Supplementary material for the article

Description of *Aliinostoc alkaliphilum* sp. nov. (Nostocales, Cyanobacteria), a new bioactive metabolite-producing strain from Salina Verde (Pantanal, Brazil) and taxonomic distribution of bioactive metabolites in *Nostoc* and *Nostoc*-like genera.

Maria Christodoulou [1,*], Jouni Jokela [1], Matti Wahlsten [1], Lyudmila Saari [1], Athena Economou-Amilli [2], Marli de Fatima Fiore [3] and Kaarina Sivonen [1]

[1] Department of Microbiology, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 56, 00014, Helsinki, Finland
[2] Department of Ecology and Systematics, Faculty of Biology, National and Kapodistrian University of Athens, Athens 15784, Greece
[3] Laboratory of Cellular and Molecular Biology, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba 13416-000, São Paulo, Brazil
* Correspondence: maria.christodoulou@helsinki.fi

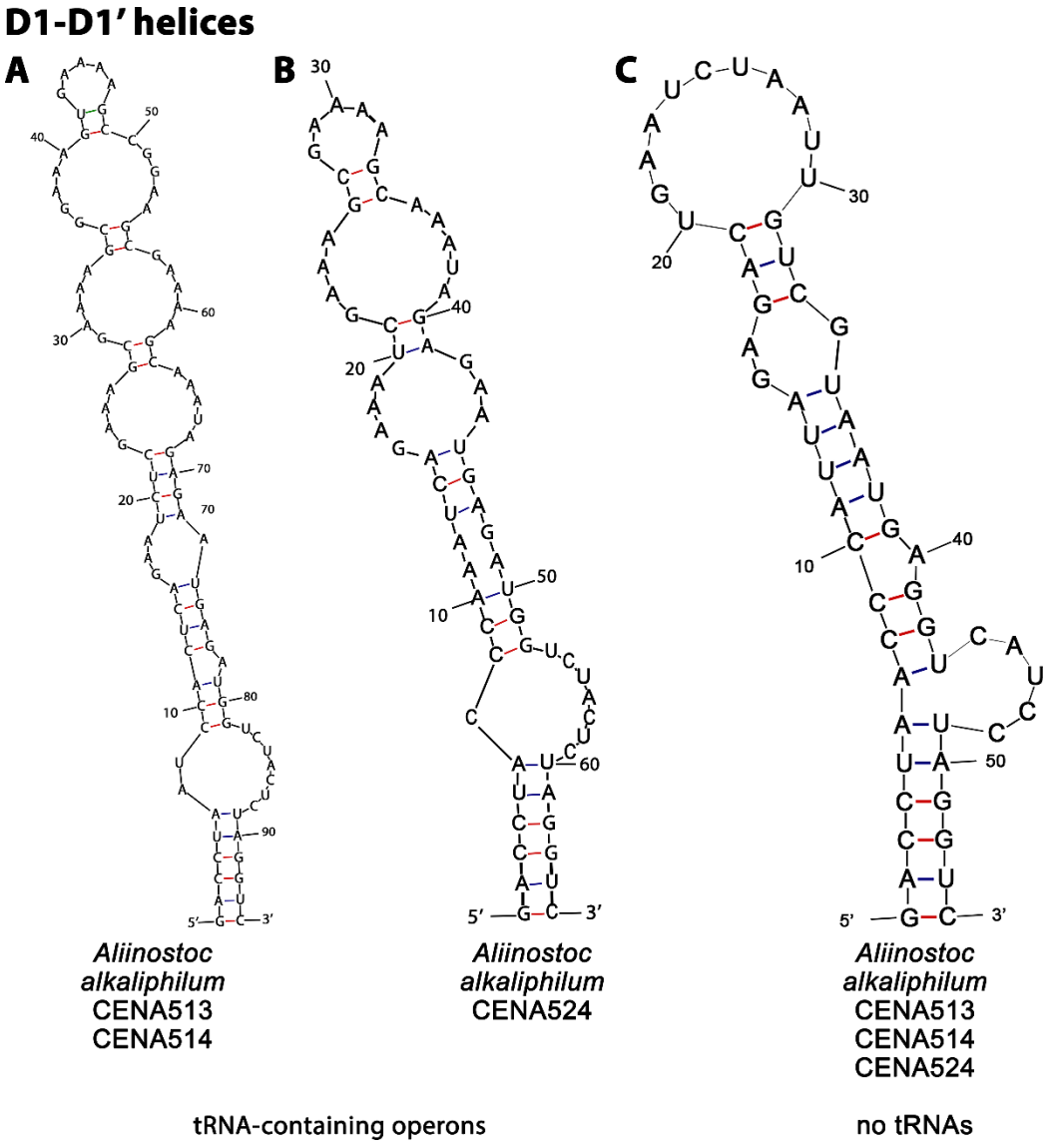

Figure S1. D1 stem region in *A. alkaliphilum* strains in tRNA-containing operons (A-B) and operons without tRNA (C). **A**; CENA513^{T} and CENA514 **(**OK042916 and OK042918, respectively), **B**; *A. alkaliphilum* CENA524 (OK042920), **C**; *A. alkaliphilum* CENA513^{T}, CENA514 and CENA524 (OK042917, OK042919 and OK042921, respectively).

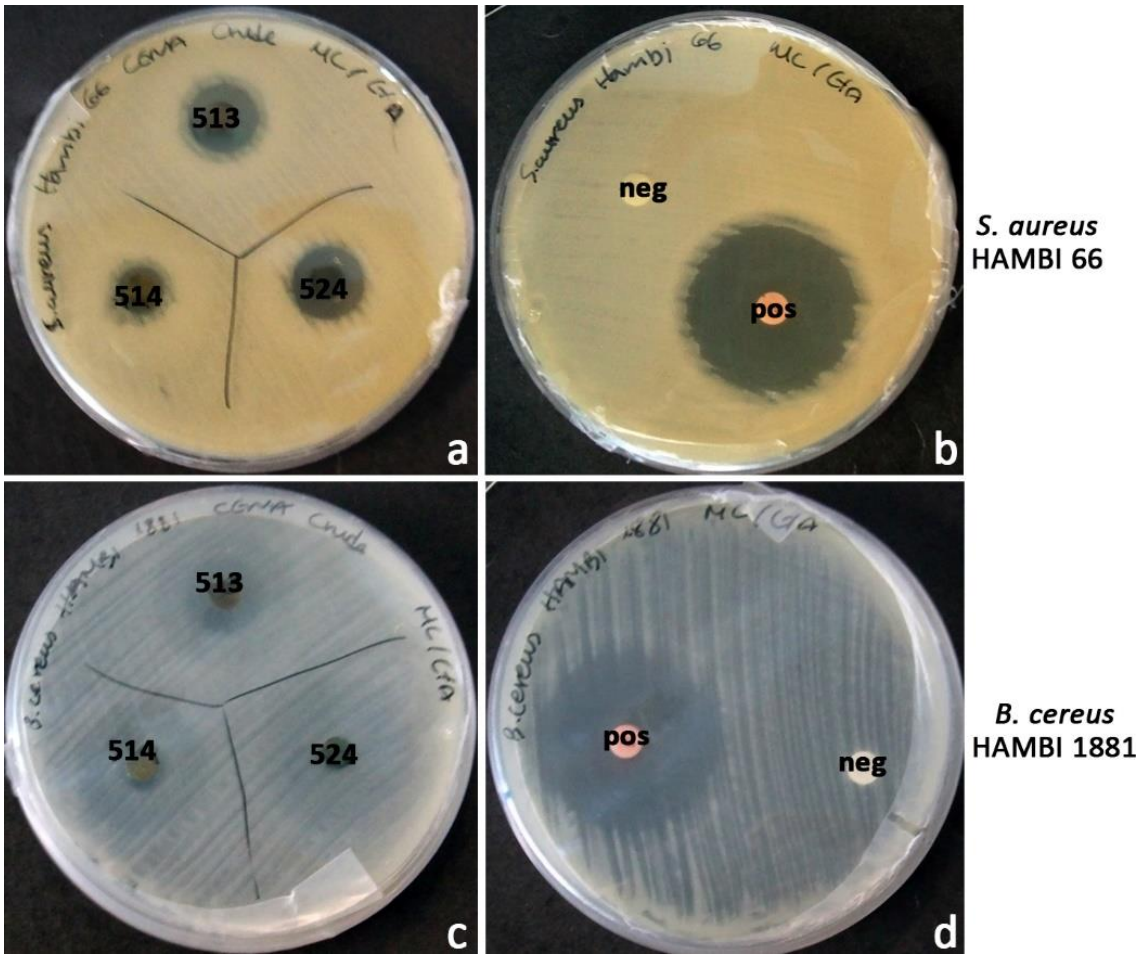

Figure S2. Antibacterial susceptibility tests performed using crude cyanobacterial extracts. The amount of sample per disk was equivalent to 30 mg of freeze-dried biomass. (a–b) *Staphylococcus aureus* HAMBI 66. (c–d) *Bacillus cereus* HAMBI 1881. Note the bacteriostatic activity of CENA514 and 524 on *B. cereus* in contrast to bacteriocidal activity of CENA513^{T} on the same pathogen. pos; positive control (Kanamycin 1000 μg per disk). neg; negative control (300 μL of 100% MeOH per disk). Disk diameter = 6 mm.

