	K	Gly	100
XPORK13A	AblA	D	L	T	K	I	G	H	V	G	K	Asx[b]	90
	AblD	D	A	W	Q	F	G	L	I	D	K	Gln	100
	AblE	D	I	L	Q	L	G	V	V	W	K	Gly	90
XPORK15F	AblA	D	L	T	K	I	G	H	V	G	K	Asx[b]	90
	AblD1	D	I	L	Q	L	G	L	I	W	K	Gly	100
	AblD2	D	I	L	Q	L	G	L	I	W	K	Gly	100

[a] Based on Stachelhaus et al. [45].
[b] Asx: prediction using NRPSpredictor2 (Asn) and PKS/NRPS Analysis (Asp) programs.

Table S8. Comparison of the first hit from BLASTp in the NCBI database of amino acids sequences from *Anabaena* strains XSPORK2A, XPORK13A and XPORK15F.

Protein		Amino acids	Predicted function	Sequence similarity
1	XSPORK2A	1048	transmembrane sensor-like protein	histidine kinase [*Nostoc* sp. PCC 7120]
	XPORK13A	558		Serine/threonine protein kinase [*Aphanizomenon flos-aquae* 2012/KM/D3]
	XPORK15F	253	Unknown function	hypothetical protein [*Cylindrospermum stagnale* PCC 7417]
2	XSPORK2A	255	hypothetical protein	hypothetical protein [*Anabaena variabilis* ATCC29413]
	XPORK13A	340		Phosphoribosylpyrophosphate synthetase [*Aphanizemonon flos-aquae* 2012/KM/D3]
	XPORK15F	522	ABC transporter	DNA repair protein RadA [*Aphanizomenon flos-aquae* NIES-81]
3	XSPORK2A	468	Lysin/chitinase	Lysin [*Tolypothrix bouteillei*]/putative chitinase [*Leptolyngbya* sp. PCC 6406]
	XPORK13A	190		Metal dependent phosphohydrolase [*Anabaena* sp. 90]
	XPORK15F	666	DNA helicase	hypothetical protein [*Acidobacterium capsulatum*]
4	XSPORK2A	111	hypothetical protein	chlorohydrolase [*Campylobacter cuniculorum* DSM 23162]
	XPORK13A	124	Unknown function	hypothetical protein [*Cylindrospermum stagnale* PCC 7417]

	XPORK15F	788	Unknown function	hypothetical protein [*Acidobacterium capsulatum*]
5	XSPORK2A	87	hypothetical protein	Peptidase M15 [*Leptolyngbya* sp. Heron Island J]
	XPORK13A	182	Unknown function	hypothetical protein [*Geitlerinema* sp. PCC 7105]
	XPORK15F	132	Cell mobility	PIN domain containing protein [Nostocaceae multispecies]
6	XSPORK2A	559	Transposase	Transposase [*Aphanizomenon flos-aquae* 2012/KM/D3]
	XPORK13A	137	Unknown function	hypothetical protein [*Cylindrospermum stagnale* PCC 7417]
	XPORK15F	98	Unknown function	hypothetical protein [*Nostocaceae* multispecies]
7	XSPORK2A	377	peptidoglycan binding protein	hypothetical protein [*Synechococcus* sp. PCC 7335].(peptidoglycan-binding protein)
	XPORK13A	1903	Unknown function	hypothetical protein [*Cylindrospermum stagnale* PCC 7417]
	XPORK15F	238	D-alaD-ala ligase	D-alanine-D-alanine ligase [*Dolichospermum circinale* AWQC310F]
CamA	XSPORK2A	669	Unknown function (SLH-SLH-glycoside hydrolase)	hypothetical protein [*Aphanizomenon flos-aquae* 2012/KM/D3]

