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Chemotyping of terrestrial *Nostoc*-like isolates from alkali grassland areas by non-targeted peptide analysis

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ABSTRACT

The *Nostoc* genus is a well-known heterocytous, filamentous cyanobacterium which can be found all over the world. The size of terrestrial and/or freshwater colonies can be microscopic and macroscopic as well. In addition, *Nostoc* species are one of the most common photosynthetic cyanobacterial partners in symbiotic interactions. Terrestrial cyanobacterial colonies were collected and isolated in this study from various alkali grassland habitats (Great Hungarian Plain). Altogether 133 colonies were isolated from the 65 collected samples. The peptide patterns of the *Nostoc*-like strains were examined using HPLC-ESI-MS/MS and 41 peptides were identified from 45 isolated *Nostoc*-like strains; these compounds belonged to 4 different peptide classes. Twelve nostoginin/microginin, 16 anabaenopeptin, 12 banyaside/suomilide variants were identified. 37% of our isolated *Nostoc*-like strains produced some of the peptide metabolites we tested. These strains showed distinct chemotypes according to their peptide patterns, and can be divided into 4 groups based on their metabolisms. Strains either contained: (1) nostoginins/microginins, (2) anabaenopeptins, (3) anabaenopeptins and banyasides or (4) banyasides as major compounds. Banyasides were present in many of our strains and showed very high intensity in some cases. A number of previously unknown banyaside variants have been identified.

1. Introduction

Cyanobacteria are a highly diverse group of photosynthetic gram-negative prokaryotes. They can be found in a wide range of habitats (freshwater, marine and terrestrial ecosystems) and are able to adapt to special circumstances such as high UV levels, extreme temperatures and pH conditions [1–4]. Their organization can be unicellular, colonial or filamentous and they can be spherical, rod and spiral shaped. Filamentous species are divided into two types, homocytous (= undifferentiated, e.g., *Oscillatoria*) and heterocytous (= differentiated, having heterocytes, e.g., *Nostoc*) [5]. Cyanobacteria are divided into five major sub-sections based on their morphological and physiological characteristics [6], but the groups are not supported by phylogenetic studies. Although several new genera and species within family *Nostocaceae* have been described in the last years including the reclassification of some *Nostoc* isolates, the taxonomic identification of *Nostoc*-like strains is still controversial due to the incongruences between morphological characters and phylogenetic data [7,8].

Nostoc is a genus of heterocytous, filamentous cyanobacteria. They can be found all over the world in almost all circumstances; the size of the colonies can be microscopic as well as macroscopic [1,9]. As a nitrogen-fixing cyanobacterium, *Nostoc* plays a role in the maintenance of soil fertility and has been used as "green manure" in many countries. In tropical and subtropical regions, rice paddies are characterized by the mass presence of *Nostoc* species when they are flooded [9]. They significantly contribute to building-up soil fertility and increase rice yields. *Nostoc* species are one of the most common photosynthetic partners in symbiotic interactions [1,9]. Symbiotic *Nostoc* strains can occur in liverwort, hornwort [10,11], ferns, cycads, angiosperms and lichens [12]. *Nostoc flagelliforme* has great economic value. Its medicinal value has been known since ancient times, and it has been used as food delicacy for about 2000 years. *Nostoc commune* is consumed as

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human food in many countries, because it is rich in fibers and proteins [13,14].

To date, several different cyanobacterial metabolites have been described with a wide range of pharmacological properties (e.g., antibacterial, anticancer, antifungal, antiviral, immunosuppressive, enzyme inhibitory, etc.). Most of these compounds are peptides and are mainly synthetized in a nonribosomal manner via peptide synthetases (NRPS) or combined with polyketide synthetases (NRPS/PKS) [15–17]. Based on their structures, cyanopeptides can be classified into seven major families: aeruginosins, microginins (MG), cyclamides, anabaenopeptins (ABP), cyanopeptolins, microcystins (MCY), and microviridins [16].

Since the 1990s, the number of publications dealing with MCYs and other cyanopeptides has greatly increased. Over the past few decades, many new cyanopeptides have been identified, but only a small part of them belong to the MCYs [18].

Most of the articles are about cyanobacteria that cause water blooms in salt and freshwater ecosystems all over the world, and they focus on the cyanotoxin (MCY, anatoxin, saxitoxin) content of the water [19–26]. Compared to this, there are much fewer studies on metabolites produced by terrestrial and symbiont cyanobacteria [11,27–29]. In several toxicological studies, the effects could not be explained simply by the MCY content of the extract. This suggested that other bioactive metabolites should be taken into account in the investigations and the risk assessment [19,30,31]. *Nostoc* species are a source of different types of metabolites which have significant medical potential [32]. A wide range of hydrocarbons, lipids, pigments, mono- and polysaccharides, terpenoids and aromatic compounds were reported from *Nostoc* species [32–36]. Terpenoids, like noscomin and comnostins have been reported to have antibacterial activity against different bacteria [37–40]. Saccharide-rich extract of the edible species *N. flagelliforme* showed antitumor activity [41,42]. Production of structurally different types of peptides and amino acid derivatives (cryptophycins, nostofungicidine, nostopeptolides, nostoginins (NGs), nostocyclin, nostocyclamides, nostopeptins, nostocyclopeptides, ABPs, MCYs, etc.) with a wide range of pharmacological activities (antimicrobial, cytotoxic, enzyme inhibitor) are characteristic to the *Nostoc* genus [32].

Grasslands are the essential elements of conventional landscapes and are very important in the perspective of biodiversity conservation. The size and species richness of natural grasslands have decreased in Europe as a result of agricultural intensification, urbanization and decline in the extensive traditional land use practices. In spite of the considerable changes in land use regimes in Central-Europe extensive stands of alkali grasslands have remained in a good nature conservation status. These areas are utilized as pastures since their soil is unsuitable for arable crops due to their low-grade soil quality and fluctuating water regime [43]. Terrestrial cyanobacteria can be found in nearly every alkali habitat. Under continental conditions, these areas are covered by water during springtime but dried out until the second half of summer, and the salt content varies from moderate to high. In Europe, these habitats cover approx. 209,152 ha, which is almost entirely (> 98%) located in Hungary. Colonies of *Nostoc* species appear at the beginning of spring, when the soil surface is flooded with water. Swollen colonies form mucous layers which then dry out during summer (Fig. 1) [44].

Beyond the well-known cyanobacterial toxin-screening approaches in this discovery work we focused on the appearance of the rarely investigated peptides also. The bioactivity, toxicology and natural consequences of the cyanobacterial peptide metabolites are in focus nowadays but for understanding the roles of these compounds it is obviously essential to study the real natural producers. In our chemotyping study we analyzed the peptide pattern of the identified *Nostoc* samples originated from a selected area with non-conventional semi-terrestrial habitats where these organisms are dominant with characteristic mats. Terrestrial cyanobacterial colonies were collected and isolated from different habitats of alkali grassland (Great Hungarian Plain). The main objective of this work was the: (i) determination of the main chemotypes of the *Nostoc* species occurring on Hungarian alkaline grasslands; and (ii) identification of well-known and new cyanotoxins and bioactive cyanopeptides produced by *Nostoc* species with non-targeted LC-MS based method.

2. Results

2.1. Description of the study sites

The study area is in the Great Hungarian Plain (East Hungary) and covers approximately 25,000 km^2. The area is characterized by a continental climate with an annual precipitation of 538 mm and mean annual temperature of 10.4 °C [45]. Even though the landscape is predominantly an agricultural one with the dominance of arable lands, large stands of alkali grasslands have been preserved in areas characterized by salt-affected soils. Nostoc species generally occur in alkali steppes (short and closed grasslands, moist in springtime and dry in summer, moderate soil salt content), open alkali grasslands (short and open grasslands, moist in springtime and dry in summer, high soil salt content) and alkali meadows (tall and closed meadows, moist habitat conditions, low soil salt content) [46] (Fig. A1). These habitats generally compose a heterogenous mosaic complex in the landscape. For the habitats present at the studied sites and their most frequent vascular plant species please refer to Table 1.

2.2. Morphology-based identification of the isolated Nostoc and other terrestrial cyanobacteria strains

Altogether 133 colonies were isolated from the 65 collected samples of dry material (Fig. 2 and Table 1). The color of the colonies varied from light green to dark brown; a gelatinous sheath was often present but gas vesicles were rarely characteristic for the colonies. Based on microscopic identification [47,48], most of the isolates (90%) belonged to the *Nostoc* genus (Fig. A2), and in addition, members of order Nostocales (*Calothrix* sp.; *Scytonema* sp.; *Tolypothrix* sp.; *Trichormus* sp.) and order Oscillatoriales (*Leptolyngbya* sp.; *Microcoleus* sp.; *Oscillatoria* sp.; *Phormidium* sp.; *Tychonema* sp.) rarely appeared.

2.3. Molecular phylogenetic analyses and fingerprinting

In the phylogenetic analysis of the isolated and peptide-producing *Nostoc* strains, 16S rRNA gene sequences of our isolates (35) and reference sequences of different *Nostoc* and *Nostoc*-like morphospecies (14) were involved (Fig. 3). Our strains formed three different lineages in the phylogenetic tree (supported with high bootstrap values) separately from the reference strains of *Nostoc*, *Aliinostoc* and *Desmonostoc*, and many of them were only distantly related with sequences of *Nostoc*-like strains available in the GenBank database (95–99% pairwise sequence similarity values, data not shown). No clear affiliation of these groups with the sampling site nor with the metabolic profile could be observed. Additionally, genetic heterogeneity of the isolated strains was examined with DNA fingerprint method. Short tandemly repeated repetitive (STRR) and enterobacterial repetitive intergenic consensus (ERIC) fingerprinting differentiated the isolated strains but no correlation with the result of the phylogenetic analysis were observed.

2.4. Identification of peptides and comparative analysis of Nostoc-like strains

The peptide patterns of the *Nostoc*-like isolates were examined using HPLC-ESI-MS/MS, and the identified compounds are summarized in Table 2. Forty-one peptides were identified from 45 isolated *Nostoc*-like strains, and these compounds belong to 4 different peptide classes.

Twelve compounds were identified as NG/MG variants. These 4–6 amino acid long linear peptides are characterized by an N-terminal octanoic acid derivative (NGs) or decanoic acid derivative (MGs).

Fig. 1. *Nostoc* colonies from the Great Hungarian Plain. Samples were taken from Ágota (location I) sampling site 6 (a); 14 (b); 15 (c) and 16 (d).

Compounds **6** and **8** were identified as NG BN 741 [49] (Fig. 4) and MG SD 755 [50]. Compounds **9** and **11** showed an identical fragmentation pattern to the previous ones, so the 16 Da mass difference in the protonated molecular mass indicates the presence of a hydroxy-tyrosine amino acid at the last position. Compounds **10** and **12** were characterized as analogues of NG BN 741 with amino-hydroxy-decanoic acid (Ahda) at position 1, and in the case of compound **12**, position 5 was occupied by hydroxy-tyrosine. Structures of compounds **4**, **5**, and **7** were derived from NG BN 741. All these variants incorporated phenylalanine at position 5 but position 1 was occupied by amino-hydroxy-octanoic acid (Ahoa), methyl-Ahoa, and Ahda, respectively. Compound **3** exhibited a 16 Da mass decrease compared to compound **4**, which can be explained with a substitution from valine to proline at position 2 and an exchange from methyl-isoleucine to isoleucine at position 3. Compounds **1** and **2** were identified as the shortened variants of compounds **8** and **10**; in both cases the last tyrosine unit is absent (Tables 2 and A1).

Sixteen ABP variants were present in our samples, which can be divided into 3 groups based on the structure of the side chain (positon 1). In the cases of seven ABPs (**13**, **14**, **16**, **18**, **21**, **22** and **24**) isoleucine was present in position 1. Compounds **13** and **14** were identified as schizopeptin (SCP) 791 and ABP 807 [51]. The structure of compound **16** was deduced from MS^3 data according to the fragmentation scheme described by Mayumi et al. [61], and the Ile-CO-[Lys-Val-Hph-MeAsn-Hph] amino acid sequence was postulated. The remaining four ABPs were identified as the congeners of **16**. Homophenylalanine at position 4 was found to be methylated in **18** and **22**, and ethylated in **21** and **24**. The 16 Da mass difference between **18** and **22** indicated an amino acid exchange from homophenylalanine to homotyrosine at position 6, and the 14 Da mass increase of **24** compared to **21** suggested a change from valine to isoleucine at position 3. In four ABPs phenylalanine was found in position 1. Compounds **17**, **19**, and **20** were previously described by Sanz et al. [52] and **15** was characterized as a de-methylated form of **17** (alanine at position 5). The remaining five compounds contained tyrosine as a side chain, and four of these were previously described. Compound **23** showed a similar ring fragmentation pattern to SCP 791 with a 26 Da mass difference, which comes from an exchange from phenylalanine to methyl-homophenylalanine at position 6. Compounds **25–28** showed very similar fragmentation patterns and were identified as ABP KB899 [53], ABP MM913 [54], ABP 915 [55] and ABP G [56] (Fig. 4, Tables 2 and A2).