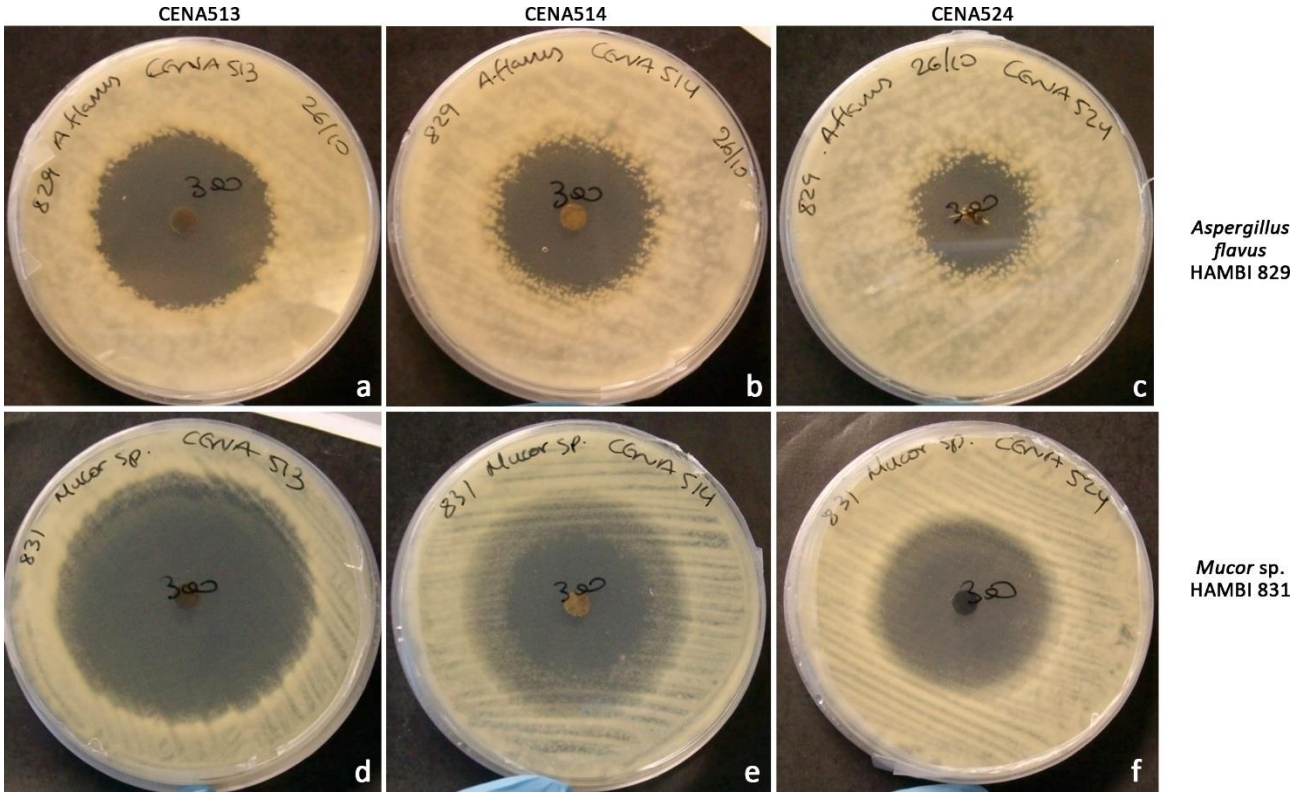

Figure S3. Antifungal activity of *Aliinostoc alkaliphilum*. CENA513 (left), CENA514 (middle), CENA524 (right) on *Aspergillus flavus* HAMBI 829 (a–c) and *Mucor* sp. HAMBI 831 (d–f). The amount of sample per disk was equivalent to 30 mg of freeze-dried biomass. Zones of inhibition in CENA513^{T} are clearly larger compared to CENA514 and CENA524. Disk diameter = 6 mm.

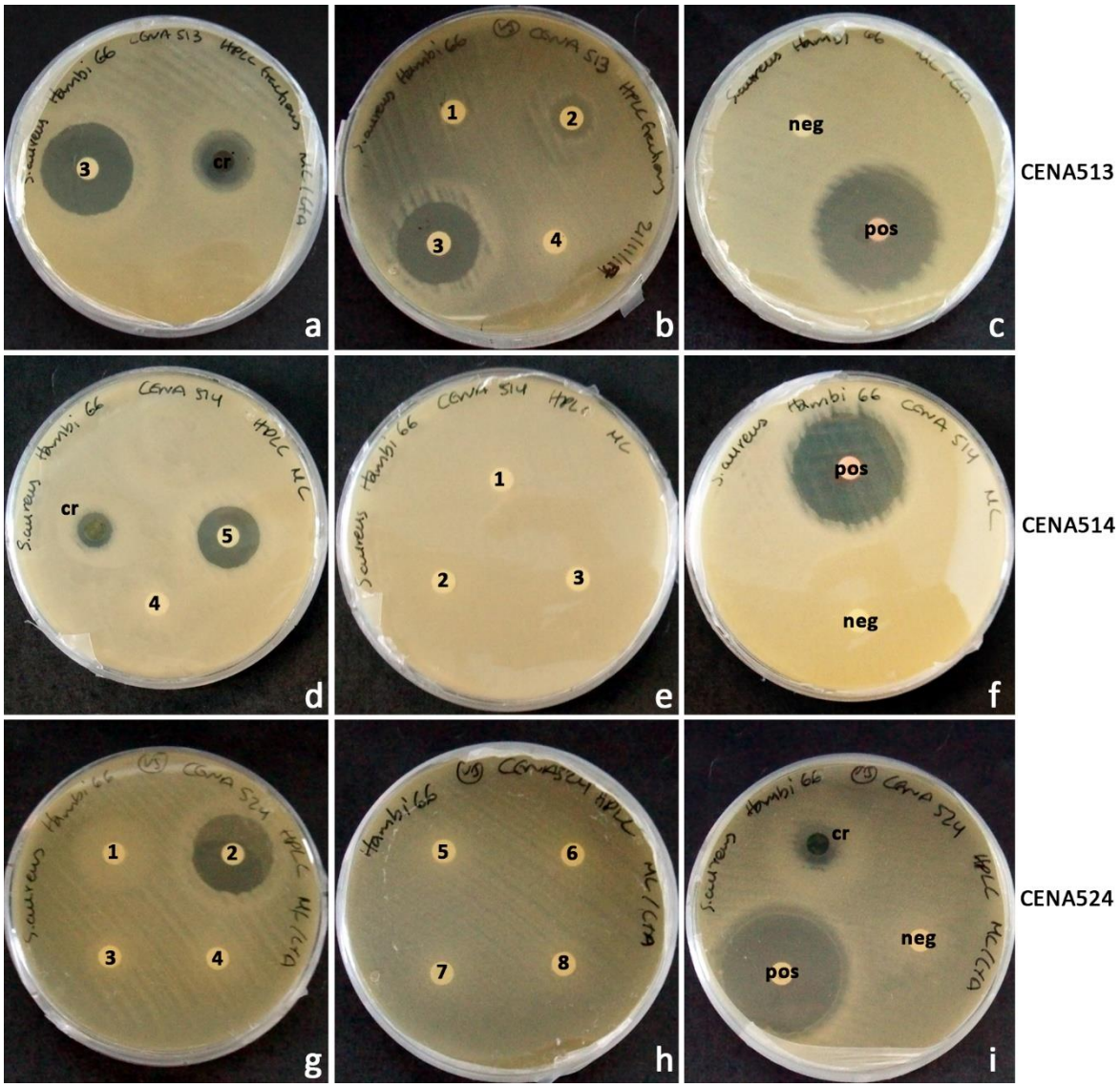

Figure S4. Antimicrobial susceptibility tests performed using HPLC fractions and crude extract of *Aliinostoc alkaliphilum* CENA513^{T} (a–c), CENA514 (d–f) and CENA524 (g–i) against *S. aureus* HAMBI 66. The concentration of metabolites in both HPLC fractions and crude extract corresponds to 30 mg of freeze-dried biomass. Numbers on disks correspond to HPLC fractions of CENA513^{T}, CENA514 and CENA524. Note that HPLC fraction 3 of CENA513^{T} is present in figures a and b for comparison reasons. cr; crude extract. pos; positive control (kanamycin 1000 μg per disk). neg; negative control (300 μL of 100% MeOH per disk). Disk diameter = 6 mm.

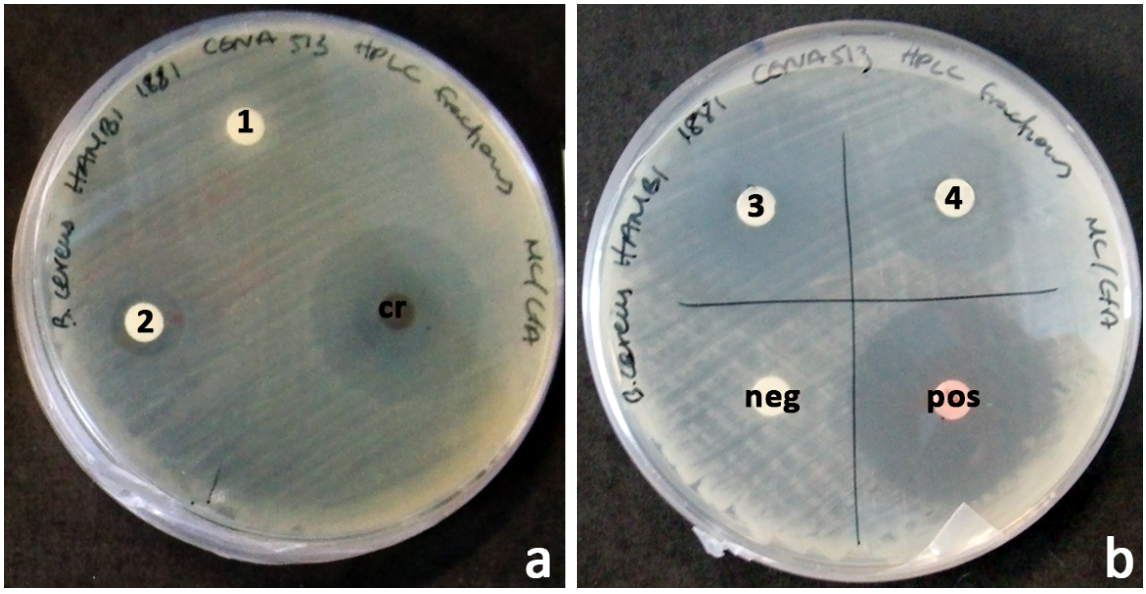

Figure S5. Antimicrobial susceptibility tests performed using HPLC fractions of *Aliinostoc alkaliphilum*. CENA513^{T} (a–b) against *B. cereus* HAMBI 1881. Note the bacteriocidal activity of HPLC fraction 513#2 and bacteriostatic activity of HPLC fractions 513#3 and 513#4 on *B. cereus*. Crude extract (cr) was also used as positive a control. The concentration of metabolites in both HPLC fractions and crude extract corresponds to 30 mg of freeze-dried biomass. 1–4; HPLC fractions. pos; positive control (kanamycin 1000 μg per disk). neg; negative control (300 μL of 100% MeOH per disk). Disk diameter = 6 mm.