	XPORK13A	669	Unknown function (SLH-SLH-glycoside hydrolase)	hypothetical protein [*Aphanizomenon flos-aquae* 2012/KM/D3]
	XPORK15F	678	Unknown function (SLH-SLH-glycoside hydrolase)	hypothetical protein [*Nostoc* sp. PCC 7120]
NRPS (AblA)	XSPORK2A	2477	NRPS	NpnB [*Nostoc* sp. 152]
	XPORK13A	2477	NRPS	NpnB [*Nostoc* sp. 152]
	XPORK15F	2468	NRPS	NpnB [*Nostoc* sp. 152]
PKS(AblB)	XSPORK2A	1542	PKS	polyketide synthase [*Mastigocoleus testarum* BC008]
	XPORK13A	1542	PKS	polyketide synthase [*Mastigocoleus testarum* BC008]
	XPORK15F	1552	PKS	polyketide synthase [*Mastigocoleus testarum* BC008]
FADS(AblC)	XSPORK2A	316	fatty-acid desaturase	fatty-acid desaturase [*Pleurocapsa minor* PCC 7327]
	XPORK13A	316	fatty-acid desaturase	fatty-acid desaturase [*Pleurocapsa minor* PCC 7327]
	XPORK15F	307	fatty-acid desaturase	fatty-acid desaturase [*Pleurocapsa minor* PCC 7327]
NRPS (AblD)	XSPORK2A	1108	NRPS	NosC [*Nostoc* sp. GSV224]

	XPORK13A	1108	NRPS	Amino acid adenylation domain [*Morea producens* 3L]
	XPORK15F	2442	NRPS	amino acid adenylation enzyme/thioester reductase family protein [*Leptolyngbya* sp. PCC7375]
NRPS (AblE)	XSPORK2A	1396	NRPS	amino acid adenylation enzyme/thioester reductase family protein [Leptolyngbya sp. PCC 7375]
	XPORK13A	1396	NRPS	amino acid adenylation enzyme/thioester reductase family protein [*Leptolyngbya* sp. PCC7375]
FADS (AblF)	XPORK15F	318	fatty-acid desaturase	fatty-acid desaturase [*Pleurocapsa minor* PCC 7327]
14	XSPORK2A	578	DNA repair protein	DNA repair protein RecN [*Anabaena* sp. 90]
	XPORK13A	578	DNA repair protein	DNA repair protein RecN [*Anabaena* sp. 90]
	XPORK15F	574	DNA repair protein	DNA repair protein RecN [*Trichormus azollae*]
15	XSPORK2A	573	Response regulator	response regulator receiver modulated diguanylate cyclase/phosphodiesterase [*Anabaena cylindrica* PCC 7122]
	XPORK13A	573	Response regulator	Two-component response regulator [*Anabaena* sp. 90]
	XPORK15F	743	Glycoside hydrolase	Glycoside hydrolase [*Aphanizomenon flos-aquae* 2012/KM/D3]
16	XSPORK2A	363	Response regulator	two-component response regulator [*Anabaena* sp. 90]
	XPORK13A	363	Response regulator	two-component response regulator [*Anabaena* sp. 90]

	XPORK15F	82	NifU	Nitrogen-fixing protein NifU [*Raphidiopsis brookii* D9]
17	XSPORK2A	404	Response regulator	hypothetical protein [*Dolichospermum circinale* AWQC310F]
	XPORK13A	404	Response regulator	hypothetical protein [*Dolichospermum circinale* AWQC310F]
	XPORK15F	1172	Filam. hemagglutinin	Filamentous hemagglutinin family domain-containing protein [*Micrococcus* sp. PCC 7113]
18	XSPORK2A	772	Response regulator	hypothetical protein [*Mastigocoleus testarum* BC008]
	XPORK13A	772	Response regulator	hypothetical protein [*Mastigocladus testarum* BC008]
	XPORK15F	960	Unknown function	Hypothetical protein [*Anabaena* sp. PCC 7108]

Table S9. BLASTn and BLASTp identities between *Anabaena* sp. XSPORK2A and strains XPORK13A and XPORK15F.

	Nucleotide (Coverage/identity)		Amino acid (Coverage/identity)	
Genes or proteins	**XSPORK2A/XPORK13A**	**XSPORK2A/XPORK15F**	**XSPORK2A/XPORK13A**	**XSPORK2A/XPORK15F**
CamA (Hypothetical protein¤)	100/99	98/76*	100/99	99/72
AblA (NRPS)	100/98	39/78	100/99	99/73
AblB (PKS)	100/98	99/75	100/99	99/72
AblC (FADS)	100/99	86/73*	100/99	93/60
AblD (NRPS)	79/90	95/80	100/76	98/75
AblE (NRPS)	100/99	93/79	100/98	97/72
AblF (FADS)	-	79/73	-	100/55

¤ SLH-SLH-glycoside hydrolase

*BLASTn optimized to show somewhat similar sequences. Other comparisons were optimized for highly similar sequences.