Twelve compounds were grouped together according to their MS/MS fragments, which are characteristic for the class of banyasides (BNS)/suomilides (SUO). Because there are both positively and negatively charged sites (guanidine and sulfate groups) in the structure, in positive ion mode positively charged ions in desulfated form ([M + H-SO_3]) can be observed. Compounds **33**, **34** and **38** showed identical MS^2 fragmentation patterns with BNS B [57] (Fig. 4), BNS A [57] and SUO [58,59]. The 98 Da mass difference between BNS A and compound **40** suggested the presence of an additional hexanoic acid on the glucose molecule, as well as in the case of SUO. In addition, congeners of BNS B and BNS A without hexanoic acid were found (compounds **29** and **30**) in our samples. The structures of the remaining six variants (**31**, **32**, **35**, **36**, **37** and **39**) were only partially defined due to insufficient data. According to the MS/MS product ions, modifications (addition or loss of methyl-, ethyl-, propyl-group) which can be observed in these compounds affected the glucose and/or the hexanoic acid unit in all cases (Tables 2 and A3).

Compound **41** was separated from the previous classes because of its very different MS/MS spectra. Although the MS/MS data set was very poor, sufficient information was obtained for the structural determination and on the basis of the data published by Liaimer et al. [62], **41** was identified as nostopeptolide A1/A3 (Fig. 4, Tables 2 and A4).

Table 1
List of the sampling sites and typical habitats and characteristic vascular plant species present on the studied sites.

No.	Sampling sites	Number of samples (codes)	Number of isolated *Nostoc* + other strains	Number of peptide producers	Habitats present on the site	Typical species
I	Ágota	21 (1–21)	35 + 8	16 (from 8 different samples)	Alkali steppe, open alkali grassland, alkali meadow	*Pholiurus pannonicus*, *Artemisia santonicum*, *Festuca pseudovina*, *Plantago tenuiflora*, *Puccinellia limosa*
II	Zám	8 (22–29)	27	7 (from 5 different samples)	Alkali steppe, open alkali grassland, alkali meadow	*Puccinellia limosa*, *Artemisia santonicum*, *Limonium gmelinii*, *Camphorosma annua*, *Plantago tenuiflora*
III	Pente_É	6 (30–35)	7	1	Open alkali grassland	*Pholiurus pannonicus*, *Artemisia santonicum*, *Puccinellia limosa*, *Camphorosma annua*, *Festuca pseudovina*
IV	Furta	2 (37–38)	3 + 1	2 (from 2 different samples)	Open alkali grassland	*Pholiurus pannonicus*, *Polygonum aviculare*, *Alopecurus geniculatus*, *Artemisia santonicum*, *Plantago tenuiflora*
V	Gyula	2 (39–40)	3 + 1	3 (from 2 different samples)	Open alkali grassland	*Artemisia santonicum*, *Hordeum hystrix*, *Limonium gmelinii*, *Plantago tenuiflora*, *Puccinellia limosa*
VI	Kígyós	4 (41–44)	7	3 (from 2 different samples)	Open alkali grassland	*Artemisia santonicum*, *Lepidium ruderale*, *Puccinellia limosa*, *Camphorosma annua*, *Plantago tenuiflora*
VII	Fülöpszállás	4 (45–48)	14	6 (from 4 different samples)	Open alkali grassland, alkali meadow	*Lepidium crassifolium*, *Agrostis stolonifera*, *Alisma lanceolata*, *Crypsis aculeata*, *Puccinellia limosa*
VIII	Pentezug	6 (49–54)	7 + 3	3 (from 3 different samples)	Open alkali grassland, alkali meadow	*Puccinellia limosa*, *Artemisia santonicum*, *Festuca pseudovina*, *Pholiurus pannonicus*, *Camphorosma annua*
IX	Egyek	2 (55–56)	2	1	Open alkali grassland	*Puccinellia limosa*, *Artemisia santonicum*, *Festuca pseudovina*, *Pholiurus pannonicus*, *Plantago tenuiflora*
X	Váncsod	2 (57–58)	2	0	Open alkali grassland	*Puccinellia limosa*, *Artemisia santonicum*, *Festuca pseudovina*, *Pholiurus pannonicus*, *Scorzonera cana*
XI	Konyár	2 (59–60)	3	0	Open alkali grassland	*Puccinellia limosa*, *Camphorosma annua*, *Plantago maritima*, *Cynodon dactylon*, *Pholiurus pannonicus*
XII	Derecske	5 (61–65)	9	3 (from 2 different samples)	Open alkali grassland	*Puccinellia limosa*, *Camphorosma annua*, *Plantago maritima*, *Cynodon dactylon*, *Artemisia santonicum*
XIII	Szálka-halom	1 (BGSD.2012)	1	1	Open alkali grassland	*Puccinellia limosa*, *Plantago maritima*, *Cynodon dactylon*

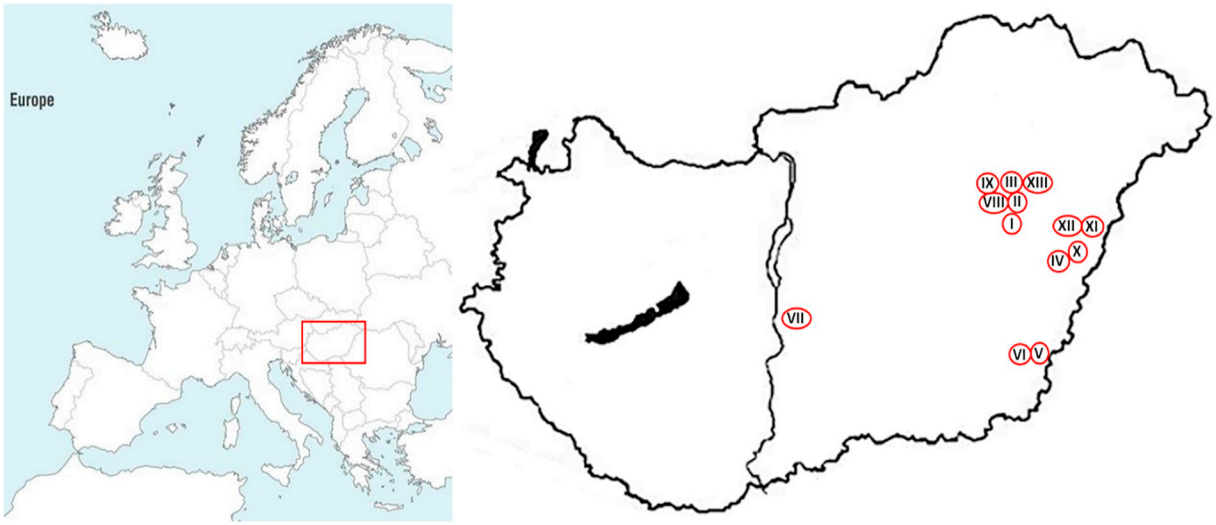

Fig. 2. Location of the sampling sites in Hungary.

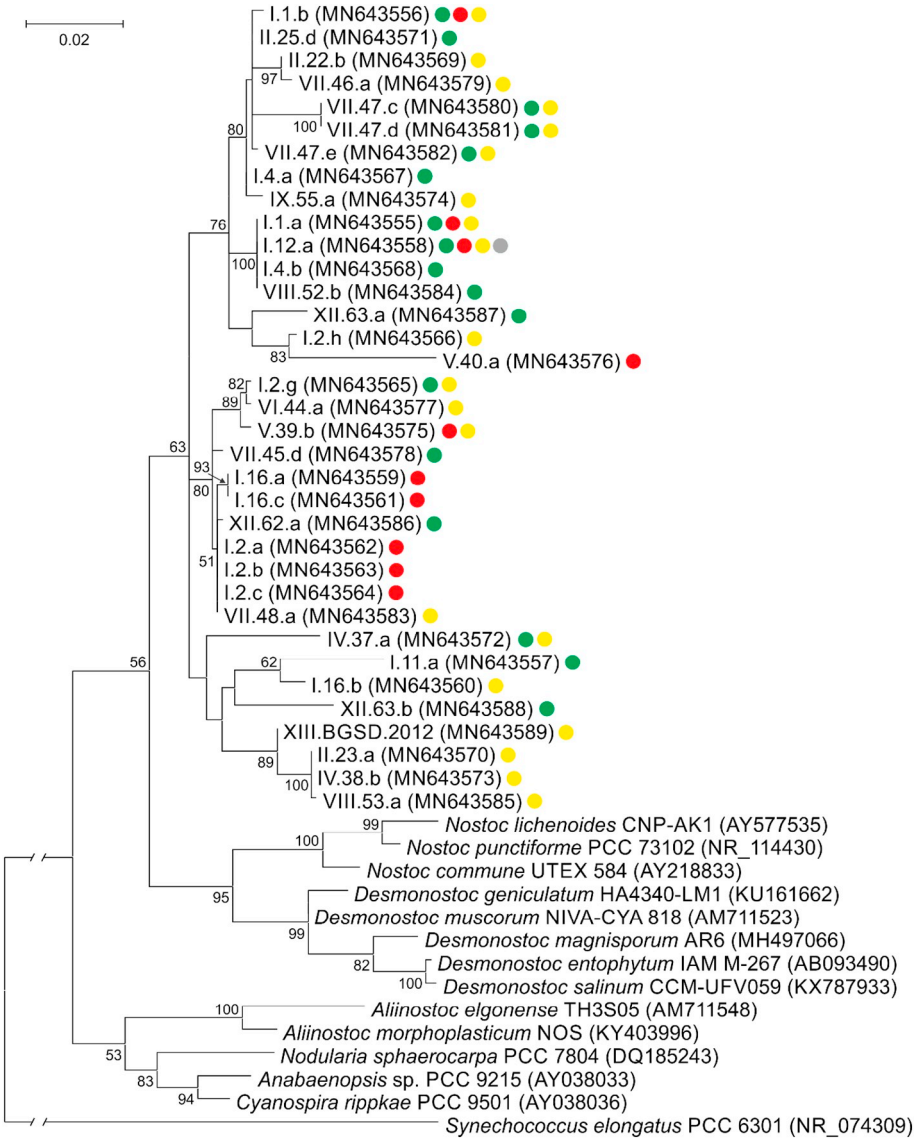

Fig. 3. Phylogenetic position of the studied *Nostoc*-like strains. The tree was constructed based on 1030 nucleotide positions of the 16S rRNA gene and the evolutionary history was inferred using the maximum likelihood method based on the Kimura 2-parameter model (K2 + G + I). Bootstrap values > 50% are given at nodes. GenBank accession numbers are shown in parentheses. Produced peptide-types are marked with colored circles: ● anabaenopeptin, ● nostoginin/microginin, ● banyaside/suomilide, ● nostopeptolide.

Table 2
Identified peptide-type metabolites from isolated *Nostoc*-like strains. Leucine and isoleucine cannot be distinguished from LC-MS/MS data, these amino acids have been deduced from the nearest literary results.

No.	Identified peptide	*m/z* [M + H] +	RT (min)	Compound	Ref.
Nostoginins/microginins (NG/MG)					
1	NG 592	593.21	5.75	MeAhoa-Val-NMeIle-NMeTyr	
2	MG 606	607.25	6.02	Ahda-Val-NMeIle-NMeTyr	
3	NG 709	710.31	6.75	Ahoa-Pro-Ile-NMeTyr-Phe	
4	NG 725	726.23	6.48	Ahoa-Val-NMeIle-NMeTyr-Phe	
5	NG 739	740.32	6.67	MeAhoa-Val-NMeIle-NMeTyr-Phe	
6	NG BN741	742.23	5.86	Ahoa-Val-NMeIle-NMeTyr-Tyr	[49]
7	MG 753	754.21	7.01	Ahda-Val-NMeIle-NMeTyr-Phe	
8	MG SD755	756.30	6.04	MeAhoa-Val-NMeIle-NMeTyr-Tyr	[50]
9	NG 757	758.23	5.78	Ahoa-Val-NMeIle-NMeTyr-OHTyr	
10	MG 769	770.31	6.34	Ahda-Val-NMeIle-NMeTyr-Tyr	
11	NG 771	772.36	5.63	MeAhoa-Val-NMeIle-NMeTyr-OHTyr	
12	MG 785	786.30	6.1	Ahda-Val-NMeIle-NMeTyr-OHTyr	
Anabaenopeptins/schizopeptins (ABP/SCP)					
13	SCP 791	792.37	8.95	Ile-CO-[Lys-Ile-Hph-MeAla-Phe]	[51]
14	ABP 807	808.22	7.54	Ile-CO-[Lys-Ile-Hty-MeAla-Phe]	[51]
15	ABP 827	828.38	7.83	Phe-CO-[Lys-Val-Hph-Ala-Hty]	
16	ABP 834	835.31	8.21	Ile-CO-[Lys-Val-Hph-MeAsn-Hph]	
17	ABP 841	842.29	7.95	Phe-CO-[Lys-Val-Hph-MeAla-Hty]	[52]
18	ABP 848	849.34	8.64	Ile-CO-[Lys-Val-MeHph-MeAsn-Hph]	
19	ABP 855	856.32	8.40	Phe-CO-[Lys-Ile-Hph-MeAla-Hty]	[52]
20	ABP 857	858.29	6.99	Phe-CO-[Lys-Val-Hty-MeAla-Hty]	[52]
21	ABP 862	863.26	8.87	Ile-CO-[Lys-Val-EtHph-MeAsn-Hph]	
22	ABP 864	865.30	7.35	Ile-CO-[Lys-Val-MeHph-MeAsn-Hty]	
23	ABP 869	870.16	8.64	Tyr-CO-[Lys-Ile-Hph-MeAla-MeHph]	
24	ABP 876	877.16	9.26	Ile-CO-[Lys-Ile-EtHph-MeAsn-Hph]	
25	ABP KB899	900.31	7.69	Tyr-CO-[Lys-Val-Hph-MeHty-Ile]	[53]
26	ABP MM913	914.41	8.10	Tyr-CO-[Lys-Ile-Hph-MeHty-Ile]	[54]
27	ABP 915	916.28	7.09	Tyr-CO-[Lys-Val-Hty-MeHty-Ile]	[55]
28	ABP G	930.35	7.36	Tyr-CO-[Lys-Ile-Hty-MeHty-Ile]	[56]
Banyasides/suomilides (BNS/SUO)[a]					
29	BNS 852	772.15	1.37	Mgs-Leu-(Glu)Abn-Aaep	
30	BNS 895	815.29	1.61	Mgs-Leu-(CGlu)Abn-Aaep	
31	BNS 909	829.34	1.86	Mgs-Leu-(CGlu + 14)Abn-Aaep	
32	BNS 979	899.36	4.25	Mgs-Leu-(Hex-CGlu)-14)Abn-Aaep	
33	BNS B	870.33	4.83	Mgs-Leu-(Hex-Glu)Abn-Aaep	[57]
34	BNS A	913.24	5.10	Mgs-Leu-(Hex-CGlu)Abn-Aaep	[57]
35	BNS 1007	927.43	5.47	Mgs-Leu-((Hex-CGlu) + 14)Abn-Aaep	
36	BNS 1020	940.43	5.66	Mgs-Leu-((diHex-Glu)-28)Abn-Aaep	
37	BNS 1035	955.98	6.84	Mgs-Leu-((Hex-CGlu) + 42)Abn-Aaep	
38	SUO	968.42	6.32	Mgs-Leu-(diHex-Glu)Abn-Aaep	[58,59]
39	BNS 1077	997.35	6.85	Mgs-Leu-((diHex-CGlu)-14)Abn-Aaep	
40	BNS 1091	1011.50	7.24	Mgs-Leu-(diHex-CGlu)Abn-Aaep	
Nostopeptolide (NPL)					
41	NPL A1/A3	1081.50	8.1	But-Ile-[Ser-mPro-LeuAc-Leu-Gly-Asn-Tyr-Pro]	[60]