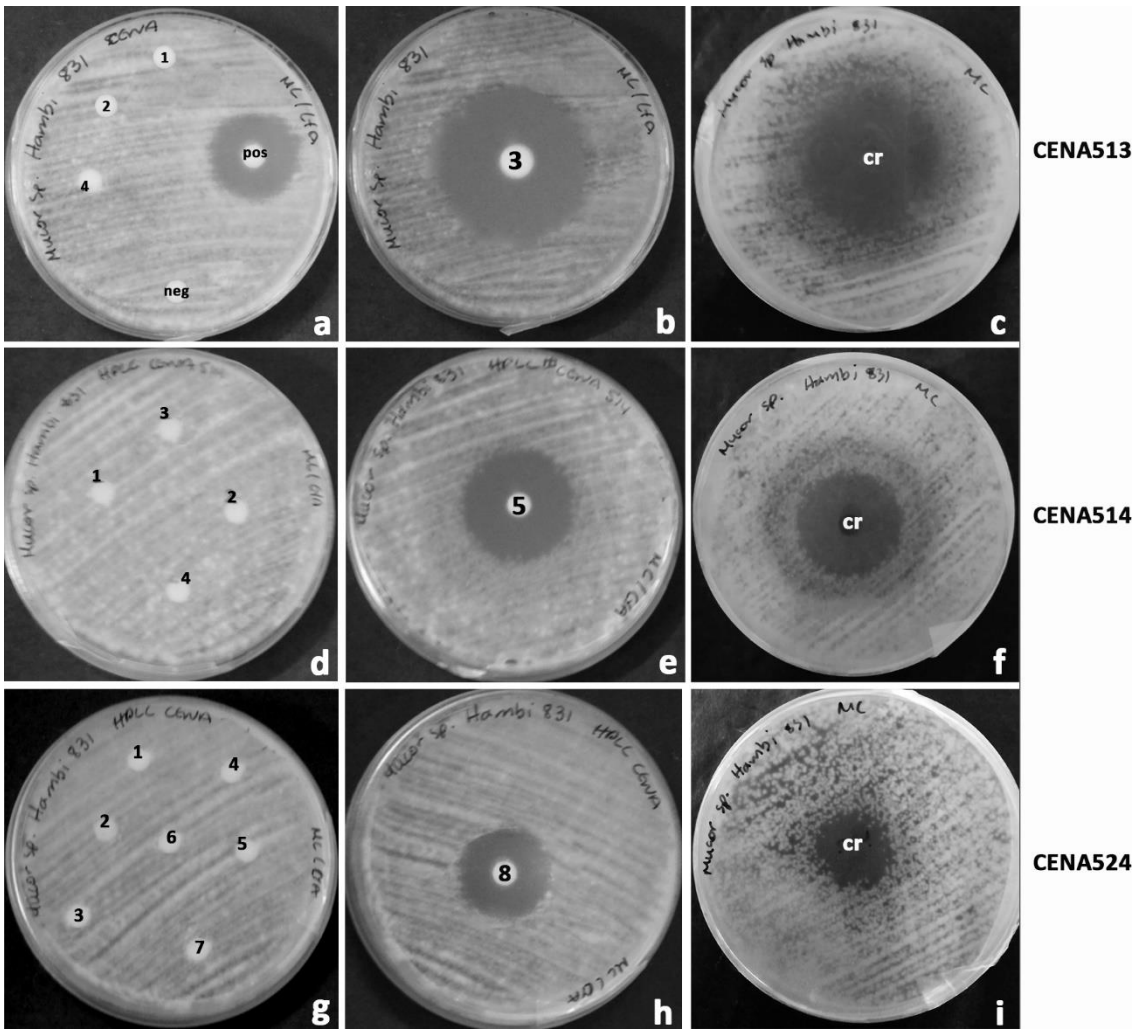

Figure S6. Antifungal susceptibility tests performed using HPLC fractions and crude extract of *Aliinostoc alkaliphilum* CENA513^{T} (a–c), CENA514 (d–f) and CENA524 (g–i) against *Mucor* sp. HAMBI 831. The concentration of metabolites in both HPLC fractions and crude extract corresponds to 10 mg of freeze-dried biomass. (c) Positive and negative controls. Numbers correspond to HPLC fractions. **cr**; crude extract. **pos**; positive control [50 μL of nystatin solution (1 mg mL^{-1})]. neg; negative control (300 μL of 100% MeOH per disk). Disk diameter = 6 mm.

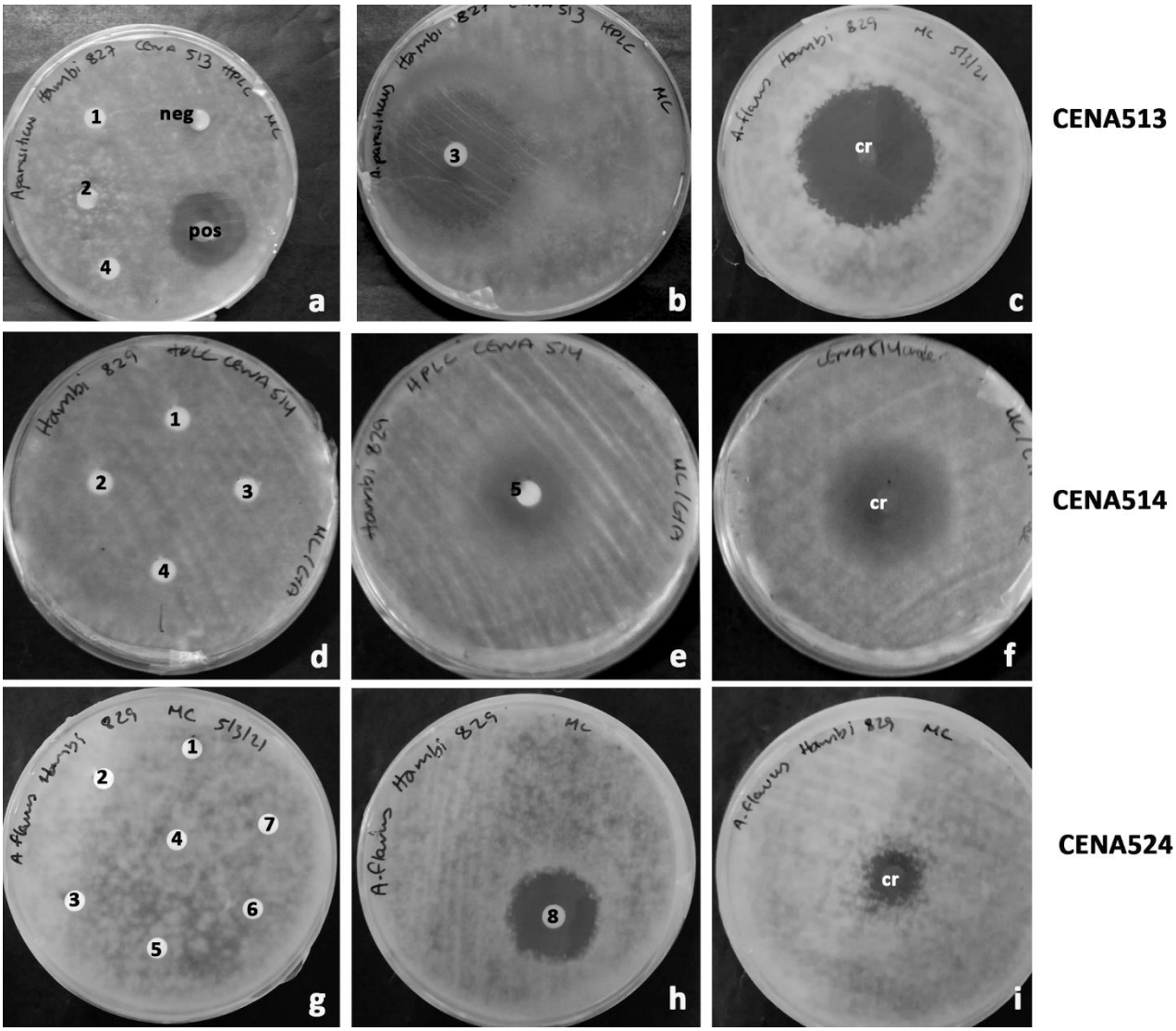

Figure S7. Antifungal susceptibility tests performed using HPLC fractions and crude extract of *Aliinostoc alkaliphilum* CENA513^{T} (a–c), CENA514 (d–f) and CENA524 (g–i) against *Aspergillus flavus*. HAMBI 829. The concentration of metabolites in both HPLC fractions and crude extract corresponds to 10 mg of freeze-dried biomass. (c) Positive and negative controls. Numbers correspond to HPLC fractions. **cr**; crude extract. **pos**; positive control [50 µL of nystatin solution (1 mg mL^{-1})]. neg; negative control (300 µL of 100% MeOH per disk). Disk diameter = 6 mm.