Table S10. Primers used for cycle sequencing the 16S rRNA gene.

Primer	Sequence (5' to 3')	Direction	Locus[a]	Reference
27F	AGAGTTTGATCMTGGCTCAG	forward	27 - 47	[46]
pC	CTACGGGAGGCAGCAGTGGG	reverse	341-361	[47]
CYA359F	GGGGAATYTTCCGCAATGGY	forward	359 – 378	[48]
16S544R	ATTCCGGATAACGCTTGC	reverse	544 - 562	[49]
CYA781Ra	GACTACTGGGGTATCTAATCCCATT	reverse	781 – 805	[48]
pE	AAACTCAAAGGAATTGACGG	forward	908 - 928	[46]
pE'	CCGTCAATTCCTTTGAGTTT	reverse	928 - 908	[46]
16S979F	CGATGCAACGCGAAGAAC	forward	979 - 998	[49]
pF	CATGGCTGTCGTCAGCTCGT	forward	1053 - 1073	[47]
pF'	ACGAGCTGACGACAGCCATG	reverse	1073 - 1053	[47]
16S1092R	GCGCTCGTTGCGGGACTT	reverse	1092 - 1110	[49]
pG'	ACGGGCGGTGTGTAC	reverse	1407 - 1392	[46]
1494Rc	TACGGCTACCTTGTTACGAC	reverse	1494 - 1513	[50]
pH'	AAGGAGGTGATCCAGCCGCA	reverse	1542 - 1522	[47]
B23S	CTTCGCCTCTGTGTGCCTAGGT	reverse	23S	[51]
M13F (-20)	GTAAAACGACGGCCAG	forward	plasmid	commercial[b]
M13R	CAGGAAACAGCTATGAC	reverse	plasmid	commercial[b]

[a] Positions in the 16S rRNA gene according to *E.coli* 16S rRNA nucleotide numbering; [b] sequences and primers as provided in the TOPO® TA Cloning® kit (Invitrogen, Life Technologies, Carslbad, United States)

3. Supporting Materials and Methods

Cyanobacterial culture, DNA extraction and sequencing

Cyanobacterial strains (SI Appendix, Table S1) were grown in Z8 medium with or without a nitrogen source [52] under constant light (8 -14 µmol $m^{-2}s^{-1}$) at 20 to 25°C for 20 to 40 days. Strains containing heterocyst are able to fix atmospheric nitrogen and have been isolated in Z8 medium lacking a combined nitrogen source. The genomic DNA was obtained using either the E.Z.N.A.® SP Plant DNA kit (Omega Bio-tek) or the DNeasy Plant Mini Kit (Qiagen Gmbh). The conditions used for amplification of the 16S rRNA sequences, as well as cloning and cycle sequencing primers [SI Appendix, Table S10] were as follows: Amplified fragments of the 16S rRNA gene were obtained with a PCR reaction containing one time Buffer solution (DyNAzyme™ PCR buffer, Finnzymes, Thermo Fischer Scientific), 0.5 µM of forward primer 27F and 0.5 µM of reverse primer B23S (Table S10), 200 µM of each dNTP, 0.5 U of Taq polymerase (DyNAzyme™ II DNA polymerase, Finnzymes), sterile water and 20 ng of template DNA in a total volume of 20 µl. The conditions of the PCR were as follows: Initial denaturation at 94°C for 5 min, 38 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 2 min, and a final extension at 72°C for 15 min. Amplified fragments of 16S rRNA gene were purified (E.Z.N.A.® MicroElute Cycle-Pure kit, Omega Bio-tek) ligated into a pCR®2.1-TOPO® plasmid and transformed into One Shot®TOPO® Chemically competent *Escherichia coli* (TOPO® TA Cloning® kit, Invitrogen). Plasmids containing the 16S rRNA gene were extracted using the QIAprep Spin Miniprep Kit (Qiagen Gmbh) and used in a sequencing PCR reaction containing a BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, Life Technologies) and primers (SI Appendix, Table S10). The contigs were the analyzed and assembled using the Phred/Phrap/Consed package (Philip Green, University of Washington, Seatle, USA). A phylogenetic tree based on 16S rRNA genes was constructed using

neighbor-joining (Kimura 2 parameter model with gamma distribution), maximum parsimony (Close-Neighbor-Interchange (CNI) on random trees) and maximum likelihood (Kimura 2-parameter model and Nearest-Neighbor-Interchange (NNI) with gamma distribution and invariant sites G+I) methods in Molecular Evolutionary Genetic Analysis (MEGA) 5 [53].