[a] *m/z* in [M + H-SO_3] + form. Ahoa: 3-amino-2-hydroxy-octanoic acid; Ahda: 3-amino-2-hydroxy-decanoic acid; Mgs: 2-*O*-methylglyceric acid 3-O-sulfate; Hex: hexanoic acid; Glu: α-glucose; CGlu: 3-carbamoyl- α-glucose; Abn: 4-Amino-5,7,9-trihydroxy-2-azabicyclo[3.3.1]nonane-3-carboxylic acid; Aaep: 1-Amidino-3-(2-aminoethyl)-3-pyrroline; But: Butyric acid; MePro: 4-methyl-proline; LeuAc: leucylacetate.

2.5. Distribution of oligopeptides and determination of chemotypes

Only 37% of our isolated *Nostoc*-like strains produced some of the peptide metabolites we tested. These strains showed distinct chemotypes according to their peptide patterns. The strains can be divided into 4 groups based on their natural product pattern. Strains either contained: (1) NGs/MGs, (2) ABPs, (3) ABPs and BNSs or (4) BNSs as major compounds (Fig. 5). While strains containing NG/MG or ABP only formed compact clusters, strains containing BNSs only could be divided into two subgroups, according to BNS subtypes: strains either contained BNS A and its congeners or BNS B and its variants (Fig. 5). Despite the fact that ABPs were present in several strains, the total intensity of these peptides was only about half of that of the NGs/MGs or BNSs. Furthermore, NGs/MGs were present in relatively few samples, but their total intensity was almost the same as that of BNSs. Although different phylogenetic clusters were defined, no correlation was found among the chemotypes and the genotypes.

3. Discussion

3.1. Phylogeny in the context of chemodiversity

Species identification is very difficult in the case of *Nostoc*-like genera, due to the limited morphological taxonomic characters and the morphological changes that occur during cultivation [63,64]. The introduction of genetic markers has raised more and more problems, leading to multiple strains being renamed several times, and resulting in the frequent use of "*Nostoc* sp." as identification. For example, *Nostoc* sp. strain PCC 7120 was originally named as *Nostoc muscorum*, then was moved to the *Anabaena* genus by Rippka et al. [6], and later, based on molecular data (DNA-DNA hybridization [65] and repetitive (STRR) DNA sequences [66]), it was renamed as *Nostoc* sp. strain PCC 7120. As a result of the polyphasic approach, heterogeneity within the *Nostoc* genus was reported in different studies [67–69] and clusters of *Nostoc*-like cyanobacteria was reported later [70–73]. *Desmonostoc* [71] and *Aliinostoc* [73] represent phylogenetically distant clades, although

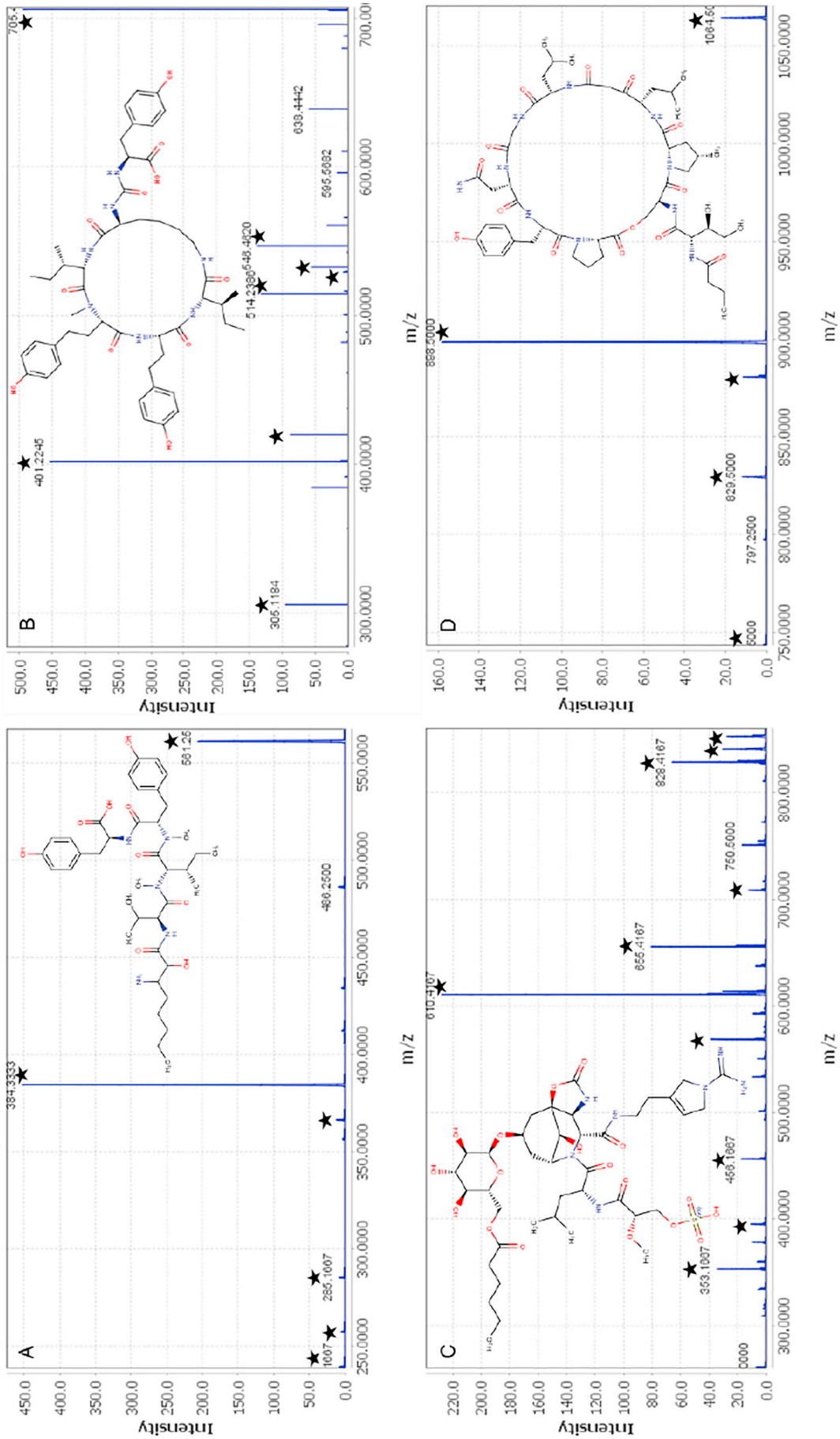

Fig. 4. Fragmentation patterns with characteristic peaks (marks) of the different peptide families. MS^2 fragmentation of nostoginin BN741 (A); MS^3 fragmentation of the ring structure of anabaenopeptin G (B); MS^2 fragmentation of banyaside B (C) and MS^2 fragmentation of nostopeptolide A1/A3 (D).

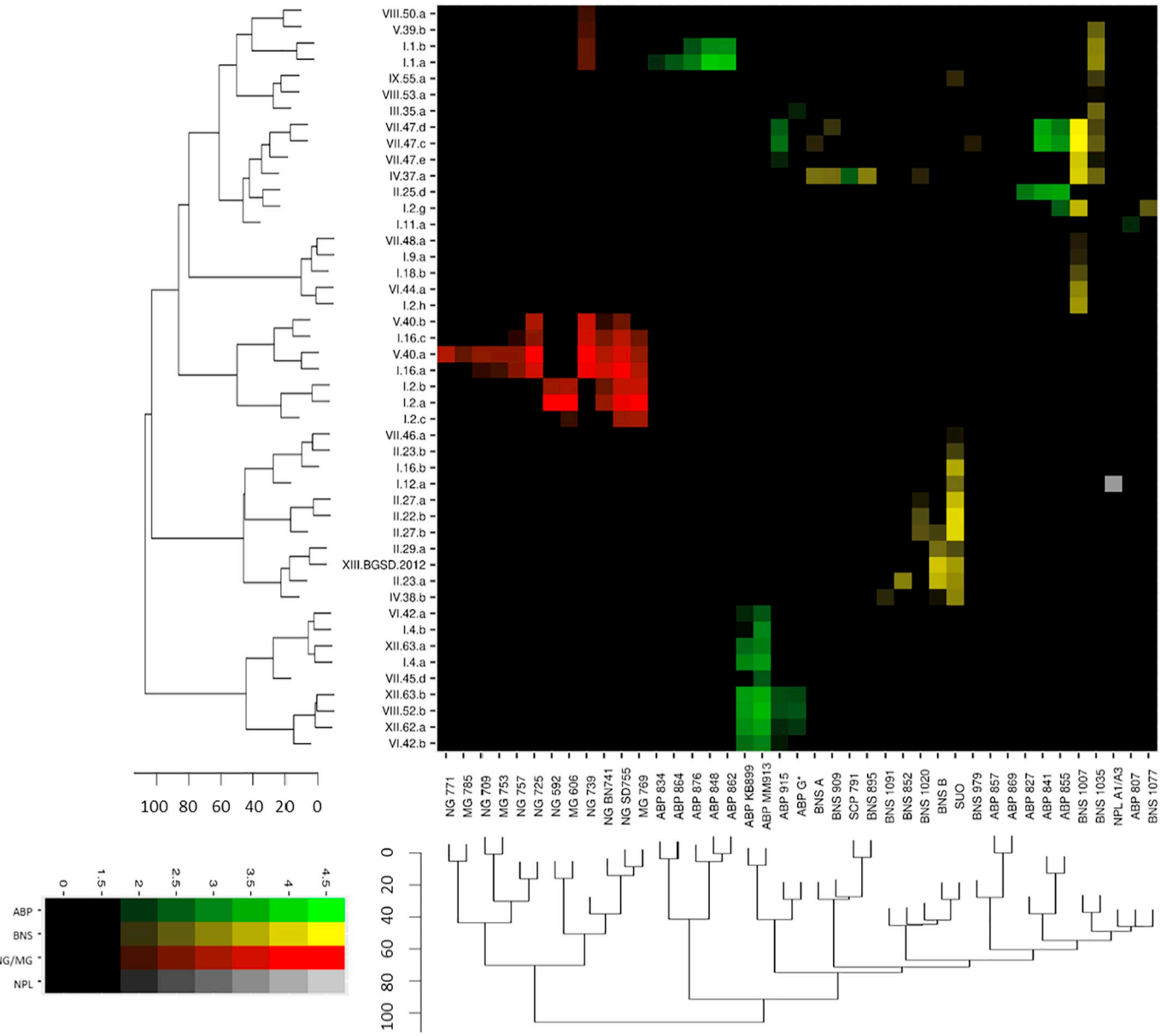

Fig. 5. Abundance heatmap of peptide metabolites from isolated *Nostoc*-like strains. Clustering was done from log10-transformed abundance data, using 'canberra' distance and 'Ward.D2' method. Color is proportional to log-10 abundance. The different identified peptide classes were assigned to different colors: anabenopeptines (ABP), green; microginins (MG)/nostoginins (NG), red; banyasides (BNS)/suomilides (SUO), yellow; nostopeptolide (NPL), grey. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

based on morphological characters these genera are nearly indistinguishable from the members of *Nostoc* sensu stricto due to the presence of huge amount of morphological plasticity within *Nostoc*-like taxa [93].

16S rRNA gene sequence analysis revealed that our strains are related to genera *Nostoc*, *Aliinostoc* and *Desmonostoc* but formed distinct lineages from them, which further extended the cryptic diversity within *Nostoc*. Solely based on 16S rRNA gene-based phylogeny, our strains are supposed to belong to a new genus (or several new genera), but the formal description is not given here, since this study focused on the produced peptides not on taxonomy.

