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ABSTRACT

Toxic benthic mats of cyanobacteria are associated with water quality problems and animal poisonings around the world. A strain of the filamentous cyanobacterial genus *Kamptonema* was isolated from a water bloom in the Baltic Sea four decades ago and later shown to produce cylindrospermopsins. However, the exact habitat of this strain remains unclear and cylindrospermopsins have not yet been reported from water blooms in the Baltic Sea. Here, we report the isolation of *Kamptonema* sp. UHCC 0994 from a benthic microbial mat collected in shallow water on the coast of Helsinki. We obtained draft genome sequences for the *Kamptonema* spp. PCC 7926 and UHCC 0994 strains that were isolated from the Baltic Sea. These genomes were 90–96% similar to previously studied *Kamptonema* sp. PCC 6506 and *Kamptonema formosum* PCC 6407, which were isolated from benthic and North American freshwater environments, respectively. The genomes of all four *Kamptonema* strains encode complete cylindrospermopsin biosynthetic gene clusters. We detected the production of cylindrospermopsin and 7-epi-cylindrospermopsin in the four *Kamptonema* strains using high-resolution liquid chromatography mass spectrometry. The four strains encode genes for producing gas vesicles distributed in two to three different regions of their genomes. *Kamptonema* spp. UHCC 0994 and PCC 7926 have both retained the ability to regulate their buoyancy when grown in liquid culture. Together this suggests that these toxic cyanobacteria may exhibit a tychoplanktic lifestyle in the Baltic Sea. This study suggests that microbial mats containing cyanobacteria could be a source of environmental toxins in the Baltic Sea.

1. Introduction

The Baltic Sea is one of the largest brackish waterbodies in the world, covering an area of 420,000 km^2, and has an important economic role for the nine countries surrounding it (HELCOM, 2018). The brackish water environment of the Baltic Sea is prone to annual massive blooms of cyanobacteria, which are commonly observed in more nutrient-depleted regions located in the offshore waters of the Baltic Sea and consequently negatively affect water quality (Löptien and Dietze, 2022). The seasonal succession of phytoplankton eventually results in the appearance of toxic blooms of cyanobacteria at the end of summer (Löptien and Dietze, 2022). Cyanobacteria often produce toxins, including microcystins, nodularins, anatoxins, saxitoxins, cylindrospermopsins and guanitoxin (Chorus and Welker, 2021a). Toxic cyanobacterial blooms in the Baltic Sea are linked to the death of wild and domestic animals and pose a health hazard in recreational water use (Sivonen 2009; Simola et al., 2012; Huisman et al., 2018). Recently, the World Health Organization issued guideline values for safe levels of toxins in drinking and recreational water (Chorus and Welker, 2021b).

The most commonly encountered cyanobacterial toxins are assembled on multi-modular enzyme complexes comprised of non-ribosomal peptide synthetase (NRPS), polyketide synthase (PKS) or hybrid NRPS-PKS enzymes (Dittmann et al., 2013). Cylindrospermopsin is a cytotoxic, hepatotoxic and neurotoxic compound and is produced by a hybrid NRPS/PKS (*cyr*) biosynthetic gene cluster (Dittmann et al., 2013). Toxic cyanobacteria have been found in both planktic and benthic aquatic environments (Kaloudis et al., 2022). Studies over the last decade have highlighted the importance of analyzing benthic

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environments for toxin production and demonstrate the danger in only monitoring planktic blooms of cyanobacteria (Wood et al., 2010a, 2010b, 2018; Faassen et al., 2012; Quiblier et al., 2013; Gaget et al., 2017, 2022; Bauer et al., 2020). The neurotoxic anatoxins and hepatotoxic microcystins can be produced by benthic cyanobacteria and have caused the death of dogs (Wood et al., 2010a, 2010b, 2018), lesser flamingos and cattle (Edwards et al., 1992; Mez et al., 1997; Hamill, 2001; Krienitz et al., 2003; Gugger et al., 2005; Wood et al., 2007; Faassen et al., 2012; Bauer et al., 2020).

The annual blooms of cyanobacteria in the Baltic Sea are invariably toxic through the production of nodularin by the diazotrophic *Nodularia spumigena* (Kankaanpää et al., 2001; Mazur-Marzec et al., 2016). *Dolichospermum* sp. has also been reported to produce microcystins in the planktic brackish water environment of the Baltic Sea (Halinen et al., 2007). Benthic cyanobacteria isolated from the Baltic Sea have been found to produce lipopeptides with cytolytic activity (Herfindal et al., 2005; Jokela et al., 2012). The ability to produce anatoxin-a has been reported for benthic and planktic cyanobacteria from the Gulf of Finland (Mazur et al., 2003; Rantala-Ylinen et al., 2011; Chernova et al., 2019). Cylindrospermopsin production was detected for the strain *Kamptonema* sp. PCC 7926 (Mazmouz et al., 2010) isolated from a surface water bloom in the southern harbour of Helsinki in July 1978 (Vaara et al., 1979). However, cylindrospermopsins have not been reported from cyanobacterial water blooms in the Baltic Sea in the subsequent four decades. Here, we report the isolation of a cylindrospermopsin-producing strain of the genus *Kamptonema* from a benthic microbial mat collected in shallow water on the coast of Helsinki. Bioinformatic analysis of the genomes of cylindrospermopsin-producing *Kamptonema* provides insights into their ecology and suggests that they may have a tychoplanktonic lifestyle in the Baltic Sea.