Genome sequencing

Anabaena spp. XSPORK2A, XPORK13A and XPORK15F were grown in Z8 with no nitrogen source at 18°C under constant light and shaking at 100 RPM or bubbling with sterile air. The *Anabaena* spp. cells were collected by centrifugation at 7,000g for 7 minutes and the genomic DNA for partial genome sequencing was obtained using the phenol-chloroform method [54, 55]. Isolated DNA from the XPORK15F, XPORK13A and XSPORK2A strains were checked using a NanoDrop 1000 spectrophotometer (Thermo Scientific) to measure the concentration and an Agilent TapeStation (Agilent Technologies) to assess the quality. XSPORK2A DNA was converted in the indexed Illumina library (Illumina TruSeq® Nano DNASample Preparation Guide), and XPORK15F and XSPORK2A strains were also prepared for 454 Paired Ends sequencing according to the 3 Kb library preparation procedure. Prior to sequencing, libraries were quantitated by Real Time PCR (qPCR) by KAPA Library Quant Kits (KAPA Biosystems) and qualitatively checked with an Agilent TapeStation. XPORK15F and XPORK13A libraries were sequenced using the Illumina MiSeq platform, with a paired ends 300 cycles run while the library XSPORK2A was sequenced on the Illumina GAIIx platform using the paired ends 85 cycles run. The 454 libraries were sequenced using the Roche/454 GS-FLX pyrosequencer.

Anabaenolysin gene cluster

After Roche/454 and Illumina sequencing, the reads for each strain were assembled in contigs using Newbler software (version 3.0). The different contigs were analyzed to detect possible NRPS and PKS biosynthetic gene clusters with antiSMASH [56] and 2metDB [57]. The function of the proteins was predicted using BLASTp and the Conserved Domain Search in the NCBI. The prediction for the amino acid incorporated by the adenylation domains within the anabaenolysin gene cluster was obtained using NRPSpredictor2 [58] and PKS/NRPS Analysis [57].

Anabaenolysin and cyclodextrin detection

Cyanobacterial strains were screened for the production of anabaenolysin with HPLC (Agilent 1100 Series, Agilent Technologies). 170 to 900 mg of cell mass was extracted with methanol and 300 mg of 0.5mm glass beads (Scientific Industries) in a homogenizer (FastPrep®-24 instrument, MP Biomedicals) for 10s at a speed of 5 ms^{-1}. The homogenization procedure was repeated three times, with incubation for one minute on ice between the treatments. The cell extracts were centrifuged at 20 000 x g for 5 min. The methanol extracts (5 µL) were analysed with the Luna C18 (2) reverse phase column (150 mm × 2 mm, particle size 5 µm, pore size 100 Å, Phenomenex), which was eluted with a methanol (solvent A) and water (solvent B) gradient (3 min 65% v/v of solvent A, 7 min 65% to 100% v/v of solvent A, 10 min 100% v/v of solvent A and 5 min 100% to 65% v/v of solvent A) at a flow rate of 0.15 ml min^{-1} at 30°C. The detection of triene UV absorption of anabaenolysin with highest absorption peak at 270 nm was used as a parameter for the screening. *Anabaena* spp.

XPORK15F and XSPORK27C methanol extracts were used as standards. Samples containing triene UV absorption were analyzed with LC-ITMS (Agilent 1100 Series LC/MSD Ion Trap XCT Plus System). The sample was run in a Luna (C18) reverse phase column (150 mm × 2 mm, particle size 5 μm, pore size 100 Å, Phenomenex) eluted with acetonitrile (solvent B) and 0.1% (v/v) aqueous formic acid (solvent A) in a gradient run from 30% to 70% v/v of solvent B in 33 min at a flow rate of 0.15 ml min^{-1} at 40°C.