Although our strains can be divided into multiple phylogenetic groups, these groups were not formed reflecting geographical origin or metabolic properties. However, regarding the diversity of the produced peptide type, the main phylogenetic groups showed differences. One group contained only ABP and BNS/SUO producing strains, while another was formed by strains able to produce ABP, NG/MG (and/) or BNS/SUO. In these two clusters only 15% of the peptide-producing strains were able to produce two different types simultaneously. Almost half (46%) of the sequenced peptide-producing strains belonged to a third phylogenetic group and many of them were able to produce several peptide types, the strain which produced all four types also belonged to this group. The observation that strains did not clustered on the phylogenetic tree based on their isolation source nor according their chemotype can be explained by the fact that the dispersal of several free-living microorganisms is not limited [74]. Similar findings regarding geographic distribution have been published earlier in the case of *Microcystis* and freshwater *Synechococcus* [75,76]. The production of different cyanobacterial oligopeptides has been extensively studied worldwide [23,52,77–82], and the lack of correlation between chemotypes and genotypes was found in a similar study on *Microcystis* in Spanish reservoirs [82]. No connection was found between the result of the fingerprinting and the phylogenetic analysis. In addition, the result of the STRR and ERIC fingerprinting showed no strict correlation with

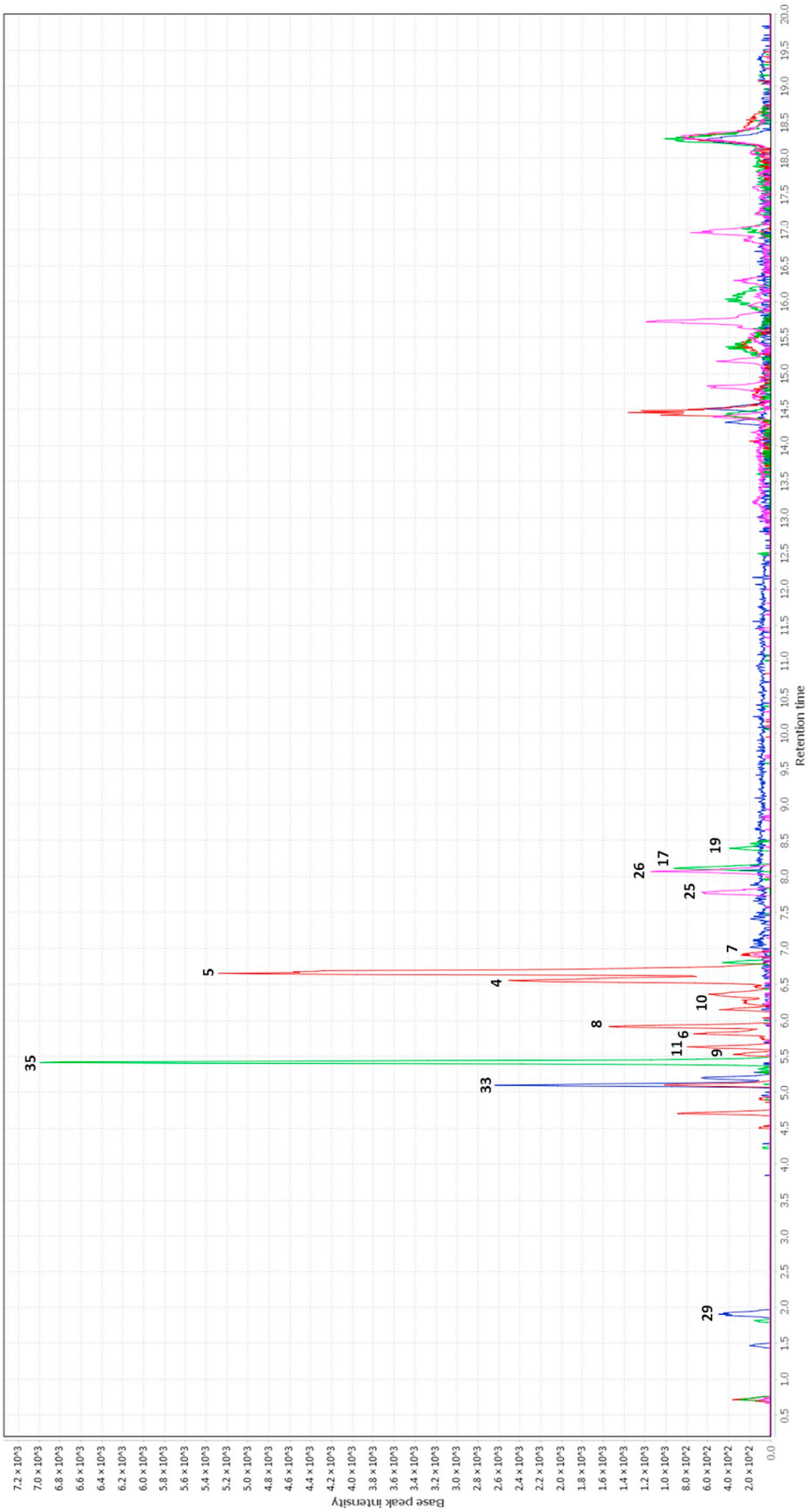

Fig. 6. LC/MS total ion chromatograms of *Nostoc*-like strains from different chemotype groups. Red: nostoginin/microginin producer strain, magenta: anabaenopeptin and banyaside producer strain, blue: banyaside producer strain. Numbers mark different compounds (see Table 2 for details). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the origin of the strains nor the produced peptide type metabolites, but most of the ABP producer strains clustered together (Fig. A3).

3.2. Chemical diversity of Nostoc-like isolates

A little more than a third of our isolates produced some kind of peptide-type metabolite(s). In this study, 41 components were identified, which were classified into 4 peptide classes. Twelve NG/MG, 16 ABP, 12 BNS/SUO and 1 NPL variants were found in the extracts of our strains (Table 2). Despite the fact that MCY and nodularin producing *Nostoc* species have been described in many cases [83–87] neither MCY nor nodularin variants were detected in the studied strains. Interestingly, our strains showed high similarity to *Nostoc* strains from GenBank, such as TAU-MAC 0799 or TAU-MAC 0899, which also did not produce any MCY variants [88]. We could not detect a connection between the sample location and the produced peptide metabolites. However, different chemotype groups can be distinguished at the level of individual strains, based on their peptide metabolite composition and content (Figs. 5 and 6).

Two known and – to the best of our knowledge – 10 new NG/MG variants were identified in our strains (Table 2). NGs/MGs are linear nonribosomal peptides with an α-hydroxy-β-amino derivate of octanoic or decanoic acid. The presence of tyrosine or phenylalanine amino acids at the C-terminal end is common. The number of known variants can be around 40–50 and still growing. These peptides are inhibitors of zinc metalloproteases and aminoproteinases and may be important candidates for the treatment of hypertension [89].

Nine previously described and – to the best of our knowledge – 7 new ABP variants were found in our analysis (Table 2). These hexapeptides are characterized by the formation of a ring structure between amino acids at position 2 and position 6. The one-amino acid side chain is attached to the lysine at position 2 via an ureido group. All the amino acid positions except for 2 are variable, and homo-amino acids and methylated amino acids are characteristic at positions 4 and 5 [16]. The numbers of known ABP variants has risen sharply in the last 15 years; Welker and von Döhren [16] mentioned 32 variants in 2005, while Spoof et al. [90] listed 97 congeners in 2015. ABPs are inhibitors of protease enzymes such as trypsin, chymotrypsin, elastase, and carboxypeptidase A. Some variants (ABP F, OSC B, OSC C) have protein phosphatase inhibitory activity [91], and in some cases calmodulin-activated brain phosphodiesterase and sarcoplasmic reticulum Ca^{2+}-ATPase inhibitory effects have been reported [92,93]. ABP B- and ABP 906-triggered relaxing activity has been detected of norepinephrine-induced contractions of rat aortic preparations [94].

The BNS/SUO is a little known group of cyanobacterial secondary metabolites (Table 2). These glycopeptides are structurally similar to aeruginosins, as they contain an arginine derivative (Aaep) at position 4 [16]. The characteristic bicyclononane moiety in position 3 is linked to an α-glucose which seems to be the only variable part. Positions 1 and 2 are occupied by methylglyceric acid sulfate and leucine [57,58]. The formerly known variants were found to be trypsin and thrombin inhibitors [57]. BNSs were present in many of our strains, which showed very high intensity in some cases, and a number of previously unknown BNS variants have been identified. In a germination test, Sonkoly et al. [44] showed that the BNS-rich extract of BGSD.2012 strain influenced (inhibited) the germination of characteristic species of alkali grasslands.

In cyanobacteria, NPLs were identified from the terrestrial strain *Nostoc* sp. GSV 224 by Golakoti et al. [60]. In the last few years, several new NPL variants and structurally similar nostoweipeptin variants have been identified, and they often occur in symbiotic *Nostoc* strains [62,95]. Each of these cyclic peptides is characterized by non-proteinogenic amino acids such as 4-methylproline or 4-hydroxyproline. Liaimer et al. [62,96] found that nostopeptolides and other polyketide compounds are involved in the life cycle regulation and transition from motile stage to vegetative growth of *Nostoc punctiforme*. Moreover NPLs and nostoweipeptins were identified as antitoxins against MCYs; they inhibited the MCY-induced apoptosis of HEK293 hepatocytes by blocking the organic anion-transporters OATP1B1/B3 which are responsible for MCY uptake [95].

The exact function of many of these metabolites is still unknown. During the examination of symbiotic *Nostoc* species, Liaimer et al. [11] found that some metabolites (e.g. MCYs) may have a role in the development of symbiotic relationships. In addition, they suggested the possibility that certain peptides may play an important role in communication, nutrient binding, or protection (due to their antibiotic activity), as they have appeared in a more significant amount in the medium than intracellularly [11]. These substances can have a significant impact on the species composition of natural communities [44]. On the other hand, these and similar cyanobacterial metabolites may have therapeutic significance [97–101]. According to our results, metabolite content of some *Nostoc* species can vary and present at high concentration values, which is consistent with the results of other experiments [11,52]. Comparing the data with our previous study [102] it can be seen that the studied terrestrial *Nostoc* spp. contained less diverse and fewer amount of peptide metabolites compared to other freshwater species (e.g. *Microcystis* sp.).

Although there are no exact comparative studies between terrestrial and a pelagic cyanobacteria (from the same genus) about the peptide producing ability/peptide content neither in the *Nostoc* nor in the other genus, our results suggest that these studied terrestrial *Nostoc* spp. contained less diverse and fewer amount of peptide metabolites compared to other freshwater species (e.g. *Microcystis* sp., *Planktothrix* sp.) [103–106].

As stated above, the exact ecophysiological function of these peptides is not known, but it is worth emphasizing that our isolates are mostly derived from characteristic plant communities. Given that the cyanotoxin-plant interaction in freshwater has been intensively researched in recent decades [107–111], and some preliminary findings [44] indicate that some terrestrial plant species are sensitive to some bioactive peptides, this raises the possibility of their role as an allelopathic metabolite at terrestrial ecosystems also. However, it cannot be excluded that these bioactive peptides play a role in control of consumers, parasites and/or in intra- and interspecific competition, as is believed for freshwater ecosystems.

4. Conclusions

In this study, we demonstrated the peptide metabolite-producing ability of terrestrial nitrogen-fixing cyanobacteria from different sites of the special alkaline habitats of Hungary. The isolated *Nostoc*-like strains could be classified into different chemotype groups based on their metabolic pattern. A total of 41 peptide-type metabolites were identified which belonged to 4 different peptide families. None of the well-studied cyanotoxins (e.g. MCYs) were found, and many previously unknown biologically active peptide metabolites were identified. Several analyses, in which cyanobacterial strains collected from environmental or bloom/mats samples were targeted, showed negative results if focusing on "just" the well-known toxin families such as like MCYs, nodularins, anatoxins, saxitoxins and β-N-methylamino-L-alanine variants. However other studies emphasized that field and laboratory bioactivity/toxicity cannot be explained (just) with the mentioned cyanotoxins and encourage to get to know new agents. The exact

role of these lesser-known or unknown metabolites remains to be clarified, and they can be significant from the medical point of view also, as they may be important in the development of potential pharmaceutically active ingredients. Our results also draw attention to the use of untargeted test methods. Among our achievements, we highlighted the significance of banyaside producers within the *Nostocaceae* family which were represented in high number in our investigated area and moreover appeared as a prominent chemotype. These glycopeptides which are structurally similar to aeruginosins and found to be protease inhibitors are much more water-soluble than several other peptides because of the sugar moiety. On the basis of the occurrence and the intensity, this peptide family needs more research and further investigation in the future.

5. Materials and methods

5.1. Sample collection and initial sample processing

Terrestrial cyanobacterial samples were collected from different parts (13 sites) of the Great Hungarian Plain in 2013 (Fig. 2 and Table 1). Dry samples were washed 3 times with sterile distilled water and incubated in nitrate-free Allen medium [112] at 26 °C under continuous illumination (100 lx m^{-2} s^{-1}). After 1 week, samples were spread onto 1.5% w/v agar-containing nitrate-free Allen plates and incubated for an additional week. Morphologically different, separate colonies were transferred into 96 wells microplates, each well containing 200 μL nitrate-free Allen medium. Colonies were streaked on plates 3 times and macro-/microscopic heterogeneity were checked. Unialgal isolates were cultured in 1 L nitrate-free Allen containing Erlenmeyer flasks for 2–3 weeks; cells were then harvested by centrifugation (4500*g*; J-10 rotor of Beckman Avanti J-25, Beckman Instruments, Palo Alto, CA, USA) and lyophilized (Christ Alpha 1-2 LD plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz; Germany). 25 mg of each sample was extracted with 80% v/v aqueous methanol. After centrifugation (21000*g*; J-18 rotor of Beckman Avanti J-25; Beckman Instruments, Palo Alto, CA, USA) the supernatant was analyzed by HPLC-ESI-MS/MS in positive ion mode.