2. Material and methods

2.1. Sampling and isolation

A mixed algal assemblage in a mat growing on mud was sampled in shallow water on the shoreline of Nuottaniemi, Uutela, located in eastern Helsinki, Finland (60°11′50.0"N 25°09′41.7"E), on July 6, 2021. Filaments that resembled *Kamptonema* (Strunecký et al., 2014) were isolated from an enrichment culture containing the benthic sample (Fig. 1A and B). The isolated strain of *Kamptonema* sp. was obtained by the isolation of a single filament from the enrichment culture to an agar plate. Once purified the strain was deposited in the University of Helsinki Culture Collection under the code UHCC 0994. The *Kamptonema* sp. UHCC 0994 was further maintained by successive transfer in liquid medium using 40 mL of Z8 medium (Kótai, 1972) in 125 mL Erlenmeyer flasks under a photon irradiance of 6 μmol m^{-2} s^{-1} (Fig. 1C and D). Other strains included in this study were obtained from the collection Pasteur Culture of Cyanobacteria (PCC). *Kamptonema* spp. PCC 6506 and PCC 6407 were maintained under the same conditions, while *Kamptonema* sp. PCC 7926 isolated and purified by T. Vaara in 1978 was maintained since by successive transfers in liquid medium BG11 (Rippka et al., 1979) at the collection PCC at 22 °C under a similar light intensity. The purity of the PCC strains was checked at each transfer by placing an aliquot of cell material provided onto a test plate made of solid growth medium supplemented with glucose (0.2%) and casamino acids (0.02%). Test plates were incubated in the dark for 3 days at room

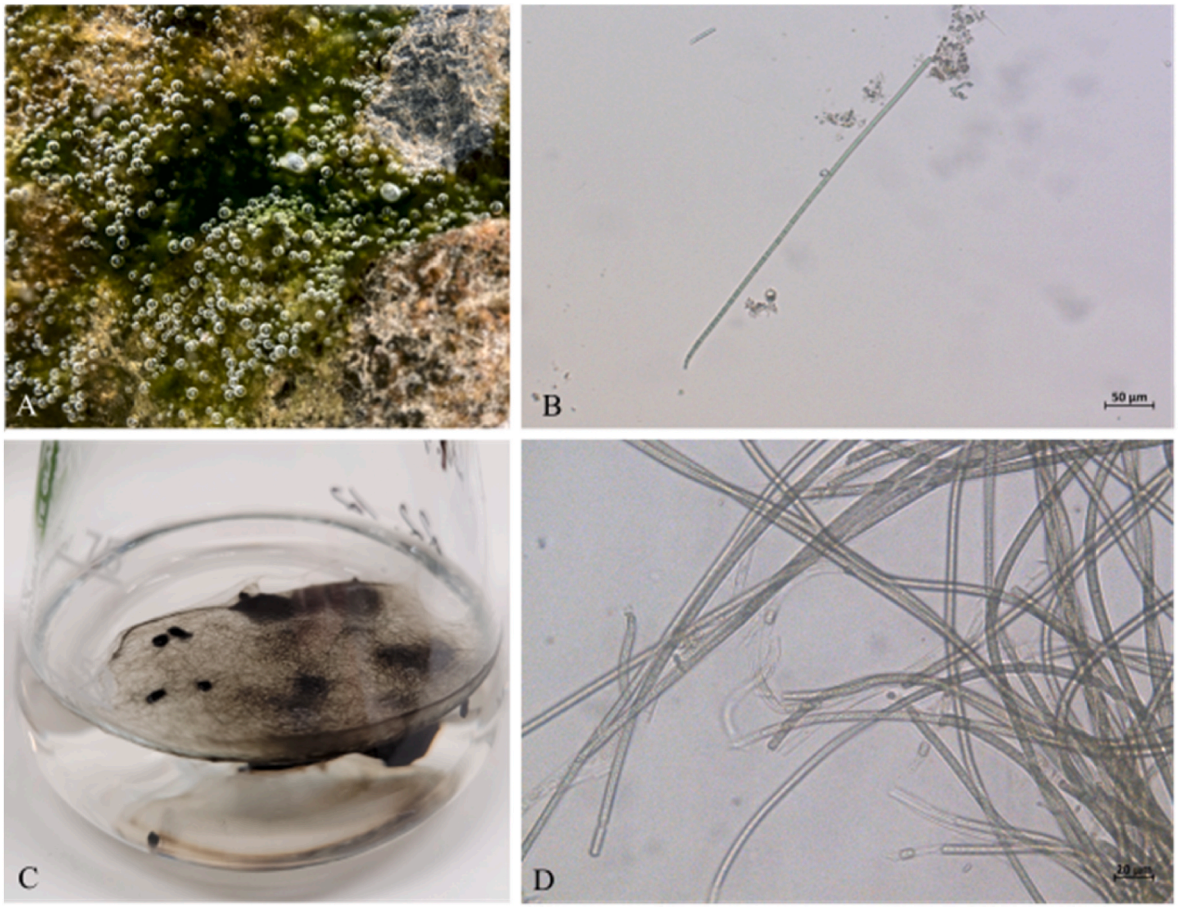

Fig. 1. (A) A benthic mat growing on rocks in shallow water on the shore of Helsinki in the Baltic Sea and (B) a photomicrograph of *Kamptonema* from an enrichment culture containing a sample of the mat. (C) *Kamptonema* sp. UHCC 0994 isolated in a Z8 medium flask and (D) a photomicrograph of the cyanobacteria culture.

temperature prior to examination either by visual inspection or by microscopy using phase contrast objectives and oil immersion. For the experiments, the cultures were cultivated in triplicate using 100 mL of Z8 medium under a constant light intensity of 8 μmol m^{-2} s^{-1} for 17 days at room temperature. The isolated cultures were kept under constant light of 6 μmol m^{-2} s^{-1} at 22 °C in Z8 medium without additional NaCl.