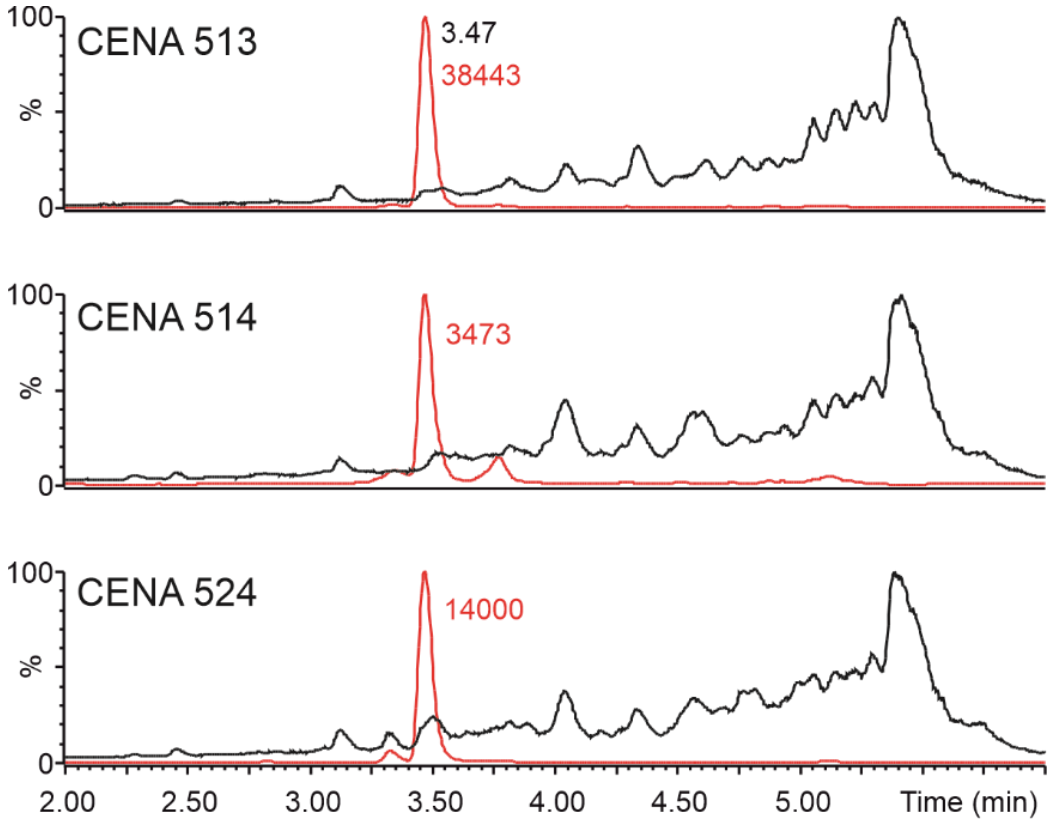

Figure S8. Total ion current (black) and extracted ion (*m*/*z* 299.23; red) chromatograms (EIC) of CENA 513^{T}, 514 and 524 strain crude extracts. Retention time in black font colour and EIC areas in red font colour.

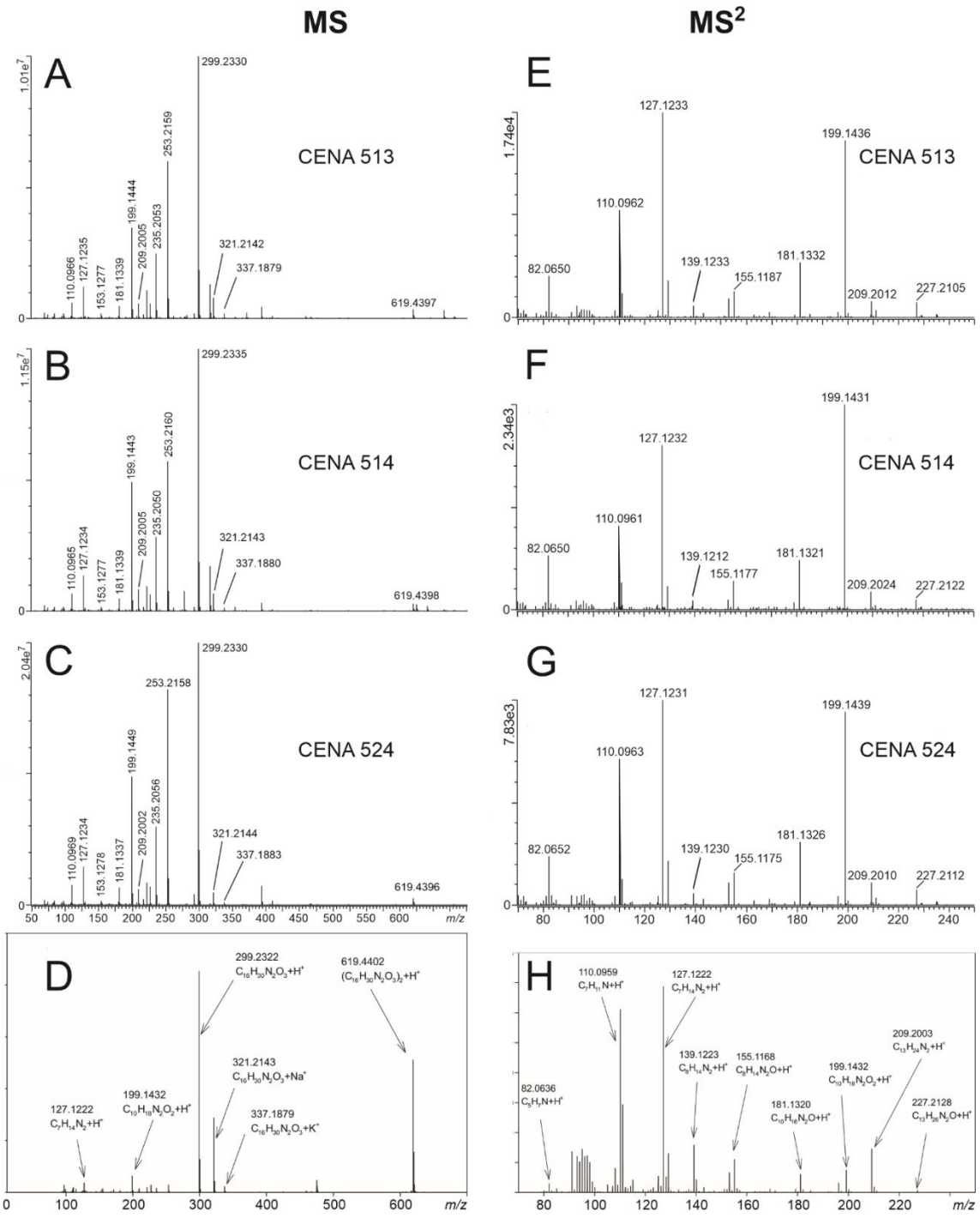

Figure S9. Mass spectra (A-C) of EIC peaks presented in Figure S8 and product ion spectra from *m/z* 299.23 (E-G) of CENA513^T, 514 and 524 extracts and nocuolin A reference spectra (D and H) by Voráčová *et al*. [60]

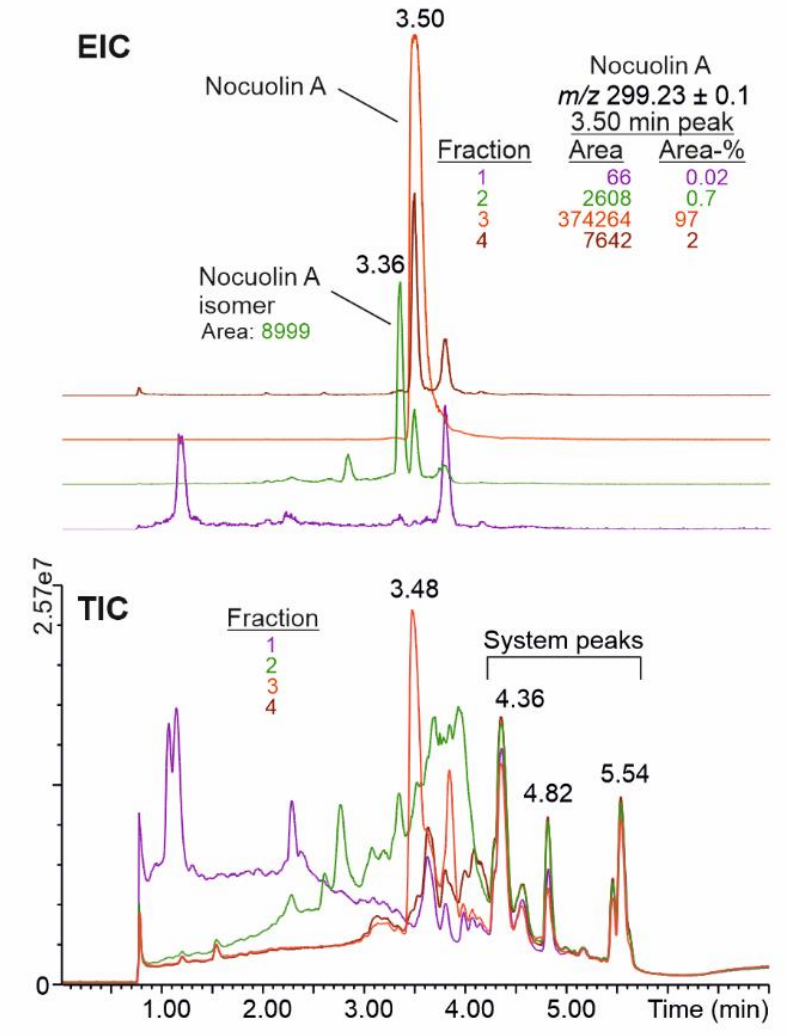

Figure S10. Total ion chromatograms (TIC) and extracted ion *m*/*z* 299.23 (protonated nocuolin A) chromatograms from *Aliinostoc alkaliphilum* CENA513^{T} extract fractionated into 4 fractions by liquid chromatography. Peaks (TIC) eluting after 4.2 min are similarly present in all fractions and hence represent irrelevant compounds causing no bioactivity.