5.2. Identification of cyanobacterial peptides

The optimal ESI ionization parameters were as follows: heater temperature, 250 °C; sheath gas, N2; flow rate, 10 arbitrary units (arb); aux gas flow rate, 5 arb; spray voltage, 5 kV; capillary temperature, 375 °C; capillary voltage, 35.00 V. Sample measurement was run in positive ion mode (MS). The LC-MS measurements were run on a Thermo Accela HPLC (Thermo Electron Corp., San Jose, CA, USA; column: Kinetex XB-C18 100 mm × 2.1 mm × 2.6 μm, Phenomenex, Torrance, CA, USA) attached to a Thermo LTQ XL Linear Ion Trap Mass Spectrometer (Thermo Electron Corp., San Jose, CA, USA). Gradient components were A, water with 0.1% v/v formic acid; B, acetonitrile with 0.1% v/v formic acid. The time program was 10–70% B: 0–10 min, 70–100% B: 10–11 min, 100% B 11–16 min, 100–10% B: 16–18 min, 10% B: 18–20 min. The injected sample amount was 1.0 μL in all cases. MS data were processed in Thermo Excalibur version 2.2 SP1.48, and MZmine 2.11 freeware [113]. Identification of secondary metabolites from the MS/MS fragmentation patterns was based on literary data [16,52,60,61,104].

5.3. Statistics

Metabolite abundance data were filtered (values below the threshold value of 1.0 × 10ˆ2 were discarded), and log10 transformed. The transformed data were used to create a distance matrix using `canberra` distance in R 3.6.0, which was used to hierarchical cluster the data using `Ward.D2` method in both directions - among samples as well as among metabolites [114].

5.4. Phylogenetic analysis

For phylogenetic analysis, DNA extracts were made from the strains. Dried cells (ca. 10 mg) were incubated at 65 °C with lysis buffer containing 100 mM TrisHCl (pH 8.0), 1.5 M NaCl, 50 mM EDTA (pH 8.0), 1% w/v SDS. After centrifugation (21000*g*; 5 min) the aqueous phase was mixed in 1:1 with TE saturated phenol three times. The aqueous phase was mixed in 1:1 with chloroform and after centrifugation was transferred into clean Eppendorf tubes. DNA was precipitated 1:3 with 96% chilled-ethanol and stored at −20 °C for at least 1 h. After centrifugation (21000*g*, 20 min, 4 °C) the DNA pellet was washed with 70% v/v chilled ethanol and dried in vacuum. The dried pellet was dissolved in nuclease-free water and stored at −20 °C. DNA concentration was spectrophotometrically measured.

For the PCR, 2× DreamTaq green PCR Master Mix (containing DreamTaq DNA polymerase in DreamTaq Green buffer, dATP, dCTP, dGTP and dTTP, 0.4 mM each, and 4 mM $MgCl_2$) and 16S rRNA gene primers CYA359F (GGG GAA TYT TCC GCA ATG GG) [115] and 1492R (TAC GGY TAC CTT GTT ACG AC) [116] were used. A total reaction volume of 25 μL contained 50 ng of DNA and 0.1 mM of each primer. The cycles were as follows: initial denaturation at 98 °C for 3 min; 32 cycles of 94 °C for 30 s, 52 °C for 45 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. Amplification was verified by agarose gel electrophoresis.

PCR products were purified with Bio Basic EZ-10 Spin Column PCR Purification Kit. Sequencing was carried out by Base Clear B.V. (Leiden, The Netherlands). Sequences were checked with the Chromas 2.6.4 software for the errors of automatic base-calling, then the sequences were subjected to an online similarity search by BLAST at the GenBank of the National Center for Biotechnology Information web site. The closest related reference strains (authentic strains, if that was possible) for phylogenetic analysis were mainly selected based on the data available on taxonomically accepted species names at the AlgaeBase website [117]. Sequences were aligned with the SINA Alignment Service [118]. Phylogenetic analysis (including the search for the best-fit model) was done using MEGA7 software [119]. Obtained sequences were submitted to GenBank under the accession numbers MN643555-MN643589.

5.5. Fingerprinting of the isolated strains

For the fingerprint analysis PCR with primers corresponding to the short tandemly repeated repetitive (STRR) and enterobacterial repetitive intergenic consensus (ERIC) sequences were made [120]. For the STRR primers, the cycles were as follows: initial denaturation at 95 °C for 6 min; 30 cycles of 94 °C for 1 min, 56 °C for 1 min, and 65 °C for 5 min; and a final extension at 65 °C for 16 min.

For the ERIC primers, the cycles were as follows: initial denaturation at 95 °C for 7 min; 30 cycles of 94 °C for 1 min, 52 °C for 1 min, and at 65 °C for 8 min; and a final extension at 65 °C for 16 min [121]. After the reaction, 5 μl of amplified DNA was separated on agarose gels, stained with ethidium bromide and recorded with UVITECH Gel Documentation System (Cambridge, UK). Gel photos were analyzed with UVI-BandMap version 11.9 software.

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CRediT authorship contribution statement

Milán Riba:Investigation, Methodology, Writing - original draft, Writing - review & editing.**Attila Kiss-Szikszai**:Formal analysis, Methodology.**Sándor Gonda**:Investigation, Methodology.**Péter Parizsa**:Investigation.**Balázs Deák**:Investigation.**Péter Török**: Investigation.**Orsolya Valkó**:Investigation.**Tamás Felföldi**: Investigation.**Gábor Vasas**:Conceptualization, Investigation, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest. No conflicts, informed consent, or human or animal rights are applicable to this study.

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Appendix A

Table A1
Product ion data for the nostoginin/microginin type peptides. Masses are given in Dalton rounded to the nearest integer. X1′ indicates core fragment of Ahoa/Ahda after abstraction of the side chain (m = 58 Da). Abbreviation: NG – nostoginin; MG – microginin; nd – not detected.

Product ion assignment	1 (*m/z*)	2 (*m/z*)	3 (*m/z*)	4 (*m/z*)	5 (*m/z*)	6 (*m/z*) [NG BN741]	7 (*m/z*)	8 (*m/z*) [MG SD755]	9 (*m/z*)	10 (*m/z*)	11 (*m/z*)	12 (*m/z*)
X1-X2 – H2O	253.25	267.25	nd	239.17	253.17	239.17	nd	253.17	239.17	267.17	253.17	nd
X1-X2	271.17	285.17	nd	257.25	271.17	257.25	nd	271.25	257.25	285.17	271.17	285.08
X1′-X2-X3 – H2O	nd	267.25	nd	267.25	267.17	nd	nd	267.25	nd	267.17	nd	nd
X1′-X2-X3	285.17	285.17	269.08	285.17	285.17	285.17	285.17	285.25	285.17	285.17	285.08	285.08
X1-X2-X3 – H2O	380.33	nd	nd	366.42	380.33	366.42	394.25	380.33	366.25	394.33	380.33	394.25
X1-X2-X3	398.25	412.33	368.25	384.25	398.25	384.33	412.33	398.25	384.25	412.42	398.25	412.33
X1′-X2-X3-X4 – H2O	nd	nd	nd	nd	448.33	nd	nd	448.17	nd	nd	nd	nd
X1′-X2-X3-X4	nd	nd	nd	nd	462.33	nd	nd	462.08	nd	462.17	462.25	nd
X1-X2-X3-X4 – H2O	nd	nd	nd	nd	557.42	nd	nd	557.34	nd	nd	nd	nd
X1-X2-X3-X4	593.21	607.25	545.33	561.25	575.42	561.25	589.50	575.33	561.25	589.33	575.42	589.50
X1-X2-X3-X4-X5 – H2O	nd	nd	nd	nd	722.58	nd	nd	738.42	nd	752.34	nd	nd
X1-X2-X3-X4-X5	nd	nd	710.31	726.23	740.32	742.23	754.21	756.30	758.23	770.31	772.36	786.30

Table A2
Product ion data for anabaenopeptin type peptides. Masses are given in Dalton rounded to the nearest integer. Abbreviation: SCP – scizopeptin; ABP – anabaenopeptin; nd – not detected.

Product ion assignment	13 (*m/z*) [SCP 791]	14 (*m/z*) [ABP 807]	16 (*m/z*)	18 (*m/z*)	21 (*m/z*)	22 (*m/z*)	24 (*m/z*)	15 (*m/z*)	17 (*m/z*)	19 (*m/z*)	20 (*m/z*)	23 (*m/z*)	25 (*m/z*) [ABP KB899]	26 (*m/z*) [ABP MM913]	27 (*m/z*) [ABP 915]	28 (*m/z*) [ABP G]
X5-X6	233.17	233.17	290.04	290.07	290.10	306.17	290.11	249.08	263.12	263.03	263.10	261.12	305.09	305.03	305.07	305.12
X2-X3-X4 – H2O	385.15	401.20	371.34	385.28	399.29	385.25	413.28	371.17	371.18	385.15	387.14	385.30	371.17	385.21	387.25	401.22
X2-X3-X4	403.16	419.22	389.36	403.24	417.29	403.29	431.29	398.15	398.21	403.16	405.18	403.30	398.24	403.30	405.26	419.41
X5-X6-X2-X3 – H-2O	456.33	456.37	499.83	499.31	499.71	515.20	513.32	458.49	472.39	486.33	472.25	484.36	484.38	498.42	500.29	514.24
X5-X6-X2-X3	474.36	474.41	517.32	517.32	517.33	533.25	531.34	476.16	490.29	504.36	490.27	502.39	502.41	516.35	518.40	532.28
X6-X2-X3-X4 – H-2O	nd	548.34	532.27	546.42	560.31	562.35	574.41	548.26	548.30	562.38	564.31	560.43	514.42	528.23	514.58	528.71
X6-X2-X3-X4	550.42	566.38	550.52	564.32	578.40	580.48	592.47	566.29	266.39	580.31	582.39	578.48	532.41	546.20	532.31	546.46
	nd	nd	nd	nd	nd	nd	nd	619.48	633.51	nd	649.51	nd	675.59	689.64	691.46	nd

(*continued on next page*)

Table A2 (*continued*)

Product ion assignment	13 (*m/z*) [SCP 791]	14 (*m/z*) [ABP 807]	16 (*m/z*)	18 (*m/z*)	21 (*m/z*)	22 (*m/z*)	24 (*m/z*)	15 (*m/z*)	17 (*m/z*)	19 (*m/z*)	20 (*m/z*)	23 (*m/z*)	25 (*m/z*) [ABP KB899]	26 (*m/z*) [ABP MM913]	27 (*m/z*) [ABP 915]	28 (*m/z*) [ABP G]
[X2-X3-X4-X5-X6] – H2O																
[X2-X3-X4-X5-X6]	635.51	651.49	678.47	692.52	706.54	708.54	720.50	637.48	651.45	665.58	667.43	663.56	693.44	707.48	709.47	723.51
CO-[X2-X3-X4-X5-X6] – H2O	nd	nd	nd	nd	nd	nd	nd	645.60	659.43	nd	675.50	nd	701.60	715.75	717.38	nd
CO-[X2-X3-X4-X5-X6]	661.42	677.51	704.42	718.54	732.48	734.47	746.44	663.50	677.42	691.49	693.43	689.67	719.52	733.51	735.44	749.59
X1-CO-[X2-X3-X4-X5-X6] – H2O	nd	nd	nd	nd	nd	nd	nd	810.67	824.53	nd	840.52	nd	882.67	896.56	898.53	912.57
X1-CO-[X2-X3-X4-X5-X6]	792.37	808.22	835.62	849.34	863.26	865.30	877.16	828.38	842.29	856.32	858.29	870.16	900.31	914.41	916.28	930.35

Table A3
Product ion data for banyaside/suomilide type peptides. Masses are given in Dalton rounded to the nearest integer. Abbreviation: BNS – banyaside; SUO – suomilide; nd – not detected.

Product ion assignment	29 (*m/z*)	30 (*m/z*)	31 (*m/z*)	32 (*m/z*)	33 (*m/z*) [BNS B]	34 (*m/z*) [BNS A]	35 (*m/z*)	36 (*m/z*)	37 (*m/z*)	38 (*m/z*) [SUO]	39 (*m/z*)	40 (*m/z*)
X2-X3	353.17	353.08	353.25	353.17	353.17	353.17	353.25	353.08	353.17	353.08	nd	353.17
X3-X4	395.17	395.17	395.17	395.08	395.25	395.17	395.25	nd	359.17	395.08	nd	nd
X1-X2-X3	456.25	456.25	456.25	456.08	456.17	456.08	456.25	nd	456.08	456.33	nd	nd
X1-X2-X3-X4 – CN2H3	568.42	568.42	568.25	568.33	568.33	568.25	568.42	568.42	568.33	568.33	568.25	568.58
X1-X2-X3-X4 – H2O	592.33	592.25	592.25	nd	592.33	592.42	592.33	nd	592.33	592.50	592.50	592.25
X1-X2-X3-X4	610.42	610.42	610.42	610.42	610.33	610.25	610.25	610.33	610.42	610.42	610.42	610.33
(S2-S1) X3-X4	557.25	600.33	614.33	684.42	655.42	698.33	712.25	725.42	740.50	753.50	782.58	796.42
X1-X2-(S2-S1)X3	nd	nd	nd	nd	716.33	nd	nd	nd	801.33	814.08	nd	857.33
X1-X2-(S2-S1)X3-X4 – C-N2H3	730.33	773.42	787.42	857.42	828.50	871.42	885.42	898.50	913.58	926.58	955.42	969.58
X1-X2-(S2-S1)X3-X4 – H2-O	754.50	797.50	811.50	881.50	852.42	895.50	909.25	922.50	937.67	950.67	979.58	993.50
X1-X2-(S2-S1)X3-X4	772.15	815.29	829.34	899.36	870.33	913.24	927.43	940.43	955.98	968.42	997.35	1011.50

Table A4
Product ion data of nostopeptolide A1/A3. Masses are given in Dalton rounded to the nearest integer.