2.2. DNA extraction and genome sequencing

DNA was extracted from *Kamptonema* sp. UHCC 0994 using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). A DNA library was prepared using Nextera XT, as described by the manufacturer, and sequenced using MiSeq (Illumina) in paired-end reads of 2 × 300 bp. DNA was extracted from strain PCC 7926 as previously described (Shih et al., 2013) and sequenced using an Illumina NextSeq 500 system. The sequencing reads were quality trimmed using Cutadapt (Martin, 2011) and PRINSEQ (Schmieder and Edwards, 2011). The quality of the raw and trimmed reads was observed using FastQC (Andrews, 2010). The genomes were assembled using SPAdes v.3.15 (Prjibelski et al., 2020) and the completeness and contamination of the genome were assessed using CheckM (Parks et al., 2015). The Whole Genome Shotgun sequences from *Kamptonema* spp. obtained in this study have been deposited at GenBank under the accession numbers JARFTR000000000 (UHCC 0994) and GCA_949342585 (PCC 7926).

2.3. Bioinformatic analysis

The genomes from *Kamptonema* spp. UHCC 0994 and PCC 7926 were annotated using Prokka v1.14.6 (Seemann, 2014). The sequence average nucleotide identity was calculated using FastANI v1.1 (Jain et al., 2018). The 16S rRNA gene sequence was retrieved with Barrnap v0.9 (Seemann, 2018), together with sequences available from the NCBI, aligned with the MUSCLE online tool (Edgar, 2004) and used for a maximum likelihood phylogenetic tree constructed using RAxML v8.0.0 (Stamatakis, 2014) with 1000 bootstraps using the GTRGAMMAI model. The genomes were analyzed for the detection of secondary metabolite biosynthetic gene clusters using antiSMASH v.6.0 (Blin et al., 2021). The cylindrospermopsin biosynthetic gene clusters were identified and compared with other biosynthetic pathways using clinker v0.0.25 (Gilchrist and Chooi, 2020). The gas vesicle genes were identified with BLAST and compared with clinker v0.0.25 (Gilchrist and Chooi, 2020). Orthologous groups of proteins were identified using Roary v3.12.0 (Page et al., 2015) with a minimum sequence identity of 85% in order to identify unique, accessory and core genes.

A phylogenomic tree was constructed with GTDB-Tk v1.3.0 using the de_novo_wf workflow (Chaumeil et al., 2019), which is based on the identification and alignment of 120 bacterial single-copy conserved marker proteins from the included genomes and genomes selected from the Genome Taxonomy Database (Parks et al., 2018), to infer the maximum-likelihood tree with FastTree v2.1.10 (Price et al., 2010). Phosphorus and nitrogen gene heatmaps were constructed using the HMM profiles provided by the KOfam database (Aramaki et al., 2020) based on KEGG references, searched against the amino acid sequences provided by gene annotations with Prokka v1.14.6 (Seemann, 2014).

2.4. UPLC-QTOF analysis

Cells and liquid media from the studied *Kamptonema* strains were freeze-dried and separated using a Christ LCS Plus Beta 2–8 LCS Plus Freeze Dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The residues were extracted using 1 mL of methanol (Honeywell Riedel-de-Haën, USA) with 0.1% of trifluoroacetic acid (VWR Chemicals, USA) and 300 mg of glass beads (0.5 mm diameter, Scientific Industries INC) in 2-mL plastic tubes, and homogenized using a cell disrupter (FastPrep-24™, MP Biomedicals) two times at a speed of 6.5 m s^{-1} for 30 s. The tubes were centrifuged at 16,000×*g* for 5 min at room temperature. The supernatant (50 μL) was mixed with acetonitrile (150 μL, Honeywell Burdick & Jackson, USA), filtered using a syringe connected to a 0.22-μm PTFE syringe filter (VWR International, USA) and added to vials (VWR International, USA).

Intracellular extracts of the samples were analyzed using a high-resolution ultra-performance liquid chromatograph-quadrupole time-of-flight mass spectrometer (UPLC-QTOF) Acquity I-Class UPLC-Synapt G2-Si HDMS (Waters Corp., USA) system with an ACQUITY UPLC BEH Amide Column (2.1 mm × 100 mm, 1.7 μm, 130 Å; Waters Corp., USA) for separation. The sample injection volume was 1.0 μL and the column temperature was 40 °C. Solvents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) were used with a flow rate of 0.3 mL min^{-1}, starting from 85% of solvent B for 7 min, then decreasing to 65% of B in 2.1 min, and thereafter returning to 85% of B for the remainder of the 15-min runtime. Samples were run in positive electrospray ionization with a capillary voltage of 2.5 kV, with the sampling cone set to 20 V, a source temperature of 120 °C and a desolvation temperature of 600 °C. The cone gas flow was 50.0 L h^{-1}, the desolvation gas flow 1000 L h^{-1}, and the nebulizer gas flow was at 6 bar. Spectra were measured in a mass range from *m/z* 50 to *m/z* 2000. The high collision energy ramp was from 20 to 50 V and the low collision energy was 10 V. QTOF was calibrated using sodium formate, and leucine enkephalin was used at 10-s intervals as a lock mass reference compound.