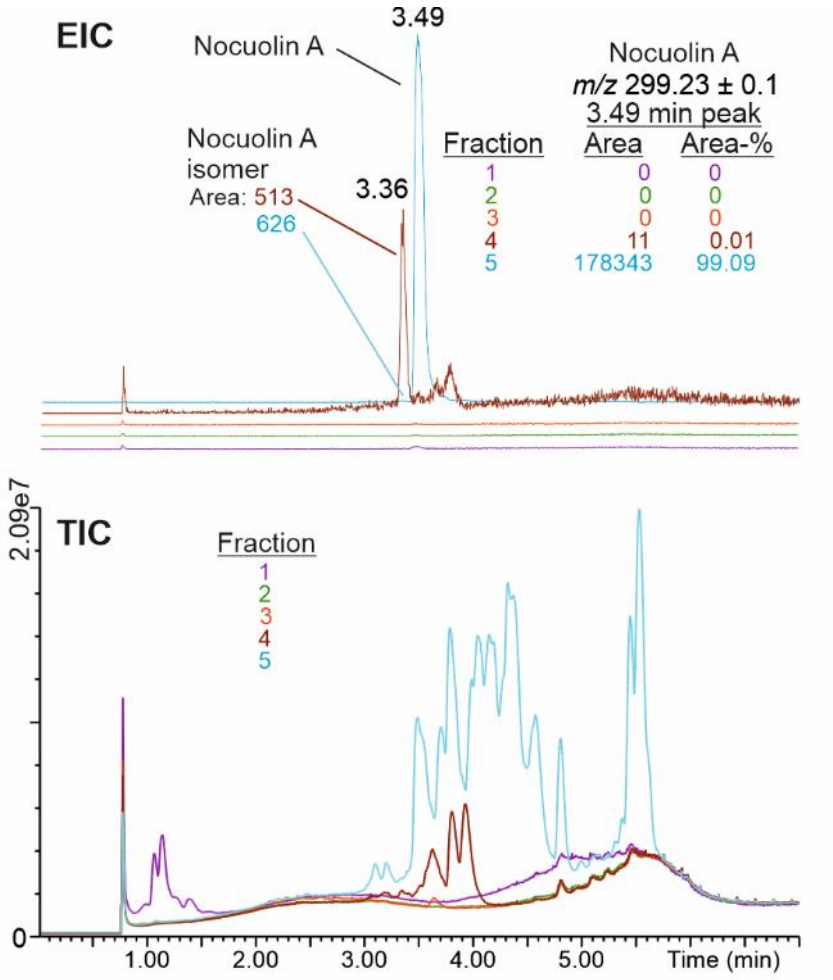

Figure S11. Total ion chromatograms (TIC) and extracted ion *m*/*z* 299.23 (protonated nocuolin A) chromatograms from *Aliinostoc alkaliphilum* CENA514 extract fractionated into 5 fractions by liquid chromatography.

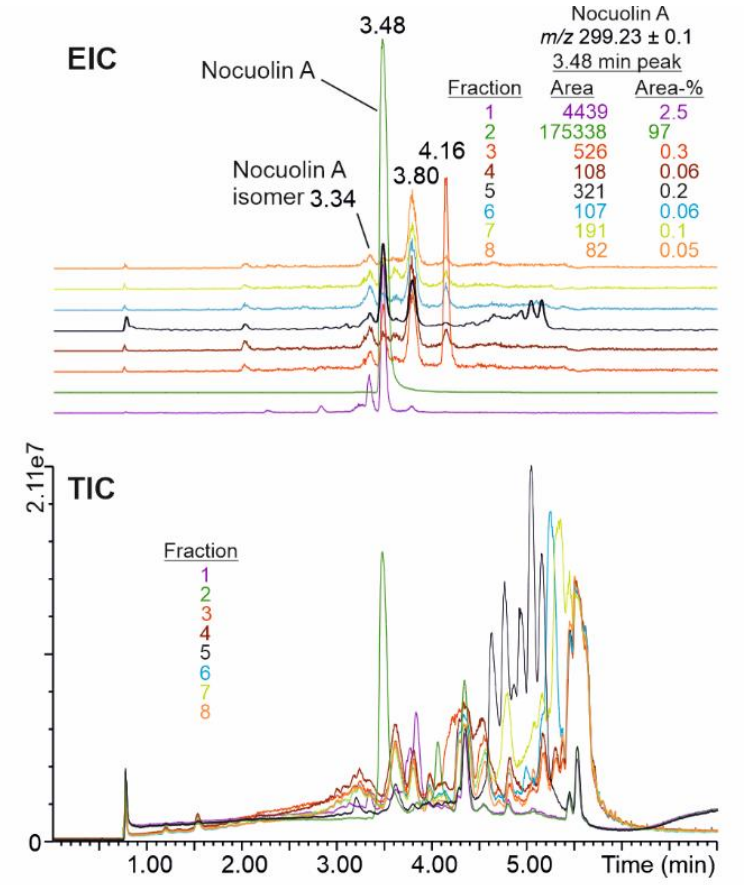

Figure S12. Total ion chromatograms (TIC) and extracted ion *m*/*z* 299.23 (protonated nocuolin A) chromatograms from *Aliinostoc alkaliphilum*. CENA524 extract fractionated into 8 fractions by liquid chromatography.

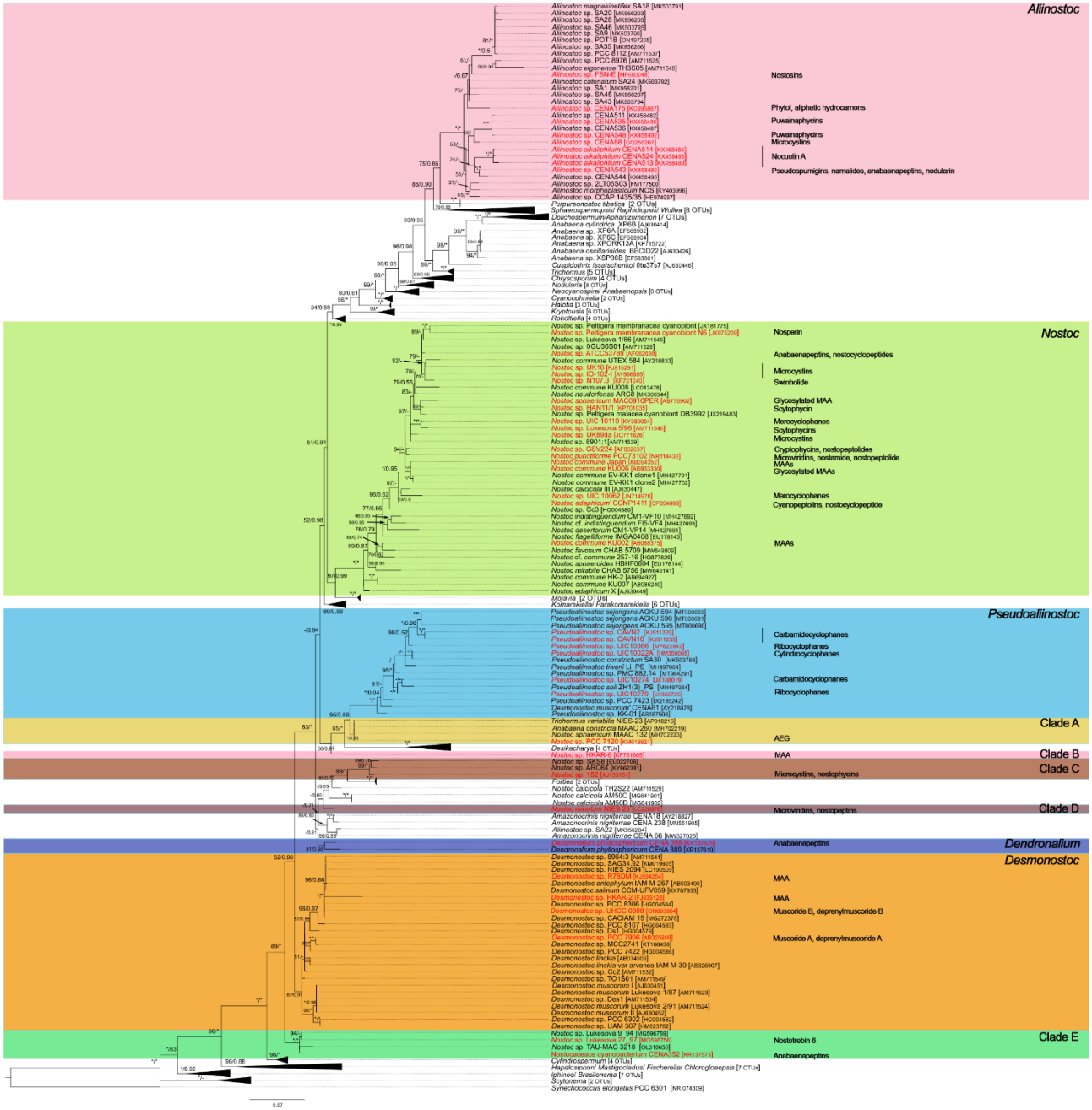

Figure S13. Phylogenetic relationships inferred from maximum likelihood analysis based on 16S rRNA sequences of *Aliinostoc* and related genera of Nostocales sensu Komarek et al. [13]. Clades hosting NP-producing strains (red font color) are shown in different font colors. All bioactive compounds produced by each strain are also shown. Numbers on nodes correspond to bootstrap values (≥50%) and posterior probabilities (≥ 0.50) obtained from maximum likelihood and bayesian analyses respectively. Asterisk (*) represents posterior probability of 1.0 for Bayesian analysis and bootstrap values of 100% for Maximum Likelihood analysis. *Synechococcus elongatus* PCC 6301 was the designated outgroup. GenBank accession numbers of sequences are given in brackets. The scale corresponds to substitutions/site.

Table S1. Bacterial and fungal strains used in this study. Growth temperature and agar-solidified growth media used for strain propagation and disk diffusion assays are also presented. MH: Mueller–Hinton, MH–GMB: Mueller-Hinton with 2% glucose and 0.5 μg ml^{-1} methylene blue, PDA: Potato Dextrose Agar. All bacterial and yeast strains were incubated overnight. Strains of filamentous fungi were incubated for 3 days.