	X9-X10-X3-X4-X5-X6-X7	X6-X7-X8-X9-X10-X3	X3-X4-X5-X6-X7-X8 – H2O	X6-X7-X8-X9-X10-X3-X4	X4-X5-X6-X7-X8-X9-X10	[X3-X4-X5-X6-X7-X8-X9-X10] – H2O	[X3-X4-X5-X6-X7-X8-X9-X10]	X1-X2-[X3-X4-X5-X6-X7-X8-X9-X10] – H2O	X1-X2-[X3-X4-X5-X6-X7-X8-X9-X10]
41 (*m/z*)	614.17	614.17	620.50	743.50	829.42	880.50	898.42	1064.58	1081.50

Fig. A1. Alkali steppes and open alkali grasslands with *Nostoc* colonies (on the top) and alkali meadows (on the bottom).

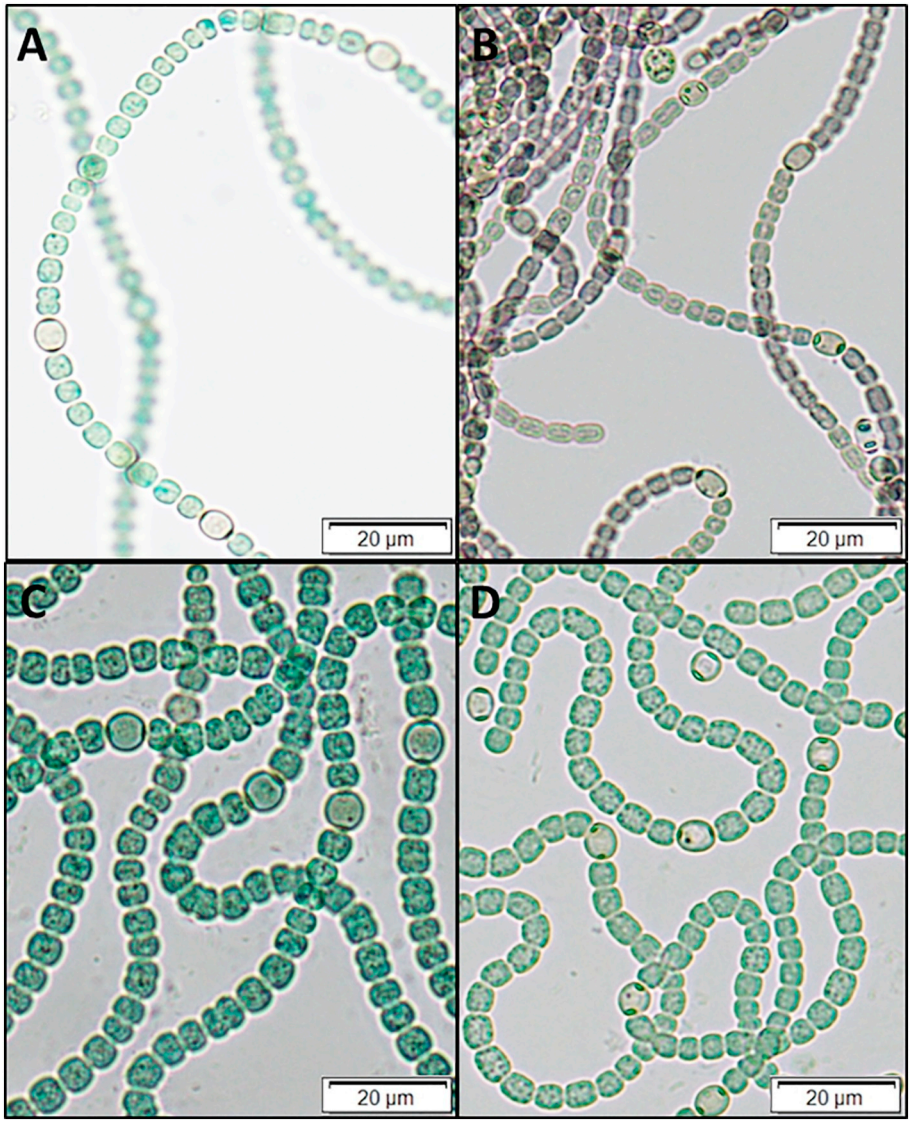

Fig. A2. Microscopic photo of selected peptide metabolite producer *Nostoc*-like strains. A: nostopeptolide A1/A3 producer (I/12/a) strain; B: anabaenopetin producer (II/25/d) strain; C: nostoginin/microginin producer (V/40/a) strain; D: banyaside producer (XIII/BGSD/2012) strain.

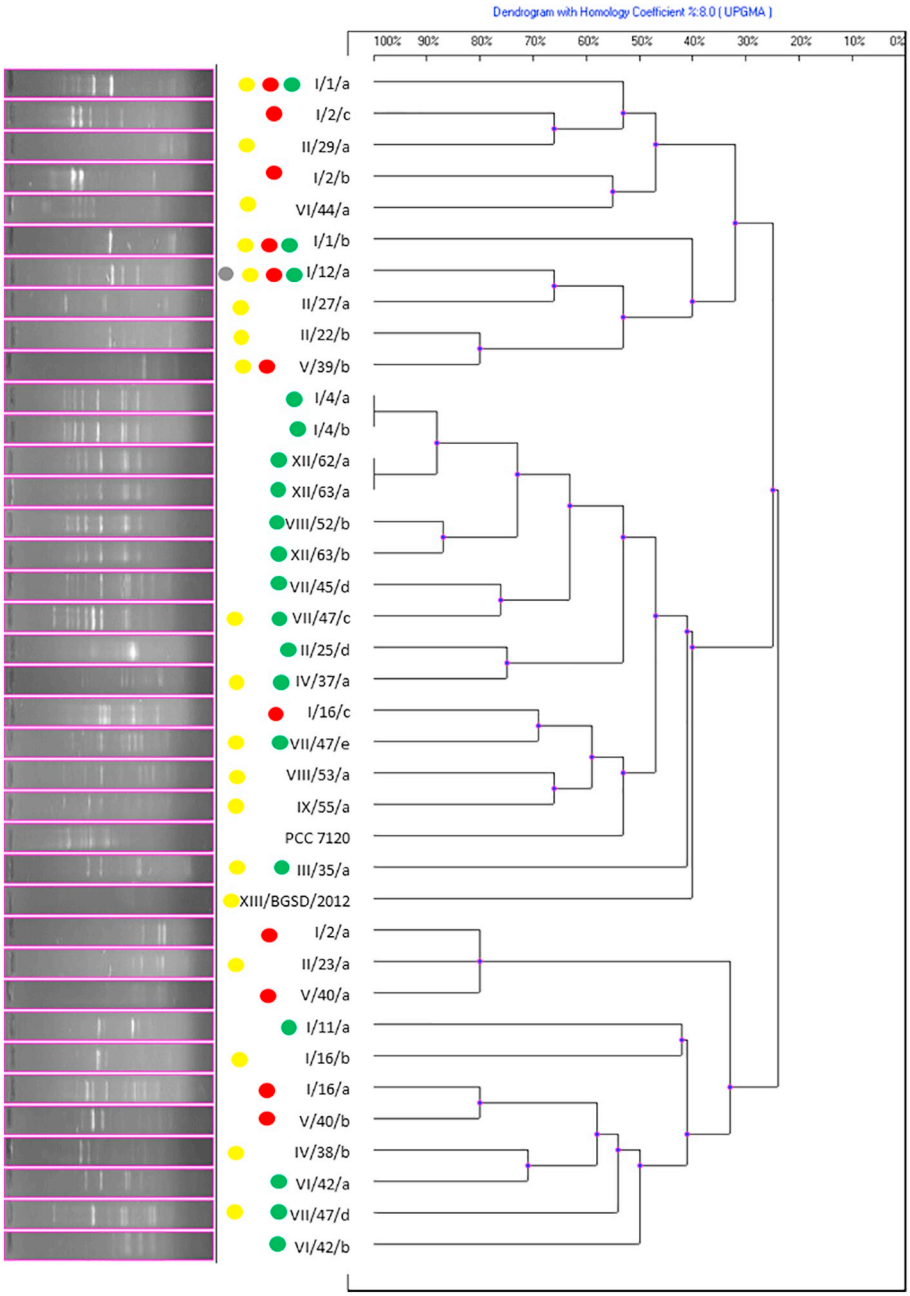

Fig. A3. STRR based dendrogram of peptide metabolite producer *Nostoc*-like strains. Strains were clustered with UPGMA method. Produced peptide-types are marked with colored circles: ● anabaenopeptin, ● nostoginin/microginin, ● banyaside/suomilide, ● nostopeptolide.

References

[1] B.A. Whitton, M. Potts (Eds.), The Ecology of Cyanobacteria, The Netherlands, Springer, 2000.

[2] R.W. Castenholz, A. Wilmotte, M. Herdman, R. Rippka, J.B. Waterbury, I. Iteman, L. Hoffmann, B.X. Phylum, Cyanobacteria. oxygenic photosynthetic bacteria, in: D.R. Boone, R.W. Castenholz, G.M. Garrity (Eds.), Bergey's Manual® of Systematic Bacteriology, Springer-Verlag, New York, NY, 2001, pp. 473–599.

[3] J. Seckbach, Algae and Cyanobacteria in Extreme Environments, 813 Springer, Netherlands, 2007XXXIV.

[4] S. Cirés, M.C. Casero, A. Quesada, Toxicity at the edge of life: a review on cyanobacterial toxins from extreme environments, Marine Drugs 15 (2017) 233.

[5] S.P. Singh, B.L. Montgomery, Determining cell shape: adaptive regulation of cyanobacterial cellular differentiation and morphology, Trends Microbiol. 19 (2011) 278–285.

[6] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, Microbiology 111 (1979) 1–61.

[7] M.F. Gugger, L. Hoffmann, Polyphyly of true branching cyanobacteria (Stigonematales), Int. J. Syst. Evol. Microbiol. 54 (2004) 349–357.

[8] M. Howard-Azzeh, L. Shamseer, H.E. Schellhorn, R.S. Gupta, Phylogenetic analysis and molecular signatures defining a monophyletic clade of heterocystous cyanobacteria and identifying its closest relatives, Photosynth. Res. 122 (2014) 171–185.

[9] W.K. Dodds, D.A. Gudder, D. Mollenhauer, The ecology of *Nostoc*, J. Phycol. 31 (1995) 2–18.

[10] J.L. Costa, P. Paulsrud, J. Rikkinen, P. Lindblad, Genetic diversity of *Nostoc* symbionts endophytically associated with two bryophyte species, Appl. Environ. Microbiol. 67 (2001) 4393–4396.

[11] A. Liaimer, J.B. Jensen, E. Dittmann, A genetic and chemical perspective on symbiotic recruitment of cyanobacteria of the genus *Nostoc* into the host plant *Blasia pusilla* L. Front. Microbiol. 7 (2016) 1693.

[12] K. Pawlowski, Prokaryotic Symbionts in Plants, 1st ed., 306 Springer-Verlag, Berlin Heidelberg, 2009VIII.

[13] X.J. Liu, F. Chen, Cell differentiation and colony alteration of an edible terrestrial cyanobacterium *Nostoc flagelliforme*, in liquid suspension cultures, Folia Microbiol. (Praha) 48 (2003) 619–626.