A volume of 10 μL of intracellular extracts from each strain was also analyzed using UPLC-QTOF with a Lux® Cellulose-2 (50 mm × 2 mm, 3 μm) LC column (Phenomenex, USA) and eluted isocratically with 5/95 of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. Mass spectral data were accumulated in positive electrospray ionization and in a scan range of *m/z* 50–2000. The original mat material was freeze-fried, extracted and analyzed as described above. Cylindrospermopsin was quantified using a standard solution (CRM-CYN-20050531, National Research Council Canada) using serial dilution with known concentrations as follows: 0.02; 0.05; 0.1; 0.2; 0.39; 0.79; 1.58 and 3.15 μg ml^{-1}, resulting in que equation y = 98302x – 2692.6 (R^2 = 0.9997) (Chen et al., 2006).

3. Results

3.1. *Kamptonema* isolated from the benthic zone of the Baltic Sea

Kamptonema sp. UHCC 0994 was isolated from a benthic microbial mat collected from shallow water in the coastal area of Helsinki, Finland (Fig. 1A). The phenotypic characteristics of this cyanobacterium included thin unbranched filaments that lacked differentiated cells (Fig. 1B and D). Trichomes with slightly narrow and bent ends were observed in the mat sample, while a thin sheath appeared in culture, with filament breakage leading to the formation of new filaments (Fig. 1B and D). The brackish strain *Kamptonema* sp. UHCC 0994 might produce sheaths to protect the trichomes when grown in the freshwater medium (data not shown). Phylogenomic analyses based on cyanobacterial genomes available in public databases indicate the grouping of *Kamptonema* spp. UHCC 0994 and PCC 7926 with *Kamptonema* spp. PCC 6505 and PCC 6407 (Fig. 2). The brackish water *Kamptonema* spp. UHCC 0994 and PCC 7926 strains formed a cluster together with other benthic *Kamptonema* strains in a phylogeny based on the 16S rRNA gene (Fig. S1). These strains were isolated from North American (PCC 6506), European freshwaters (PCC 6407 and SAG 1459/6), a puddle on a road in India (INDIA92), a Brazilian botanical garden (CCALA 771), and a French thermal spring (type species *Kamptonema animale* CCALA 139 and the holotype for the genus *Kamptonema* Strunecký, Komárek & Smarda) (Fig. S1). Interestingly, the brackish water strain PCC 7926 is most closely related to the benthic freshwater *Kamptonema*, while the brackish UHCC strain appears to be the most distant strain of the *Kamptonema* cluster (Fig. S1). Nevertheless, BLASTn analyses of the 16S rRNA gene of strain UHCC 0994 indicated 99.04% identity (100%