HAMBI accession number	Strain name	Solidified growth media	Growth temperature (°C)
Bacteria			
66	*Staphylococcus aureus*	MH	37
1821	*Enterococcus faecium*	MH	37
1881	*Bacillus cereus*	MH	28
2688	*Micrococcus luteus*	MH	28
25	*Pseudomonas aeruginosa*	MH	37
1760	*Acinetobacter baumannii*	MH	28
1898	*Enterobacter aerogenes*	MH	28
2331	*Salmonella enterica*	MH	37
Fungi			
484	*Candida albicans*	MH-GMB	28
486	*Candida krusei*	MH-GMB	28
487	*Candida parapsilosis*	MH-GMB	28
488	*Cryptococcus neoformans*	MH-GMB	28
829	*Aspergillus flavus*	PDA	28
831	*Mucor* sp.	PDA	28

Table S2. Taxa included in the phylogenetic analyses and references for bioactive metabolite-producing strains

Taxon name	GenBank accession number
Aliinostoc morphoplasticum NOS	KY403996
Aliinostoc magnakinetifex SA18	MK503791
Aliinostoc catenatum SA24	MK503792
***Aliinostoc alkaliphilum* CENA513**	KX458483
***Aliinostoc alkaliphilum* CENA514**	KX458484
***Aliinostoc alkaliphilum* CENA524**	KX458485
Aliinostoc elgonense TH3S05	AM711548
Aliinostoc sp. CENA88	GQ259207
Aliinostoc sp. CENA175	KC695867
Aliinostoc sp. CENA511	KX458482
Aliinostoc sp. CENA535	KX458486
Aliinostoc sp. CENA536	KX458487
Aliinostoc sp. CENA543	KX458489
Aliinostoc sp. CENA544	KX458490
Aliinostoc sp. CENA548	KX458492
Aliinostoc sp.FSN-E	MF680048
Aliinostoc sp. SA20	MK956203
Aliinostoc sp. SA28	MK956205
Aliinostoc sp. SA35	MK956206
Aliinostoc sp. SA45	MK956207
Aliinostoc sp. SA9	MK503790
Aliinostoc sp. SA43	MK503794
Aliinostoc sp. SA46	MK503795
Aliinostoc sp. SA1	MK956201
Aliinostoc sp. POT1B	ON197205
Aliinostoc sp. CCAP 1453/35	HE974997
Aliinostoc sp. 2LT05S03	FM177500
Aliinostoc sp. PCC 8976	AM711525
Aliinostoc sp. PCC 8112	AM711537
Pseudoaliinostoc sejongens ACKU 594	MT000689
Pseudoaliinostoc sejongens ACKU 595	MT000690
Pseudoaliinostoc sejongens ACKU 596	MT000691
Pseudoaliinostoc tiwarii Li_PS	MH497064
Pseudoaliinostoc soli ZH13_PS	MH497065
Pseudoaliinostoc constrictum SA30	MK503793
Pseudoaliinostoc sp. PMC 882.14	MT984291
Pseudoaliinostoc sp. UIC 10022A	HM359085
Pseudoaliinostoc sp. UIC 10366	MF622942
Pseudoaliinostoc sp. UIC 10279	JX962720
Pseudoaliinostoc sp. UIC 10274	JX188019
Pseudoaliinostoc sp. CAVN2	KJ511229
Pseudoaliinostoc sp. CAVN10	KJ511235
Pseudoaliinostoc sp. KK 01	AB187508
Nostoc muscorum CENA61	AY218828
Pseudoaliinostoc sp. PCC 7423	DQ185242
Nostoc commune EV1-KK1 clone 1	MH427701
Nostoc commune EV1-KK1 clone 2	MH427702
Nostoc commune KU002	AB088375
Nostoc sp. UIC 10110	KY380004

Table S2-cont.

Taxon name	GenBank accession number
Nostoc sp. UIC 10062	JN714978
Nostoc commune Japan Ishikawa Kanazawa Asano River	AB094352
Nostoc sphaericum MAC0910PER	AB775902
Nostoc commune KU006	AB933330
Nostoc sp. GSV224	AF062637
Nostoc sp. ATCC53789	AF062638
Nostoc sp. IO.102.I	AY566855
Nostoc edaphicum CCNP1411	CP054698
Nostoc sp. Lukesova 5/96	AM711546
Nostoc sp. Peltigera membranacea cyanobiont N6	JX975209
Nostoc sp. HAN 11/1	KP701035
Nostoc sp. N107.3	KP701040
Nostoc punctiforme PCC73102	NR_114430
Nostoc sp. UK18	FJ815291
Nostoc sp. UK89IIa	JQ771626
Nostoc commune HK-02	AB694927
Nostoc commune KU007	AB986249
Nostoc calcicola III	AJ630447
Nostoc edaphicum X	AJ630449
Nostoc sp. 0GU36S01	AM711526
Nostoc sp. 8901:1	AM711539
Nostoc sp. Lukesova 1/86	AM711545
Nostoc commune UTEX 584	AY218833
Nostoc flagelliforme IMGA0408	EU178143
Nostoc sphaeroides HBHF0604	EU178144
Nostoc sp. Cc3	HG004580
Nostoc cf. *commune* 257-16	HQ877826
Nostoc mirabile CHAB 5756	MW649141
Nostoc favosum CHAB 5709	MW649809
Nostoc sp. Peltigera membranacea cyanobiont	JX181775
Nostoc sp. Peltigera malacea DB3992 cyanobiont	JX219483
Nostoc commune KU008	LC013478
Nostoc desertorum CM1-VF14	MH427691
Nostoc indistinguendum CM1-VF10	MH427692
Nostoc indistinguendum FI5-VF4	MH427693
Nostoc neudorfense ARC8	MK300544
Desmonostoc sp. HKAR 2	FJ939126
Desmonostoc linckia	AB074503
Desmonostoc entophytum IAM M-267	AB093490
Desmonostoc muscorum I	AJ630451
Desmonostoc muscorum II	AJ630452
Desmonostoc muscorum Lukesova 1/87	AM711523
Desmonostoc muscorum Lukesova 2/91	AM711524
Desmonostoc sp. Cc2	AM711532
Desmonostoc sp. De1	AM711534
Desmonostoc sp. 8964:3	AM711541
Desmonostoc sp. Ds1	HG004579
Desmonostoc sp. PCC 6302	HG004582
Desmonostoc sp. PCC 8107	HG004583

Table S2-cont.

Taxon name	GenBank accession number
Desmonostoc sp. PCC 8306	HG004584
Desmonostoc sp. PCC 7422	HG004586
Desmonostoc sp. UAM 307	HM623782
Desmonoostoc sp. MCC2741	KT166436
Desmonostoc salinum CCM-UFV059	KX787933
Desmonostoc sp. NIES 2094	LC190509
Desmonostoc sp. R76DM	KJ994254
Desmonostoc sp. SAG 34.92	KM019925
Desmonostoc linckia var *arvense* IAM M-30	AB325907
Desmonostoc sp. TO1S01	AM711549
Desmonostoc sp. PCC 7906	AB325908
Desmonostoc sp. UHCC0398	ON693864
Desmonostoc sp. CACIAM 19	MG272378
Dendronalium phyllosphericum CENA358	KR137579
Dendronalium phyllosphericum CENA389	KR137610
Amazonocrinis nigriterrae CENA66	MW327025
Amazonocrinis sp. SA22	MK956204
Amazonocrinis nigriterrae CENA18	AY218827
Amazonocrinis nigriterrae CENA238	MN551905
Nostoc sp. Lukesova 9_94	MG596759
Nostoc sp. Lukesova 27/97	MG596756
Nostocaceae cyanobacterium CENA352	KR137573
Nostoc sp. TAU MAC 3218	OL310650
Nostoc sp. PCC 7120	KM019921
Nostoc sphaericum MACC 132	MH702223
Trichormus variabilis NIES 23	AP018216
Anabaena constricta MACC 260	MH702219
Nostoc sp. HKAR 6	KF751605
Nostoc calcicola TH2S22	AM711529
Nostoc calcicola AM50C	MG641901
Nostoc calcicola AM50D	MG641902
Nostoc minutum NIES 26	LC228976
Nostoc sp. SKS8	EU022706
Nostoc sp. ARC 64	KY662381
Nostoc sp. strain 152	AJ133161
Fortiea sp. HA4221-MV2 clone B4 p3C	HQ847569
Fortiea sp. HA4221-MV2 clone B4 p3D	HQ847570
Purpureonostoc tibetica CHAB5880 clone 1	MN381942
Purpureonostoc tibetica CHAB5880 clone 2	MN381943
Cyanocohniella crotaloides PJ-S45	MN243143
Cyanocohniella hyphalmyra TAU-MAC 3117	OL310605
Anabaenopsis sp. CENA549	KX458493
Anabaenopsis elenkinii SAG 25280	KM020015
Neocyanospira rippkae CR86F5	FR774773
Neocyanospira rippkae CR86F7	FR774774
Neoyanospira capsulata 9NAT	FR774776
Neocyanospira capsulata CCAX	FR774777

Table S2-cont.

Taxon name	GenBank accession number
Anabaenopsis sp. PCC 9215	AY038033
Neoyanospira rippkae	AY038036
Cylindrospermum stagnale PCC 7417	NR_114701
Cylindrospermum stagnale	NR_102462
Cylindrospermum licheniforme UTEX B 2014 strain ATCC 29412	KX014846
Cylindrospermum alatosporum SAG 43.79	GQ287650
Halotia branconii CENA186	KC695877
Halotia branconii CENA390	KJ843310
Halotia branconii CENA392	KJ843312
Kryptousia microlepis CENA329	KY508607
Kryptousia microlepis CENA334	KY508608
Kryptousia macronema CENA336	KY508609
Kryptousia macronema CENA338	KY508610
Kryptousia microlepis CENA343	KY508611
Kryptousia microlepis CENA354	NR_157979
Roholtiella fluviatilis UAM 332	HM751847
Roholtiella fluviatilis UAM 334	HM751849
Roholtiella edaphica AR6 clone 1	MF002050
Roholtiella edaphica AR6 clone 1	MF002051
Nodularia sphaerocarpa Fae19	AJ781144
Nodularia spumigena PCC 73104	NR_115707
Nodularia spumigena PCC73104	NR_114565
Nodularia spumigena strain AV63	AJ781138
Nodularia harveyana Bo53	AJ781143
Nodularia baltica BY1	AJ133177
Nodularia spumigena isolate BCNOD9427	AJ224447
Nodularia harveyana Lukesova 18/94	AM711554
Komarekiella atlantica CCIBT 3307	KX638483
Komarekiella atlantica CCIBT 3483	KX638487
Komarekiella atlantica CCIBT 3487	KX638488
Komarekiella atlantica CCIBT 3486	KX638489
Parakomarekiella sesnandensis COI00088998	MT044191
Parakomarekiella sesnandensis COI00088999	MT044192
Desikacharya nostocoides BHU1-PS	MH036167
Desikacharya soli BHU2-PS	MH036168
Desikacharya constricta SA10	MK354274
Mojavia sp. CMT-3FDIN-NPC3 clone CN6 4	KU161676
Mojavia sp. CMT-3FDIN-NPC3 clone CN6 2	KU161675
Trichormus variabilis PMC 1148.19	MW405041
Trichormus variabilis KCTC AG10180	DQ234832
Trichormus variabilis KCTC AG10269	DQ234833
Trichormus variabilis HINDAK 2001/4	AJ630456
Trichormus variabilis GREIFSWALD	AJ630457
Anabaena cylindrica XP6B	AJ630414
Anabaena oscillarioides BECID22	AJ630426
Anabaena sp. XP6C	EF568904
Anabaena sp. XSP36B	EF583861
Anabaena sp. XP6A	EF568902

Table S2-cont.