[14] R.M.M. Abed, S. Dobretsov, K. Sudesh, Applications of cyanobacteria in biotechnology, J. Appl. Microbiol. 106 (2009) 1–12.
[15] A.M. Burja, B. Banaigs, E. Abou-Mansour, J. Grant Burgess, P.C. Wright, Marine cyanobacteria—a prolific source of natural products, Tetrahedron 57 (2001) 9347–9377.
[16] M. Welker, H. von Döhren, Cyanobacterial peptides - nature's own combinatorial biosynthesis, FEMS Microbiol.Lett. 30 (2006) 530–563.
[17] G.E. Chlipala, S. Mo, J. Orjala, Chemodiversity in freshwater and terrestrial cyanobacteria - a source for drug discovery, Curr. Drug Targets 12 (2011) 1654–1673.
[18] E.M. Janssen, Cyanobacterial peptides beyond microcystins - a review on co-occurrence, toxicity, and challenges for risk assessment, Water Res. 151 (2019) 488–499.
[19] H.I. Baumann, F. Jüttner, Inter-annual stability of oligopeptide patterns of *Planktothrix rubescens* blooms and mass mortality of Daphnia in Lake Hallwilersee, Limnologica 38 (2008) 350–359.
[20] S. Gkelis, T. Lanaras, K. Sivonen, Cyanobacterial toxic and bioactive peptides in freshwater bodies of Greece: concentrations, occurrence patterns, and implications for human health, Mar. Drugs 13 (2015) 6319–6335.
[21] R. Kurmayer, E. Schober, L. Tonk, P.M. Visser, G. Christiansen, Spatial divergence in the proportions of genes encoding toxic peptide synthesis among populations of the cyanobacterium *Planktothrix* in European lakes, FEMS Microbiol. Lett. 317 (2011) 127–137.
[22] V.R. Lopes, V. Ramos, A. Martins, M. Sousa, M. Welker, A. Antunes, V.M. Vasconcelos, Phylogenetic, chemical and morphological diversity of cyanobacteria from Portuguese temperate estuaries, Mar. Environ. Res. 73 (2012) 7–16.
[23] H. Mazur-Marzec, M.J. Kaczkowska, A. Blaszczyk, R. Akcaalan, L. Spoof, J. Meriluoto, Diversity of peptides produced by *Nodularia spumigena* from various geographical regions, Marine Drugs 11 (2013) 1–19.
[24] P. Ferranti, S. Fabbrocino, E. Chiaravalle, M. Bruno, A. Basile, L. Serpe, P. Gallo, Profiling *Microcystin* contamination in a water reservoir by MALDI-TOF and liquid chromatography coupled to Q/TOF tandem mass spectrometry, Food Res. Int. 54 (2013) 1321–1330.
[25] M. Grabowska, J. Kobos, A. Torunska-Sitarz, H. Mazur-Marzec, Non-ribosomal peptides produced by *Planktothrix agardhii* from Siemianowka Dam Reservoir SDR (Northeast Poland), Arch. Microbiol. 196 (2014) 697–707.
[26] D. Jancula, L. Strakova, J. Sadilek, B. Marsalek, P. Babica, Survey of cyanobacterial toxins in Czech water reservoirs—the first observation of neurotoxic saxitoxins, Environ. Sci. Pollut. Res. Int. 21 (2014) 8006–8015.
[27] T. Golakoti, W.Y. Yoshida, S. Chaganty, R.E. Moore, Isolation and structure determination of nostocyclopeptides A1 and A2 from the terrestrial cyanobacterium *Nostoc* sp. ATCC53789, J. Nat. Prod. 64 (2001) 54–59.
[28] P. Hrouzek, P. Tomek, A. Lukesova, J. Urban, L. Voloshko, B. Pushparaj, S. Ventura, J. Lukavsky, D. Stys, J. Kopecky, Cytotoxicity and secondary metabolites production in terrestrial *Nostoc* strains, originating from different climatic/geographic regions and habitats: is their cytotoxicity environmentally dependent? Environ. Toxicol. 26 (2011) 345–358.
[29] U. Kaasalainen, D.P. Fewer, J. Jokela, M. Wahlsten, K. Sivonen, J. Rikkinen, Cyanobacteria produce a high variety of hepatotoxic peptides in lichen symbiosis, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 5886.
[30] S. Le Manach, N. Khenfech, H. Huet, Q. Qiao, C. Duval, A. Marie, G. Bolbach, G. Clodic, C. Djediat, C. Bernard, et al., Gender-specific toxicological effects of chronic exposure to pure microcystin-LR or complex *Microcystis aeruginosa* extracts on adult medaka fish, Environ. Sci. Technol. 50 (2016) 8324–8334.
[31] M. Smutná, P. Babica, S. Jarque, K. Hilscherová, B. Maršálek, M. Haeba, L. Bláha, Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in Daphnia magna and the role of microcystins, Toxicon 79 (2014) 11–18.
[32] V.M. Dembitsky, T. Rezanka, Metabolites produced by nitrogen-fixing *Nostoc* species, Folia Microbiol. (Praha) 50 (2005) 363–391.
[33] V.M. Dembitsky, I. Shkrob, I. Dor, Separation and identification of hydrocarbons and other volatile compounds from cultured blue-green alga *Nostoc* sp. by gas chromatography–mass spectrometry using serially coupled capillary columns with consecutive nonpolar and semipolar stationary phases, J. Chromatogr. A 862 (1999) 221–229.
[34] A. Liu, T. Zhu, X. Lu, L. Song, Hydrocarbon profiles and phylogenetic analyses of diversified cyanobacterial species, Appl. Energy 111 (2013) 383–393.
[35] M. Temina, H. Rezankova, T. Rezanka, V.M. Dembitsky, Diversity of the fatty acids of the *Nostoc* species and their statistical analysis, Microbiol. Res. 162 (2007) 308–321.
[36] K. Sharathchandra, M. Rajashekhar, Total lipid and fatty acid composition in some freshwater cyanobacteria, Journal of Algal Biomass Utilization 2 (2011) 83–97.
[37] B. Jaki, J. Orjala, O. Sticher, A novel extracellular diterpenoid with antibacterial activity from the cyanobacterium *Nostoc commune*, J. Nat. Prod. 62 (1999) 502–503.
[38] B. Jaki, J. Heilmann, O. Sticher, New antibacterial metabolites from the cyanobacterium *Nostoc commune* (EAWAG 122b), J. Nat. Prod. 63 (2000) 1283–1285.
[39] B. Jaki, J. Orjala, J. Heilmann, A. Linden, B. Vogler, O. Sticher, Novel extracellular diterpenoids with biological activity from the cyanobacterium *Nostoc commune*, J. Nat. Prod. 63 (2000) 339–343.
[40] A. Ploutno, S. Carmeli, Nostocyclyne A, a novel antimicrobial cyclophane from the cyanobacterium *Nostoc* sp. J. Nat. Prod. 63 (2000) 1524–1526.
[41] H. Takenaka, Y. Yamaguchi, S. Sakaki, K. Watarai, N. Tanaka, M. Hori, H. Seki, M. Tsuchida, A. Yamada, T. Nishimori, et al., Safety evaluation of *Nostoc flagelliforme* (Nostocales, Cyanophyceae) as a potential food, Food Chem. Toxicol. 36 (1998) 1073–1077.
[42] Z. Huang, Y. Liu, B.S. Paulsen, D. Klaveness, Studies on polysaccharides from three edible species of *Nostoc* (cyanobacteria) with different colony morphologies: comparison of monosaccharide compositions and viscosities of polysaccharides from field colonies and suspension cultures, J. Phycol. 34 (1998) 962–968.
[43] P. Török, I. Kapocsi, B. Deák, Conservation and management of alkali grassland biodiversity in Central-Europe, in: W.J. Zhang (Ed.), Grasslands: Types, Biodiversity and Impacts, Nova Science Publishers Inc., New York, NY, USA, 2012, pp. 109–118.
[44] J. Sonkoly, O. Valkó, B. Deák, T. Miglécz, K. Tóth, S. Radócz, A. Kelemen, M. Riba, G. Vasas, B. Tóthmérész, et al., A new aspect of grassland vegetation dynamics: cyanobacterium colonies affect establishment success of plants, J. Veg. Sci. 28 (2017) 475–483.
[45] S.E. Fick, R.J. Hijmans, WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas, Int. J. Climatol. 37 (2017) 4302–4315.
[46] B. Deák, O. Valkó, C. Alexander, W. Mücke, A. Kania, J. Tamás, H. Heilmeier, Fine-scale vertical position as an indicator of vegetation in alkali grasslands – case study based on remotely sensed data, Flora - Morphology, Distribution, Functional Ecology of Plants 209 (2014) 693–697.
[47] J. Komárek, K. Anagnostidis, Cyanoprokaryota, 2. Teil/2nd part: oscillatoriales, in: B. Büdel, L. Krienitz, G. Gärtner, M. Schagerl (Eds.), Süßwasserflora Von Mitteleuropa, Bd. 19/2, Elsevier/Spektrum, Heidelberg, 2005.
[48] J. Komárek, Cyanoprokaryota, 3. Teil/3rd part: heterocytous genera, in: B. Büdel, G. Gärtner, L. Krienitz, M. Schagerl (Eds.), Süßwasserflora Von Mitteleuropa, Bd. 19/3, Elsevier/Spektrum, Heidelberg, 2013.
[49] A. Ploutno, S. Carmeli, Modified peptides from a water bloom of the cyanobacterium *Nostoc* sp. Tetrahedron 58 (2002) 9949–9957.
[50] V. Reshef, S. Carmeli, Protease inhibitors from a water bloom of the cyanobacterium *Microcystis aeruginosa*, Tetrahedron 57 (2001) 2885–2894.
[51] T.K. Shishido, J. Jokela, D.P. Fewer, M. Wahlsten, M.F. Fiore, K. Sivonen, Simultaneous production of anabaenopeptins and namalides by the cyanobacterium *Nostoc* sp. CENA543, ACS Chem. Biol. 12 (2017) 2746–2755.
[52] M. Sanz, A.P. Andreote, M.F. Fiore, F.A. Dorr, E. Pinto, Structural characterization of new peptide variants produced by cyanobacteria from the Brazilian Atlantic coastal Forest using liquid chromatography coupled to quadrupole time-of-flight tandem mass spectrometry, Mar. Drugs 13 (2015) 3892–3919.
[53] S. Elkobi-Peer, S. Carmeli, New prenylated aeruginosin, microphycin, anabaenopeptin and micropeptin analogues from a *Microcystis* bloom material collected in Kibbutz Kfar Blum, Israel, Mar. Drugs 13 (2015) 2347–2375.
[54] E. Zafrir-Ilan, S. Carmeli, Eight novel serine proteases inhibitors from a water bloom of the cyanobacterium *Microcystis* sp. Tetrahedron 66 (2010) 9194–9202.
[55] H.S. Okumura, B. Philmus, C. Portmann, T.K. Hemscheidt, Homotyrosine-containing cyanopeptolins 880 and 960 and anabaenopeptins 908 and 915 from *Planktothrix agardhii* CYA 126/8, J. Nat. Prod. 72 (2009) 172–176.
[56] Y. Itou, S. Suzuki, K. Ishida, M. Murakami, Anabaenopeptins G and H, potent carboxypeptidase A inhibitors from the cyanobacterium *Oscillatoria agardhii* (NIES-595), Bioorg. Med. Chem. Lett. 9 (1999) 1243–1246.
[57] A. Pluotno, S. Carmeli, Banyasin A and Banyasides A and B, three novel modified peptides from a water bloom of the cyanobacterium *Nostoc* sp. Tetrahedron 61 (2005) 575–583.
[58] K. Fujii, K. Sivonen, K. Adachi, K. Noguchi, Y. Shimizu, H. Sano, K. Hirayama, M. Suzuki, K. Harada, Comparative study of toxic and non-toxic cyanobacterial products: a novel glycoside, suomilide, from non-toxic *Nodularia spumigena* HKVV, Tetrahedron Lett. 38 (1997) 5529–5532.
[59] K. Fujii, T. Mayumi, K. Noguchi, T. Kashiwagi, S. Akashi, K. Sivonen, K. Hirayama, K. Harada, Mass spectrometric studies of peptides from cyanobacteria under FAB MS/MS conditions, Journal of the Mass Spectrometry Society of Japan 48 (2000) 56–64.
[60] T. Golakoti, W.Y. Yoshida, S. Chaganty, R.E. Moore, Isolation and structures of nostopeptolides A1, A2 and A3 from the cyanobacterium *Nostoc* sp. GSV224, Tetrahedron 56 (2000) 9093–9102.
[61] T. Mayumi, H. Kato, Y. Kawasaki, K. Harada, Formation of diagnostic product ions from cyanobacterial cyclic peptides by the two-bond fission mechanism using ion trap liquid chromatography/multi-stage mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 1025–1033.
[62] A. Liaimer, E.J.N. Helfrich, K. Hinrichs, A. Guljamow, K. Ishida, C. Hertweck, E. Dittmann, Nostopeptolide plays a governing role during cellular differentiation of the symbiotic cyanobacterium *Nostoc punctiforme*, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 1862.
[63] N. Keshari, S.K. Das, S.P. Adhikary, Identification of cyanobacterial species with overlapping morphological features by 16S rRNA gene sequencing, Eur. J. Phycol. 50 (2015) 395–399.
[64] M.M. Svenning, T. Eriksson, U. Rasmussen, Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA sequence analyses, Arch. Microbiol. 183 (2005) 19–26.
[65] M. Lachance, Genetic relatedness of heterocystous cyanobacteria by deoxyribonucleic acid-deoxyribonucleic acid reassociation, Int. J. Syst. Bacteriol. 31 (1981) 139–147.
[66] D. Mazel, J. Houmard, A.M. Castets, N. Tandeau de Marsac, Highly repetitive DNA sequences in cyanobacterial genomes, J. Bacteriol. 172 (1990) 2755–2761.
[67] P. Rajaniemi, J. Komárek, R. Willame, P. Hrouzek, K. Kaštovská, L. Hoffmann, K. Sivonen, Taxonomic consequences from the combined molecular and phenotype evaluation of selected *Anabaena* and *Aphanizomenon* strains, Algological Studies/Archiv Für Hydrobiologie, Supplement Volumes 117 (2005) 371–391.
[68] P. Hrouzek, S. Ventura, A. Lukesova, M. Angela Mugnai, S. Turicchia, J. Komárek, Diversity of soil *Nostoc* strains: phylogenetic and phenotypic variability, Algol. Stud. 117 (2005) 251–264.
[69] M.A. Fernandez-Martinez, A. de Los Rios, L.G. Sancho, S. Perez-Ortega, Diversity of endosymbiotic *Nostoc* in *Gunnera Magellanica* from Tierra Del Fuego, Chile,