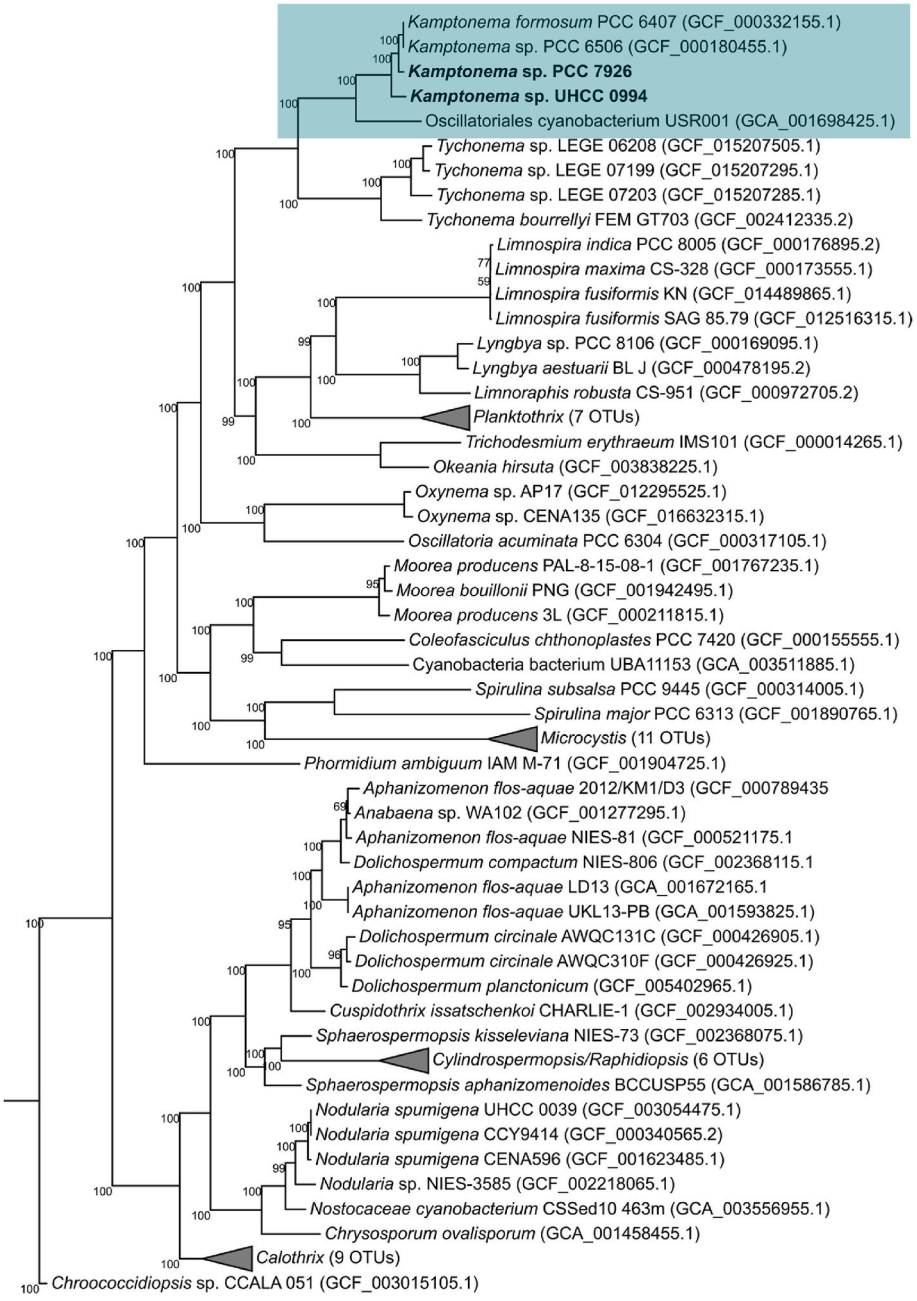

Fig. 2. Phylogenomic analysis using available genomes of cyanobacteria and the genomes of *Kamptonema* spp. UHCC 0994 and PCC 7926 obtained in this study.

coverage) to *Kamptonema animale* SAG 1459/6 (EF654087).

3.2. Genome sequence analysis

The genome sequences of *Kamptonema* spp. UHCC 0994 and PCC 7926 were obtained to gain insights into the ecology and habitat of *Kamptonema* in the Baltic Sea. The genome sequence of *Kamptonema* sp. UHCC 0994 has 6,588,613 bp divided into 319 contigs (≥1000 bp) with a GC content of 43.20%. The largest contig has 152,511 bp, the N50 is 33,292 bp and the L50 is 57 (Table S1). This genome has a completeness of 99.56% and a contamination value of 0.44%, while the genome of *Kamptonema* sp. PCC 7926 revealed 98.36% completeness and 0.66% contamination, consistent with the large number of contigs (Table S2). The genome of *Kamptonema* sp. UHCC 0994 contains 5516 CDS, 5591 genes, 5 rRNA genes, 6 repeat regions and 69 tRNA genes, while that of *Kamptonema* sp. PCC 7926 contains 5605 CDS, 5679 genes, 4 rRNA genes, 7 repeat regions, 69 tRNA genes and 1 tmRNA gene (Table S1). A comparison of the average nucleotide identity (ANI) indicated that these two new genomes of *Kamptonema* share 90.81% identity and belong to different species (Fig. 3). The ANI analysis confirmed that *Kamptonema* sp. PCC 7926 was more closely related to the genomes of *Kamptonema formosa* PCC 6407 and *Kamptonema* sp. PCC 6506 (>96.4%), and that these three strains may belong to the same species (Fig. 3A). In addition, one metagenome-assembled genome (MAG) assigned as Oscillatoriales cyanobacterium USR001 grouped on the basis of the *Kamptonema* clade in the genomic and 16S rRNA gene phylogenetic trees (Fig. 2 and S1), but it appeared much smaller (5.8 Mb) than the *Kamptonema* genomes (6.6–6.9 Mb). The small ANI with *Kamptonema* strains and lower number of orthologous proteins (Fig. 3A and B) corroborates that the MAG Oscillatoriales cyanobacterium USR001 belongs to another genus.

We identified gas vesicle gene clusters in the genomes of *Kamptonema* spp. UHCC 0994, PCC 7926, PCC 6506 and PCC 6407, and MAG

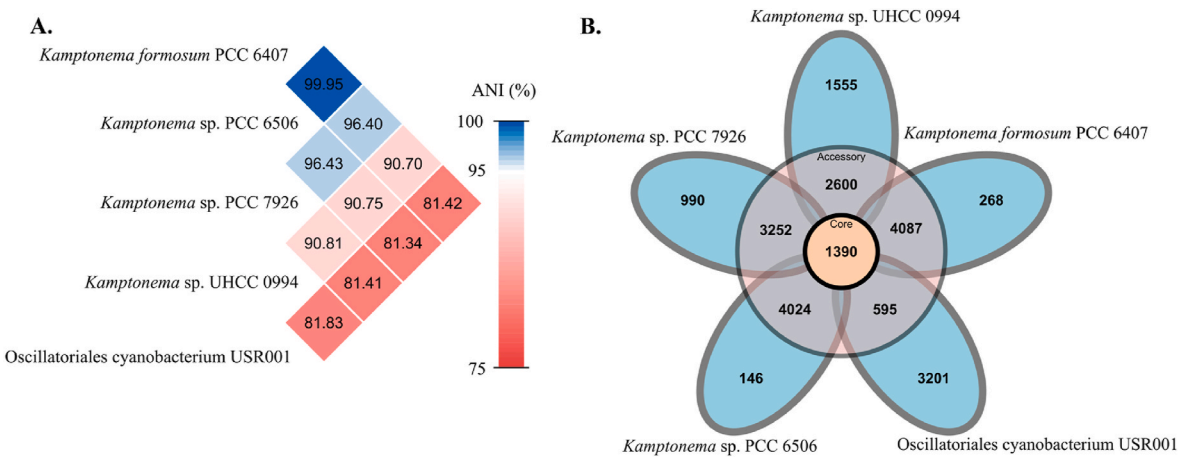

Fig. 3. Comparison of nucleotide sequences of *Kamptonema* spp. and closely related MAG. (A) Average nucleotide identity (ANI). (B) A schematic diagram with clusters of orthologous proteins representing the core, accessory and unique genes across the five strains.