In the cyclodextrin HPLC-ITMS (Agilent 1100 Series LC/MSD Ion Trap XCT Plus System) analysis, 10 μl of extracts were injected into a Luna C8 (2) column (pore size 100 Å, 150 mm × 2 mm, particle size 5 μm, Phenomenex) eluted from 100% of 0.1% HCOOH to 70% of 0.1% HCOOH in isopropanol (+ 0.1% HCOOH) in 30 min at a flow rate of 0.15 ml min^{-1} at 40°C. Mass spectra were recorded using positive electrospray ionization in ultra-scan mode with a scan range of *m/z* 50–1700. Regioisomer structures (acetyl group positions relative to each other) were solved from the ion intensities of the product ion spectra of the sodiated cyclodextrins according to a statistical method (SI Appendix, Table S5) [59].

High-resolution UPLC-QTOF analyses were performed with Acquity I-Class UPLC-Synapt G2-Si HDMS (Waters Corp., Milford, MA, USA) system. In anabaenolysin (Abl) analyses, a 1 μl sample was injected into an Acquity UPLC BEH C_{18} column (2.1 x 50 mm, 1.7 μm, Waters) eluted at 40°C with a flow rate of 0.3 ml min^{-1} from 20% acetonitrile (+ 0.1% HCOOH) (solvent B) in 0.1% HCOOH to 70% of B in 5 mins, then to 95% of B in 0.01 mins, where it remained for 1.99 mins, before returning to 20% of B in 0.5 mins, where it finally remained for 2.5 mins before the next run. In cyclodextrin (CD) analyses a 1 μl sample was injected into an Acquity UPLC HSS T3 column (2.1 x 50 mm, 1.8 μm, Waters) eluted at 40°C with a flow rate of 0.3 ml min^{-1}: first isocratically for 1 min with 0.1% HCOOH, then with 0% acetonitrile (+ 0.1% HCOOH) (solvent B) in 0.1% HCOOH to 95% of B in 5 mins, where it remained for 2 mins, before returning to 0% of B in 0.5 mins, where it finally remained for 2.5 mins before the next run. The QTOF was calibrated with sodium iodide, producing a calibrated mass range from *m/z* 322.879-2121.335 (Abl) or 322.839-2870.289 (CD). Leucine enkephalin at 10 s intervals served as a lock mass reference compound. Mass spectral data accumulated in positive electrospray ionization Resolution Mode at a scan range of *m/z* 200-2200 (Abl) or 300-3000 (CD).

Antifungal activity

The disc diffusion bioassay was performed as described in [14] and [60]. Discs containing 200 μL of crude extract obtained with methanol were used. The negative control contained 100 μL of methanol, and the positive control 15 μg of nystatin (Sigma-Aldrich). Plates for *Aspergillus flavus* or *Aspergillus parasiticus* were incubated for 48h at 28°C, and for *Candida albicans* HAMBI 261 or HAMBI484, 35°C. To test the anabaenolysin synergistic activity, the methanol extract obtained from 200 mg of freeze-dried cells from strain *Anabaena* sp. XPORK1D was mixed with dichloromethane and water in a proportion of 1:1:1 (v/v). The sample was shaken and centrifuged at 10 000 *x* g for 5 min. This allowed the hydrophilic compounds in the water and methanol phases to separate from the hydrophobic compounds in the dichloromethane phase. Both phases were dried separately and re-

suspended in 1.5 mL of 65% (v/v) aqueous methanol. Compound separation took place in a Luna C18(2) reverse phase column (150 mm × 2 mm, particle size 5 µm, pore size 100 Å, Phenomenex) eluted with acetonitrile (solvent A, E CHROMASOLV®, Sigma-Aldrich) and a 0.1% (v/v) aqueous (Milli-Q purity, Millipore, Merck KGaA, Darmstadt, Germany) formic acid (Sigma-Aldrich) gradient (49 min 30% v/v of solvent A to 70% v/v A, 20 min 70% v/v of solvent B) at a flow rate of 0.15 ml min^{-1} at a temperature of 30°C. The isolated anabaenolysin B and the fraction containing the cyclodextrins were dried and re-suspended in 200 µL of methanol. Half of this volume was added to each disc to demonstrate the synergistic activity of the compounds from *Anabaena* sp. XPORK1D.