Microb. Ecol. 66 (2013) 335–350.
[70] K. Rehakova, J. Johansen, D. Casamatta, L. Xuesong, J. Vincent, Morphological and molecular characterization of selected desert soil cyanobacteria: three species new to science including *Mojavia pulchra* gen. et sp. nov. Phycologia 46 (2007) 481–502.
[71] P. Hrouzek, A. Lukesova, J. Mares, S. Ventura, Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus Nostoc, Fottea 13 (2013) 201–213.
[72] D.B. Genuario, M.G. Vaz, G.S. Hentschke, C.L. Sant'Anna, M.F. Fiore, *Halotia* gen. nov., a phylogenetically and physiologically coherent cyanobacterial genus isolated from marine coastal environments, Int. J. Syst. Evol. Microbiol. 65 (2015) 663–675.
[73] S.N. Bagchi, N. Dubey, P. Singh, Phylogenetically distant clade of Nostoc-like taxa with the description of *Aliinostoc* gen. nov. and *Aliinostoc morphoplasticum* sp. nov. Int. J. Syst. Evol. Microbiol. 67 (2017) 3329–3338.
[74] I. van Gremberghe, F. Leliaert, J. Mergeay, P. Vanormelingen, K. Van der Gucht, A.E. Debeer, G. Lacerot, L. De Meester, W. Vyverman, Lack of phylogeographic structure in the freshwater cyanobacterium *Microcystis aeruginosa* suggests global dispersal, PLoS One 6 (2011) e19561.
[75] M.C. Bittencourt-Oliveira, M.C. Oliveira, C.J.S. Bolch, Genetic variability of Brazilian strains of the *Microcystis aeruginosa* complex (Cyanobacteria/Cyanophyceae) using the phycocyanin intergenic spacer and flanking regions (cpcBA), J. Phycol. 37 (2001) 810–818.
[76] T. Felföldi, B. Somogyi, K. Márialigeti, L. Vörös, Notes on the biogeography of non-marine planktonic picocyanobacteria, J. Plankton Res. 33 (2011) 1622–1626.
[77] R.P. Rastogi, D. Madamwar, A. Incharoensakdi, Bloom dynamics of cyanobacteria and their toxins: environmental health impacts and mitigation strategies, Front. Microbiol. 6 (2015) 1254.
[78] J. Fastner, M. Erhard, H. von Dohren, Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption ionization-time of flight mass spectrometry, Appl. Environ. Microbiol. 67 (2001) 5069–5076.
[79] D. Harustiakova, M. Welker, Chemotype diversity in *Planktothrix rubescens* (Cyanobacteria) populations is correlated to lake depth, Environ. Microbiol. Rep. 9 (2017) 158–168.
[80] G. Vasas, O. Farkas, G. Borics, T. Felföldi, G. Sramkó, G. Batta, I. Bácsi, S. Gonda, Appearance of *Planktothrix rubescens* bloom with D-Asp3, Mdha7]MC-RR in gravel pit pond of a shallow lake-dominated area, Toxins 5 (2013) 2434–2455.
[81] D. Sanchis, C. Padilla, F.F. Del Campo, A. Quesada, S. Sanz-Alférez, Phylogenetic and morphological analyses of *Microcystis* strains (Cyanophyta/Cyanobacteria) from a Spanish water reservoir, Nova Hedwigia 81 (2005) 431–448.
[82] M. Lezcano, R. Agha, S. Cirés, A. Quesada, Spatial-temporal survey of *Microcystis* oligopeptide chemotypes in reservoirs with dissimilar waterbody features and their relation to genetic variation, Harmful Algae 81 (2019) 77–85.
[83] K. Sivonen, M. Namikoshi, W.R. Evans, M. Fardig, W.W. Carmichael, K.L. Rinehart, Three new microcystins, cyclic heptapeptide hepatotoxins, from *Nostoc* sp. strain 152, Chem. Res. Toxicol. 5 (1992) 464–469.
[84] I. Oksanen, J. Jokela, D.P. Fewer, M. Wahlsten, J. Rikkinen, K. Sivonen, Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp. strain IO-102-I, Appl. Environ. Microbiol. 70 (2004) 5756–5763.
[85] U. Kaasalainen, J. Jokela, D.P. Fewer, K. Sivonen, J. Rikkinen, Microcystin production in the tripartite cyanolichen *Peltigera leucophlebia*, Mol. Plant-Microbe Interact. 22 (2009) 695–702.
[86] R. Kurmayer, The toxic cyanobacterium *Nostoc* sp. strain 152 produces highest amounts of microcystin and nostophycin under stress conditions, J. Phycol. 47 (2011) 200–207.
[87] M.M. Gehringer, L. Adler, A.A. Roberts, M.C. Moffitt, T.K. Mihali, T.J.T. Mills, C. Fieker, B.A. Neilan, Nodularin, a cyanobacterial toxin, is synthesized in planta by symbiotic *Nostoc* sp. The Isme Journal 6 (2012) 1834.
[88] S. Gkelis, M. Panou, D. Konstantinou, P. Apostolidis, A. Kasampali, S. Papadimitriou, D. Kati, G.M. Di Lorenzo, S. Ioakeim, S.-K. Zervou, C. Christophoridis, T.M. Triantis, T. Kaloudis, A. Hiskia, M. Arsenakis, Diversity, cyanotoxin production, and bioactivities of cyanobacteria isolated from freshwaters of Greece, Toxins 11 (2019) 436.
[89] A. Lodin-Friedman, S. Carmeli, Microginins from a *Microcystis* sp. bloom material collected from the Kishon Reservoir, Israel, Marine Drugs 16 (2018) 78.
[90] L. Spoof, A. Blaszczyk, J. Meriluoto, M. Ceglowska, H. Mazur-Marzec, Structures and activity of new anabaenopeptins produced by Baltic Sea cyanobacteria, Mar. Drugs 14 (2015) 8.
[91] T. Sano, T. Usui, K. Ueda, H. Osada, K. Kaya, Isolation of new protein phosphatase inhibitors from two cyanobacteria species, *Planktothrix* spp. J. Nat. Prod. 64 (2001) 1052–1055.
[92] J. Kobayashi, M. Sato, M. Ishibashi, H. Shigemori, T. Nakamura, Y. Ohizumi, Keramamide A, a novel peptide from the Okinawan marine sponge *Theonella* sp. J. Chem. Soc., Perkin Trans. 1 (1991) 2609–2611.
[93] J. Kobayashi, M. Sato, T. Murayama, M. Ishibashi, M. Kanai, J. Shoji, Y. Ohizumi, Konbamide, a novel peptide with calmoduiin antagonistic activity from the Okinawan marine sponge *Theonella* sp. J. Chem. Soc. Chem. Commun. (1991) 1050–1052.
[94] K.I. Harada, T. Mayumi, T. Shimada, M. Suzuki, F. Kondo, M.F. Watanabe, Occurrence of four depsipeptides, aeruginopeptins, together with microcystins from toxic cyanobacteria, Tetrahedron Lett. 34 (1993) 6091–6094.
[95] L. Liu, J. Jokela, L. Herfindal, M. Wahlsten, J. Sinkkonen, P. Permi, D.P. Fewer, S.O. Doskeland, K. Sivonen, 4-Methylproline guided natural product discovery: co-occurrence of 4-hydroxy- and 4-methylprolines in nostoweipeptins and nostopeptolides, ACS Chem. Biol. 9 (2014) 2646–2655.
[96] A. Liaimer, H. Jenke-Kodama, K. Ishida, K. Hinrichs, J. Stangeland, C. Hertweck, E. Dittmann, A polyketide interferes with cellular differentiation in the symbiotic cyanobacterium *Nostoc punctiforme*, Environ. Microbiol. Rep. 3 (2011) 550–558.
[97] R.P. Rastogi, R.P. Sinha, Biotechnological and industrial significance of cyanobacterial secondary metabolites, Biotechnol. Adv. 27 (2009) 521–539.
[98] M. Nagarajan, V. Maruthanayagam, M. Sundararaman, A review of pharmacological and toxicological potentials of marine cyanobacterial metabolites, J. Appl. Toxicol. 32 (2012) 153–185.
[99] S. Vijayakumar, M. Menakha, Pharmaceutical applications of cyanobacteria—a review, Journal of Acute Medicine 5 (2015) 15–23.
[100] B. Nowruzi, S. Haghighat, H. Fahimi, E. Mohammadi, *Nostoc* cyanobacteria species: a new and rich source of novel bioactive compounds with pharmaceutical potential, J. Pharm. Health Serv. Res. 9 (2018) 5–12.
[101] R. Singh, P. Parihar, M. Singh, A. Bajguz, J. Kumar, S. Singh, V.P. Singh, S.M. Prasad, Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: current status and future prospects, Front. Microbiol. 8 (2017) 515.
[102] M. Riba, A. Kiss-Szikszai, S. Gonda, G. Boros, Z. Vitál, A.K. Borsodi, G. Krett, G. Borics, A.Z. Ujvárosi, G. Vasas, *Microcystis* chemotype diversity in the alimentary tract of bigheaded carp, Toxins 11 (2019) 288.
[103] M. Welker, M. Brunke, K. Preussel, I. Lippert, H. von Döhren, Diversity and distribution of *Microcystis* (cyanobacteria) oligopeptide chemotypes from natural communities studied by single-colony mass spectrometry, Microbiology 150 (2004) 1785–1796.
[104] M. Welker, B. Maršálek, L. Šejnohová, H. von Döhren, Detection and identification of oligopeptides in *Microcystis* (cyanobacteria) colonies: toward an understanding of metabolic diversity, Peptides 27 (2006) 2090–2103.
[105] S. Kim Tiam, M. Gugger, J. Demay, S. Le Manach, C. Duval, C. Bernard, B. Marie, Insights into the diversity of secondary metabolites of *Planktothrix* using a biphasic approach combining global genomics and metabolomics, Toxins 11 (2019) 498.
[106] J. Meriluoto, L. Spoof, G.A. Codd (Eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis.; John Wiley & Sons, Inc, Chichester, West Sussex, UK, 2016.
[107] M. M.-Hamvas, C. Máthé, G. Vasas, K. Jámbrik, M. Papp, D. Beyer, I. Mészáros, G. Borbély, Cylindrospermopsin and microcystin-LR alter the growth, development and peroxidase enzyme activity of white mustard (*Sinapis alba* L.) seedlings, a comparative analysis, Acta Biol. Hung. 61 (2010) 55–68.
[108] A.Z. Ujvarosi, M. Riba, T. Garda, G. Gyemant, G. Vereb, M. M-Hamvas, G. Vasas, C. Mathe, Attack of *Microcystis aeruginosa* bloom on a *Ceratophyllum submersum* field: ecotoxicological measurements in real environment with real microcystin exposure, Sci. Total Environ. 662 (2019) 735–745.
[109] D. Beyer, G. Surányi, G. Vasas, J. Roszik, F. Erdődi, M. M-Hamvas, I. Bácsi, R. Bátori, Z. Serfőző, Z.M. Szigeti, et al., Cylindrospermopsin induces alterations of root histology and microtubule organization in common reed (*Phragmites australis*) plantlets cultured in vitro, Toxicon 54 (2009) 440–449.
[110] Z.M. Szigeti, K. Jámbrik, J. Roszik, M. M-Hamvas, I. Tándor, D. Beyer, G. Vasas, G. Vereb, G. Surányi, C. Máthé, Cytoskeletal and developmental alterations in *Ceratophyllum demersum* induced by microcystin-LR, a cyanobacterial toxin, Aquat. Bot. 92 (2010) 179–184.
[111] C. Máthé, D. Beyer, F. Erdődi, Z. Serfőző, L. Székvölgyi, G. Vasas, M. M-Hamvas, K. Jámbrik, S. Gonda, A. Kiss, et al., Microcystin-LR induces abnormal root development by altering microtubule organization in tissue-cultured common reed (*Phragmites australis*) plantlets, Aquat. Toxicol. 92 (2009) 122–130.
[112] M.M. Allen, Simple conditions for growth of unicellular blue-green algae on plates, J. Phycol. 4 (1968) 1–4.
[113] T. Pluskal, S. Castillo, A. Villar-Briones, M. Orešič, MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data, BMC Bioinformatics 11 (2010) 395.
[114] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, 2014.
[115] U. Nübel, F. Garcia-Pichel, G. Muyzer, PCR primers to amplify 16S rRNA genes from cyanobacteria, Appl. Environ. Microbiol. 63 (1997) 3327–3332.
[116] D.J. Lane, 16S/23S rRNA sequencing, in: E. Stackebrandt, M. Goodfellow (Eds.), Nucleic Acid Techniques in Bacterial Systematic, John Wiley and Sons, New York, 1991, pp. 115–175.
[117] M.D. Guiry, G.M. Guiry, AlgaeBase, World-Wide Electronic Publication, National University of Ireland, Galway, 2019.
[118] E. Pruesse, J. Peplies, F.O. Glockner, SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes, Bioinformatics 28 (2012) 1823–1829.
[119] S. Kumar, G. Stecher, K. Tamura, MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874.
[120] U. Rasmussen, M.M. Svenning, Fingerprinting of cyanobacteria based on PCR with primers derived from short and long tandemly repeated repetitive sequences, Appl. Environ. Microbiol. 64 (1998) 265–272.
[121] F.J. de Bruijn, Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergeneric consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria, Appl. Environ. Microbiol. 58 (1992) 2180–2187.