Oscillatoriales cyanobacterium USR001 (gas vesicle protein gene cluster or *gvp;* Fig. 4). The gas vesicle genes were encoded in two separate regions of the genome in the isolated *Kamptonema* spp. and in MAG USR001, except for *Kamptonema* sp. UHCC 0994, which encoded *gvpF* in another region of the genome and lacked the *gvpG* gene (Fig. 4). Noticeably, all *Kamptonema* genomes encode an unusually long *gvpC* gene and a single copy of the *gvpA* gene (Fig. 4). The GvpC protein from *Kamptonema* sp. UHCC 0994 shared just 27.42% sequence identity (66% coverage) with the homologs from other cyanobacterial genera (data not shown).

Protein sequences retrieved from the Kyoto Encyclopaedia of Genes and Genomes (KEGG) indicated the presence of a phosphate-specific transport system (PstSACB), phosphate starvation-inducible protein (PhoH), polyphosphate formation (Ppk), oxidative phosphorylation (Ppa), exopolyphosphatase (Ppx-GppA) and glycerophospholipid metabolism (UgpQ) in all *Kamptonema* genomes and the related MAG analyzed (Fig. S2A). Protein sequences suggesting the ability to fix nitrogen (NifHKD) were encoded in most *Kamptonema* strains, except for *Kamptonema* sp. UHCC 0994 (Fig. S2B). All *Kamptonema* genomes included ferredoxin-nitrate reductase (NarB), ferredoxin-nitrite reductase (NirA) and organic degradation and synthesis (UreABC, GltBS, GlnA; Fig. S2B).

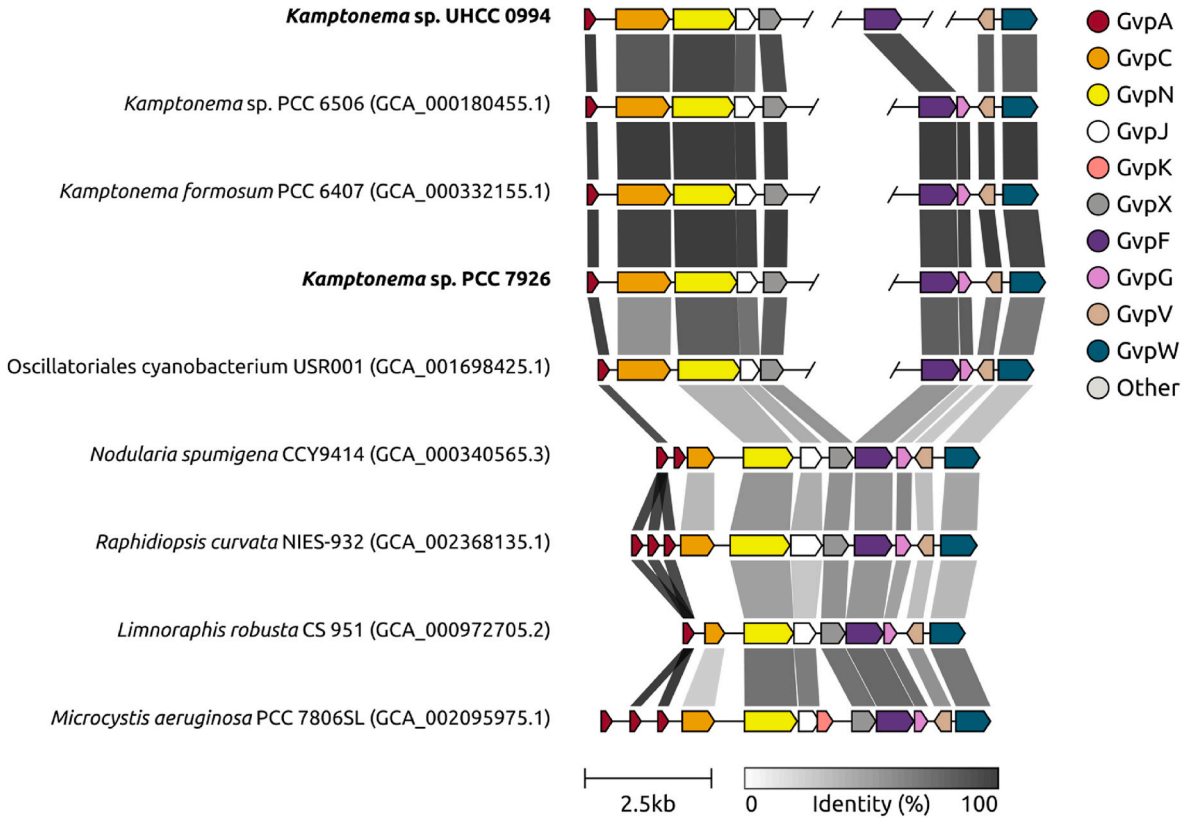

Fig. 4. Comparison of genes involved in the synthesis of gas vesicles encoded in the genome of the studied *Kamptonema* spp. UHCC 0994 and PCC 7926 together with other cyanobacteria strains.