New anabaenolysins C and D

Amino acid analysis of the anabaenolysin C based on hydrolysis and derivatization using Marfey's reagent (FDAA, Pierce, Thermo Fisher Scientific) was performed as described in [61]. The derivatized amino acids of anabaenolysin C and commercial glutamine and glycine were analyzed by LC-MS as described previously [61]. Anabaenolysin C was isolated from 2.4 g of freeze-dried *Anabaena* sp. XPORK13A cell mass. Each batch of 100 mg of freeze-dried cells were extracted twice per 1 mL of methanol and glass beads in a FastPrep (6.5 ms^{-1} per 30 s three times). The samples were centrifuged at 10 000 *x* g for 5 min at room temperature and combined into a single tube. Anabaenolysin C was either first isolated with HPLC using a Luna C8 column (150 x 4.6 mm, Phenomenex, Torrance, CA) in an isocratic run of acetonitrile:water (50:50 v/v) at a flow of 1 or 0.75 mL min^{-1} at 30°C or prepurified using a conditioned solid phase extraction cartridge (Strata C18-E, 55 µM, 70Å, 5g/mL, Phenomenex) as previously described [16]. The prepurified anabaenolysin C underwent further purification with HPLC on a Luna C18 column (250 x 10 mm, Phenomenex) in an isocratic run consisting of acetonitrile:water (45:55 v/v) at a flow rate of 5 mL min^{-1} at 30°C as previously described [16]. The solvent was evaporated using a rotary evaporator (Büchi rotavapor R-200), and the sample in water was freeze-dried, yielding a total of 1.94 mg of anabaenolysin C.

Cyclodextrin isolation

Freeze-dried cells from *Anabaena* sp. XSPORK2A (21 x 100 mg) were extracted using methanol as described in the previous sub-section. A mixture of cyclodextrins were isolated in HPLC on a Luna C8 column (150 x 4.6 mm, particle size 5 µm, Phenomenex) in a gradient run of isopropanol:water (from 0 to 100% isopropanol v/v) for 25 min at 30°C. The fractions were combined and the solvent was evaporated using the rotary evaporator (Büchi rotavapor R-200). The sample was freeze-dried, yielding a 10.8 mg mixture of cyclodextrins stored at -80°C.

NMR spectroscopy

For the NMR data collection, anabaenolysin C was dissolved in d_6-DMSO. All NMR measurements were carried out at 25°C on a Varian Inova NMR spectrometer operating at 800 MHz of ^{1}H frequency and equipped with a cryogenically cooled triple-resonance ^{1}H, ^{13}C, and ^{15}N probehead. One-

dimensional ^{1}H spectrum together with several two-dimensional homo- and heteronuclear NMR spectra were collected. ^{1}H spectrum was acquired with 24 k complex points, which translated to an acquisition time of 2 s. Two-dimensional double-quantum filtered COSY and TOCSY experiments were collected using 256 (256) complex points in t_1 (t_2) dimension, corresponding to acquisition time of 256 ms. The isotropic mixing in the TOCSY experiment required 60 ms. Heteronuclear ^{13}C-HSQC experiments optimized for establishing aliphatic ^{1}H-^{13}C (aromatic ^{1}H-^{13}C) connectivities were collected using 128 and 852 complex points in t_1 and t_2 dimension, yielding acquisition times of 4.6 and 85.2 ms (10.7 and 85.2 ms), respectively. Long-range ^{1}H-^{13}C connectivities were established using a ^{13}C-HMBC experiment optimized for 7 Hz (^{1}H-^{13}C transfer delay 71.4 ms) and 5 Hz (^{1}H-^{13}C transfer delay 100 ms) couplings. Data were collected using 128 and 1280 complex points in t_1 and t_2, corresponding to acquisition times of 3.2 ms and 128 ms, respectively. ^{15}N-HSQC experiment for correlating one-bond ^{1}H-^{15}N connectivities were measured using 32 and 1020 complex points in dimensions t_1 and t_2, which translates to acquisition times of 10.7 and 85 ms, respectively. For structural analysis of the cyclodextrins from *Anabaena* sp. XSPORK2A, the cyclodextrin sample was dissolved into d_6-DMSO and identical sets of NMR experiments for the anabaenolysin C NMR analysis, excluding the ^{15}N-HSQC, were collected at 25 °C on Varian Inova NMR 800 NMR spectrometer.

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