Taxon name	GenBank accession number
Anabaena sp. XPORK13A	KP715722
Chrysosporum bergii	AF160256
Chrysosporum ovalisporum 1LT27S04	FM177485
Chrysosporum ovalisporum FAS-AP1	EU076457
Chrysosporum bergii ANA360D	MW219692
Dolichospermum mucosum 1tu35s5	AJ630425
Dolichospermum brachiatum WB20619 B1 clone Z25	MT535756
Dolichospermum brachiatum WB20619 B3 clone Z26	MT535757
Dolichospermum affine NIES-40	AF247591
Aphanizomenon flos-aquae 1tu26s2	AJ630443
Aphanizomenon gracile HEANEY Camb/1986 140/1/1	AJ630444
Aphanizomenon gracile 1tu26s16	AJ630445
Cuspidothrix issatschenkoi 0tu37s7	AJ630446
Sphaerospermopsis aphanizomenoides 04-43	FM161350
Sphaerospermopsis crassa CHAB4404	KT583658
Sphaerospermopsis reniformis 06-01	FM161348
Sphaerospermopsis reniformis 07-01	FM161349
Wollea saccata ACCS 045	GU434226
Raphidiopsis curvata HB1	AY763116
Raphidiopsis mediterranea FSS1-150-1	HQ730899
Raphidiopsis mediterranea HB2	AY763117
Brasilonema bromeliae SPC951	NR_115807
Brasilonema octagenarum UFV-E1	NR_115956
Brasilonema terrestre CENA116	NR_116034
Brasilonema angustatum HA4187-MV1	NR_125582
Brasilonema angustatum HA4187-MV1	NR_125583
Iphinoe spelaeobios LO2-B1	HM748317
Iphinoe spelaeobios strain LO2-B1	NR_117880
Scytonema hofmanni PCC 7110	AM709637
Scytonema sp. CG23	KT222810
Hapalosiphon welwitschii UH IC-52-3	KJ767019
Mastigocladus laminosus Greenland 8	DQ431003
Hapalosiphon hibernicus BZ-3-1	EU151900
Chlorogloeopsis fritschii PCC 6718	AF132777
Hapalosiphon delicatulus IAM M-266	AB093484
Fischerella major NIES 592	AB093487
Hapalosiphon welwitschii	AY034793
Synechococcus elongatus PCC 6301	NR_074309

Table S3. Morphological comparison and habitat preferences of all known *Aliinostoc* species

Strain name	Macroscopic features in natural habitat	Filaments	Sheaths	Vegetative cell color, shape & size (μm)	Heterocytes	Akinetes (μm)	Hormogonia	Habitat	Origin	Reference
Aliinostoc morphoplasticum	Macroscopic mats on benthic rocks	Loosely arranged with variable tendencies for coiling	Thin, colorless	Brownish Barrel-shaped to spherical to oblong L:2.62–5.20 W: 2.71–3.78	Spherical to elliptical to ovate to oblong	Oblong L:5.69–6.11 W:4.48–4.63	Motile, containing gas vesicles	Eutrophic, polluted pond with slightly alkaline pH	Sihora, Jabalpur, India	Bagchi et al 2017
Aliinostoc catenatum	Thick bluish green mats with significant amount of mucilage around them	Loosely arranged with a slight tendency for coiling	Lightly colored	Bright greenish-blue Cylindrical to barrel shaped L:3.2–9.5 W: 2.4–4.0	Spherical to cylindrical	Mostly oval L: 3.4–11.3 W: 3.3–6.4	No information provided	Clay-loam soil with slightly alkaline pH	Semeska-ndeh, Sari, Mazandar-an, Iran	Kabirnataj et al 2020
Aliinostoc magnakinetifex	Greenish blue discrete colonies with tough mucilaginous texture	Straight to coiled (old cultures)	Mucilaginous, lightly colored	Greenish Spherical to squarish or even cylindrical L:1.4–4.3 W: 2.9–4.1	Spherical to cylindrical	Large spherical L: 6.3–10.1 W: 5.2–8.4	No information provided	Clay-loam soil with slightly alkaline pH	Semeska-ndeh, Sari, Mazandar-an, Iran	Kabirnataj et al 2020
Aliinostoc alkaliphilum	Planktic, no colonies are formed in nature	Long, flexuous, with variable tendencies for coiling	Very thin, colorless	Brown cylindrical with rounded ends to oval or barrel-shaped L:(3.3) 3.5–6.6 W: 2.6–3.5	Cylindrical, cylindrical to oval, spherical or oblong	Spherical to oval Up to 12 μm	Motile, containing gas vesicles	Alkaline lake (Salina Verde)	Pantanal wetland, Brazil	This study

Table S5. Detailed description of the ITS secondary structures of *Aliinostoc* species

	Strain name	ITS Secondary structure description
D1-D1′ helices	*A. morphoplasticum* NOS	6-residue basal stem (GACCUA-UAGGUC) + 8-residue asymmetrical internal loop (C-UUUACUC) + 9-residue stem region (CCAAAUCAG-CUGAGAUGG) + 5-residue asymmetrical internal loop (AAA-GA) + 2-residue stem region (UC-GA) + 9-residue asymmetrical internal loop (GAAA-AAAUA) + 2-residue stem region (GC-GC) + 9-residue asymmetrical internal loop (AGAAA-GAAA) + 3-residue stem region (GGC-GCC) + 4-residue symmetrical internal loop (AGAA-AAAA) + 3-residue stem region (GGC-GCC) + 4-residue terminal hairpin (GAAA)
	A. catenatum SA24	6-residue basal stem (GACCUA-UAGGUC) + 8-residue asymmetrical internal loop (C-UCUACUC) + 9-residue stem region (CCAAAUCAG-CUGAGAUGG) + 5-residue asymmetrical internal loop (AAA-GA) + 2-residue stem region (UC-GA) + 9-residue asymmetrical internal loop (GAAA-AAAUA) + 3-residue stem region (GCU-AGC) + 6-residue symmetrical internal loop (CAA-CAA) + 4-residue stem region (AGCU-AGCU) + 9-residue asymmetrical internal loop (AGAAG-AAAA) + 2-residue stem region (GC-GC) + 4-residue terminal hairpin (GAAA)
	A. magnakinetifex SA18	6-residue basal stem (GACCUA-UAGGUC) + 8-residue asymmetrical internal loop (AU-UCUAGC) + 7-residue stem region (CCACUCA-UGAGAGG) +2-residue left bulge (GA) + 4-residue stem region (UCUC-GAGA) + 7-residue asymmetrical internal loop (GAA-AAUA) + 2-residue stem region (GC-GC) + 5-residue terminal hairpin (AAACA)
	A. alkaliphilum CENA513 CENA514 (both tRNA genes)	6-residue basal stem (GACCUA-UAGGUC) + 9-residue asymmetrical internal loop (AU-UCUACUC) +3-residue stem region (CCA-UGG) + 1-residue right bulge (A) + 4-residue stem region (CUCA-UGAG) + 4-residue asymmetrical internal loop (GAA-A) + 4-residue stem region (UCUC-GAGA) + 9-residue asymmetrical internal loop (GAAA-AAAUA) + 2-residue stem region (GC-GC) + 5-residue symmetrical internal loop (GAAAA-GAAAA) + 2-residue stem region (GC-GC) + 5-residue symmetrical internal loop (GGAAA-CGGAA) + 2-residue stem region (GU-GC) + 5-residue terminal hairpin (GAAAA)
	A. alkaliphilum CENA524 (both tRNA genes)	6-residue basal stem (GACCUA-UAGGUC) + 8-residue asymmetrical internal loop (C-UCUACUC) +8-residue stem region (CCAAAUCA-UGAGAUGG) + 7-residue asymmetrical internal loop (GAAA-GAA) + 2-residue stem region (UC-GA) + 9-residue asymmetrical internal loop (GAAA-AAAUA) + 2-residue stem region (GC-GC) + 5-residue terminal hairpin (GAAAA)
	A. alkaliphilum CENA513, 514, 524 (no tRNA genes)	6-residue basal stem (GACCUA-UAGGUC) + 5-residue right bulge (CAUCC) +3-residue stem region (ACC-GGU) + 1-residue right bulge (A) + 5-residue stem region (CAUUA-UAAUG) + 3-residue asymmetrical internal loop (GA-G) +3-residue stem region (GAC-GUC) + 11-residue terminal hairpin (UGAAUCUAAUU)
Box B helices	*A. morphoplasticum* NOS	4-residue basal stem (AGCA-UGCU) + 3-residue asymmetrical internal loop (A-AC) + 5-residue stem region (CUAAC-GUUAG) + 4-residue terminal hairpin (AAUU)
	A. catenatum SA24	5-residue basal stem (AGCAG-CUGCU) + 4-residue asymmetrical internal loop (C-AGA) + 4-residue stem region (GUCA-UGAU) + 4-residue terminal hairpin (CGCU)
	A. magnakinetifex SA18	5-residue basal stem (AGCAC-GUGCU) + 4-residue asymmetrical internal loop (U-CAC) + 3-residue stem region (GAU-GUC) + 5-residue asymmetrical internal loop (GA-AAA) + 2-residue stem region (GU-AU) + 4-residue terminal hairpin (CGAG)
	A. alkaliphilum CENA513, 514, 524 (both tRNAs & no tRNAs)	4-residue basal stem (AGCA-UGCU) + 3-residue asymmetrical internal loop (A-AC) + 5-residue stem region (CUAGC-GUUAG) + 4-residue terminal hairpin (GAAA)

Table S6. Nocuolin A specific ion *m/z* values and formulas by Voráčová et al. [60] and difference (Δ) compared to calculated (Calc) values from MS spectra of CENA 513, 514 and 524 strains. Mass of ions 14 and 16 were inaccurate but accurate in product ion spectra from *m/z* 299.23.