3.3. Bioinformatic analysis of secondary metabolite biosynthetic gene clusters

The genomes from *Kamptonema* spp. presented between 14 and 18 biosynthetic gene clusters potentially involved in the synthesis of natural products (Table S3). Among the biosynthetic pathways detected, 8 to 10 corresponded to nonribosomal peptide synthetase (NRPS), polyketide synthase (PKS) and hybrids of thereof observed in the isolated cyanobacterial genomes (Table S3). A detailed analysis of one of these hybrid biosynthetic gene clusters from *Kamptonema* sp. UHCC 0994 revealed 44% similarity to BGC0000981 (*Aphanizomenon* sp. 10E6) and 78% to BGC0000978 (*Cylindrospermopsis raciborskii* AWT205), which are assigned to the cylindrospermopsin biosynthetic gene cluster (*cyr*) in the Minimum Information about a Biosynthetic Gene Cluster (MIBiG). In a genetic comparison of the *cyr* biosynthetic gene clusters, the four *Kamptonema* strains presented a conserved gene organization but lacked the terminal *cyrN* and *cyrO* genes only found in *Cylindrospermopsis raciborski* genomes of CS-505 and AWT205 (Fig. 5). Despite the gene rearrangements of the cylindrospermopsin biosynthetic gene cluster in *Kamptonema* when compared to the reference clusters of *Cylindrospermopsis* and *Aphanizomenon* strains, the gene content and modules were found to be conserved (60–98%), with core genes having at least 80% identity, while lower similarity was recorded for the transposases (36%) (Fig. 5). *Kamptonema* spp. PCC 6506 and PCC 6407 produce anatoxin-a (Mazmouz et al., 2010). However, the anatoxin-a biosynthetic pathway was not encoded in the genomes of *Kamptonema* spp. UHCC 0994 or PCC 7926.

3.4. Cylindrospermopsin-producing *Kamptonema*

Chemical analysis based on UPLC-QTOF detected the production of cylindrospermopsins by all four analyzed *Kamptonema* spp. (Fig. 6). Cylindrospermopsins were more abundant intracellularly than in the spent culture medium in all *Kamptonema* strains analyzed, with the exception of desulfo-deoxy-cylindrospermopsin (Fig. S3A). All *Kamptonema* strains produced higher amounts of cylindrospermopsin and 7-epi-cylindrospermopsin, followed by 7-deoxy-cylindrospermopsin and 7-desulfo-deoxy-cylindrospermopsin (Fig. 6 and S3A). 7-Deoxy-desulfo-cylindrospermopsin (1) is the least polar of the variants, eluting first from the HILIC column. The ion mass average of the protonated molecules was *m/z* 320.1708 (Δ −5.7−1.3 ppm for C15H22N5O3+). 7-Deoxy-cylindrospermopsin (2) eluted next in lower concentrations and was mostly present in the extracellular extracts, with an average protonated ion mass of *m/z* 400.1301 (Δ 2.9–6.2 ppm for C15H22N5O6S+) (Fig. 6). Cylindrospermopsin (3) and 7-epi-cylindrospermopsin (4) eluted very close to each other, but they could be separated using the chiral Lux Cellulose-2 column (not shown). The protonated ion mass for cylindrospermopsin and 7-epi-cylindrospermopsin from UHCC 0994

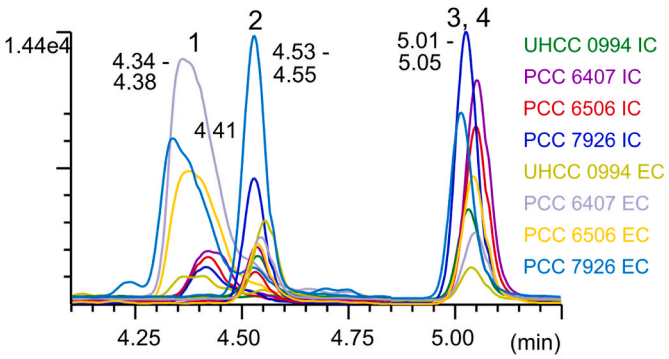

Fig. 6. Extracted ion chromatograms (EICs) of intracellular (IC) and extracellular (EC) cylindrospermopsins (CYNs) produced by *Kamptonema* spp. UHCC 0994, PCC 7926, PCC 6506 and PCC 6407.1: 7-deoxy-desulfo-CYN, 2: 7-deoxy-CYN, 3: CYN and, 4: 7-epi-CYN. EIC *m/z* values: 320.17 (7 x multiplied intensity, protonated 7-deoxy-desulfo-CYN), 400.13 (4 x multiplied intensity, protonated 7-deoxy-CYN), 416.12 (protonated CYN and 7-epi-CYN).