				CENA 513		CENA 514		CENA 524	
No	Name	Ion formula	Calc *m/z*	Exp *m/z*	Δ ppm	Exp *m/z*	Δ ppm	Exp *m/z*	Δ ppm
1	Noc A dimer	$[(C_{16}H_{30}N_2O_3)_2+Na]^+$	619.4405	619.4397	-1.4	619.4398	-1.2	619.4396	-1.5
2	Noc A	$[C_{16}H_{30}N_2O_3+K]^+$	337.1888	337.1879	-2.8	337.1880	-2.5	337.1883	-1.6
3	Noc A	$[C_{16}H_{30}N_2O_3+Na]^+$	321.2149	321.2142	-2.2	321.2143	-1.9	321.2144	-1.6
4	Noc A	$[C_{16}H_{30}N_2O_3+H]^+$	299.2329	299.2330	0.1	299.2335	1.8	299.2330	0.1
5	Fragment	$[C_{16}H_{28}O_2+H]^+$	253.2162	253.2159	-1.4	253.2160	-1.0	253.2158	-1.8
6	-"-	$[C_{16}H_{26}O+H]^+$	235.2056	235.2053	-1.7	235.2050	-2.9	235.2056	-0.4
7	-"-	$[C_{13}H_{26}N_2O+H]^+$	227.2118	227.2111	-3.3	227.2118	-0.2	227.2115	-1.5
8	-"-	$[C_{15}H_{26}O+H]^+$	223.2056	223.2051	-2.6	223.2056	-0.4	223.2054	-1.3
9	-"-	$[C_{13}H_{24}N_2+H]^+$	209.2012	209.2005	-3.7	209.2005	-3.7	209.2002	-5.1
10	-"-	$[C_{10}H_{15}N_2O_2+H]^+$	199.1441	199.1444	1.3	199.1443	0.8	199.1449	3.8
11	-"-	$[C_{10}H_{16}N_2O+H]^+$	181.1335	181.1339	1.7	181.1339	1.7	181.1337	0.6
12	-"-	$[C_{12}H_{18}O+H]^+$	179.1430	179.1432	0.6	179.1435	2.6	179.1429	-1.1
13	-"-	$[C_{11}H_{16}O+H]^+$	165.1274	165.1274	-0.2	165.1276	1.0	165.1279	2.8
14	-"-	$[C_8H_{14}N_2O+H]^+$	155.1179	155.1138	-26.7	155.1131	-31.2	155.1122	-37.0
15	-"-	$[C_{10}H_{16}O+H]^+$	153.1274	153.1277	1.7	153.1277	1.7	153.1278	2.4
16	-"-	$[C_8H_{14}N_2+H]^+$	139.1230	139.1188	-30.3	139.1183	-33.9	139.1185	-32.5
17	-"-	$[C_7H_{14}N_2+H]^+$	127.1230	127.1235	3.8	127.1234	3.0	127.1234	3.0
18	-"-	$[C_7H_{11}N+H]^+$	110.0964	110.0966	1.2	110.0965	0.3	110.0969	3.9
19	-"-	$[C_3H_4N_2O+H]^+$	85.0396	85.0397	0.1	85.0395	-2.2	85.0396	-1.1
20	-"-	$[C_5H_7N+H]^+$	82.0651	82.0657	6.5	82.0654	2.8	82.0657	6.5

Table S7. Bioactive metabolite-producing strains and their GenBank accession numbers

Taxon name	Clade	GenBank accession number	Natural products
***Aliinostoc alkaliphilum* CENA513**	*Aliinostoc*	KX458483	Nocuolin A
***Aliinostoc alkaliphilum* CENA514**	*Aliinostoc*	KX458484	Nocuolin A
***Aliinostoc alkaliphilum* CENA524**	*Aliinostoc*	KX458485	Nocuolin A
Aliinostoc sp. CENA88	*Aliinostoc*	GQ259207	Microcystin
Aliinostoc sp. CENA175	*Aliinostoc*	KC695867	Volatile compounds
Aliinostoc sp. CENA535	*Aliinostoc*	KX458486	Puwainaphycins
Aliinostoc sp. CENA543	*Aliinostoc*	KX458489	Pseudospumigins, nodularin, nostamides, namalides, anabaenapeptins
Aliinostoc sp. CENA548	*Aliinostoc*	KX458492	Puwainaphycins
Aliinostoc sp.FSN-E	*Aliinostoc*	MF680048	Nostosins
Pseudoaliinostoc sp. UIC 10022A	*Pseudoaliinostoc*	HM359085	Cylindrocyclophanes
Pseudoaliinostoc sp. UIC 10366	*Pseudoaliinostoc*	MF622942	Ribocyclophanes
Pseudoaliinostoc sp. UIC 10279	*Pseudoaliinostoc*	JX962720	Ribocyclophanes
Pseudoaliinostoc sp. UIC 10274	*Pseudoaliinostoc*	JX188019	Carbamidocyclophanes
Pseudoaliinostoc sp. CAVN2	*Pseudoaliinostoc*	KJ511229	Carbamidocyclophanes
Pseudoaliinostoc sp. CAVN10	*Pseudoaliinostoc*	KJ511235	Carbamidocyclophanes
Nostoc commune KU002	*Nostoc*	AB088375	1050-Da MAA, Pentose-bound shinorine
Nostoc sp. UIC 10110	*Nostoc*	KY380004	Merocyclophanes
Nostoc sp. UIC 10062	*Nostoc*	JN714978	Merocyclophanes
Nostoc commune (Japan)	*Nostoc*	AB094352	Glycosylated Palythine-threonine, hexose-bound palythine-threonine, hexose-bound porphyra-334
Nostoc sphaericum MAC0910PER	*Nostoc*	AB775902	13-O-(β-galactosyl)-porphyra-334
Nostoc commune KU006	*Nostoc*	AB933330	478-Da MAA, mycosporine-GABA, nostoc-756
Nostoc sp. GSV224	*Nostoc*	AF062637	Cryptophycins, nostopeptolides
Nostoc sp. ATCC53789	*Nostoc*	AF062638	Cryptophycins, nostocyclopeptide, anabaenapeptins
Nostoc sp. IO.102.I	*Nostoc*	AY566855	Microcystins
Nostoc edaphicum CCNP1411	*Nostoc*	CP054698	Nostocyclopeptides, cyanopeptolins
Nostoc sp. Lukesova 5/96	*Nostoc*	AM711546	Scytophycins
Nostoc sp. *P. membranacea* cyanobiont N6	*Nostoc*	JX975209	Nosperin
Nostoc sp. HAN 11/1	*Nostoc*	KP701035	6-OH-7-Ome-15-O-deMe-scytophycin B
Nostoc sp. N107.3	*Nostoc*	KP701040	Swinholide A
Nostoc punctiforme PCC73102	*Nostoc*	NR_114430	Microviridins, nostamide A, nostopeptolide 1052
Nostoc sp. UK18	*Nostoc*	FJ815291	Microcystins
Nostoc sp. UK89IIa	*Nostoc*	JQ771626	Microcystins
Desmonostoc sp. HKAR 2	*Desmonostoc*	FJ939126	Porphyra-334
Desmonostoc muscorum IAM M-14 (= *Nostoc* sp. PCC 7906)	*Desmonostoc*	AB325908	Muscoride A, deprenylmuscoride A
Desmonostoc sp. UHCC0398	*Desmonostoc*	ON693864	Muscoride B, deprenylmuscoride B
Desmonostoc sp. R76DM	*Desmonostoc*	KJ994254	Palythine
Dendronalium phyllosphericum CENA358	*Dendronalium*	KR137579	Anabaenopeptin 882 and 897
Nostoc sp. PCC 7120	Clade A	KM019921	N-(2-aminoethyl)-glycine
Nostoc sp. HKAR 6	Clade B	KF751605	Porphyra-334
Nostoc sp. strain 152	Clade C	AJ133161	Nostophycins A-C, Microcystins
Nostoc minutum NIES 26	Clade D	LC228976	Microviridins G-H, nostopeptins A-B
Nostoc sp. Lukesova 27/97	Clade E	MG596756	Nostotrebin 6
Nostocaceae cyanobacterium CENA352	Clade E	KR137573	Anabaenopeptin 857 and 871
Nostoc linckia UTEX B1932	No 16S rRNA sequencing data available		Nostocyclophanes A-D
Nostoc sp. XSPORK13A			Nostocyclopeptide M1
Nostoc linckia			Borophycin
Nostoc calcicola CBT158			Anabaenopeptins SA1,7,8
Nostoc commune EAWAG 122b			Noscomin, Comnostins A-E, 4,5-dihydroxy-1-methyl-anthraquinone
Nostoc commune			Nostofungicine
Nostoc ellipsosporum			Glucosylsucrose, Glucosylsucrose derivatives 2-4
Nostoc insulare CBT163			Anabaenopeptin SA4-5
Nostoc sp CCAP 1453/38			Nocuolin A

Table S7-*cont*

Taxon name	Clade	GenBank accession number	Natural products
Nostoc sp TAU IL-220	No 16S rRNA sequencing data available		Nostocyclyne A
Nostoc sp. CBT599			Anabaenopeptin SA6
Nostoc sp TAU IL-235			Banyascyclamide A-C, Nostoginins, Nostopeptin BN920, Banyasides A-B, Banyasin A
Nostoc sp. 31			Nostocyclamides A and M
Nostoc sp. 78-12A			Nostocarboline
Nostoc sp. CCIBt 3329			Mycosporine-ornithine
Nostoc sp. DUN901			Microcystins, Nostocyclin
Nostoc sp. (coralloid roots)			β-N-methylamino-L-alanine
Nostoc sp. LTPNA DBG 94			Palythine–threonine
Nostoc sp. Lukešová 30/93			Aeruginosin 865
Nostoc sp. UK2aImI			Nostopeptolide L1-4
Nostoc sp. XPORK5A			Nostoweipeptin W1-7
Nostoc spongiaforme TAU IL-184-6			Tenuecyclamide A-D
Nostoc sp. Peltigera canina symbiot			Nostoclide 1-2
Nostoc sp BEA-0956*		MG543678*	Cybastacine A

**Nucleotide sequence too short to be included in the phylogenetic analyses*