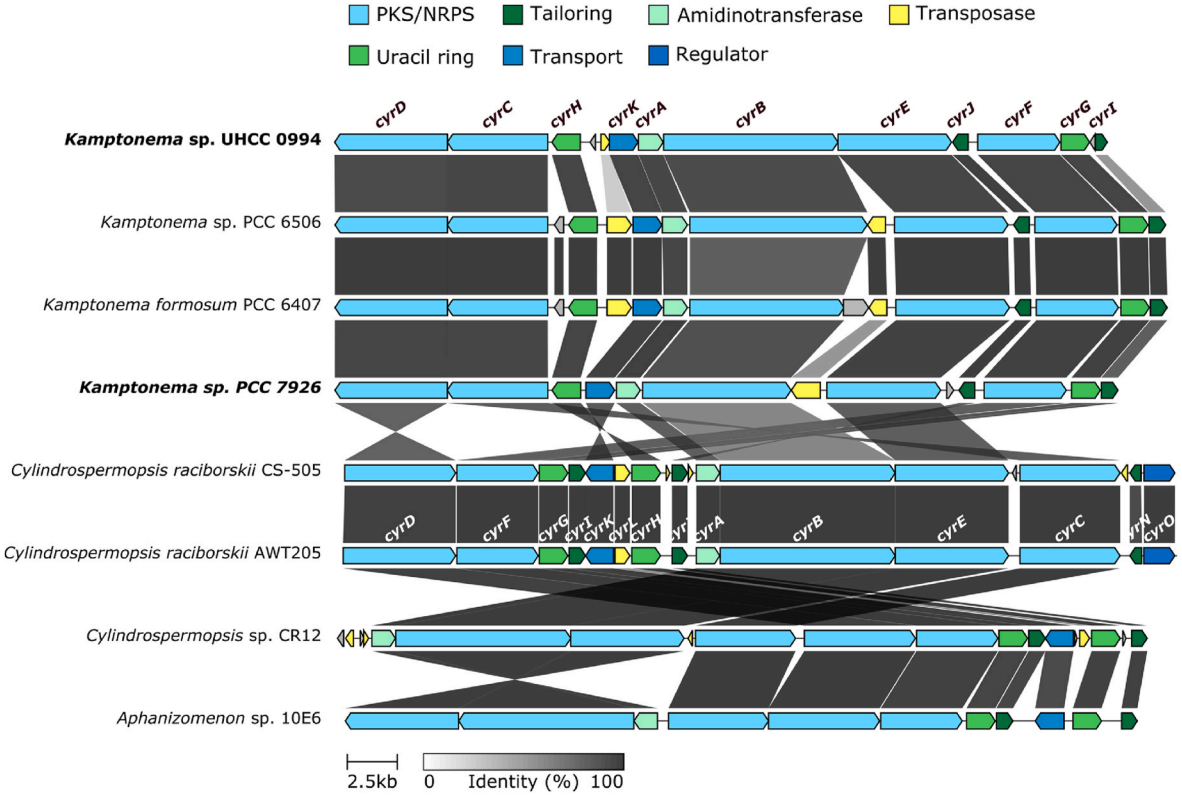

Fig. 5. Comparison of cylindrospermopsin biosynthetic gene clusters of the studied *Kamptonema* spp. UHCC 0994 and PCC 7926 and other strains.

was 416.1233 and 416.1268, respectively (Δ −0.4 and 8.1 ppm, respectively, for C15H22N5O7S+). *Kamptonema* sp. UHCC 0994 MSE spectra from the chromatographic peaks of cylindrospermopsin (3) and 7-epi-cylindrospermopsin (4) were highly similar compared to the reference strains PCC 7926, PCC 6506 and PCC 6407 (Fig. S4). Arg-cylindrospermopsin (5) was identified from *Kamptonema* spp. UHCC 0994 based on the retention time and MSE comparison with *Kamptonema* PCC 6506 (Fig. S5). The ion mass of protonated Arg-cylindrospermopsin was *m/z* 434.2498 (Δ −2.8 ppm for C20H32N7O4+). Arg-cylindrospermopsin was detected from the culture medium, while intracellular Arg-cylindrospermopsin was almost absent. Cylindrospermopsin concentration varied from 0.04 to 0.092 μg mg^{-1} of freeze-dried cells in the intracellular and from 0.004 to 0.03 μg mg^{-1} in the extracellular fraction (Fig. S3B). The original freeze-dried mat sample was analyzed in the same manner as the *Kamptonema* cultures, however, cylindrospermopsin variants were not detected (data not shown).

4. Discussion

Toxic benthic mats of cyanobacteria are associated with animal poisonings around the world but they are underexplored in terms of their microbial diversity and toxin production compared to the cyanobacteria living in planktic zones. Cyanobacterial toxins are linked to the death of dogs and young cattle in the Baltic Sea (Sivonen, 2009; Simola et al., 2012; Huisman et al., 2018). Here, we isolated a cylindrospermopsin-producing strain of the genus *Kamptonema* from a microbial mat collected in shallow water in the Baltic Sea and sequenced a draft genome (Fig. 1). We also obtained a genome sequence for *Kamptonema* sp. PCC 7926, which was isolated over 40 years ago from a surface water bloom in the southern harbour of Helsinki (Vaara et al., 1979). Phylogenomic analysis and phenotypic characteristics of these two strains were consistent with other members of the genus *Kamptonema* (Strunecký et al., 2014). *Kamptonema* spp. UHCC 0994 and PCC 7926 are the first representatives of the genus *Kamptonema* from a brackish water environment. Other *Kamptonema* genomes are from the benthic strain PCC 6506 and the freshwater strain PCC 6407 (Méjean et al., 2010; Shih et al., 2013). The MAG Oscillatoriales cyanobacterium was retrieved from a co-culture originating from a tropical freshwater lake. In phylogenomic analysis, the latter grouped at the root of the clade containing the four *Kamptonema*, but the low ANI level as well as the CDS and gene contents indicated that it belongs to another genus (Fig. 3).

Benthic cyanobacteria have been reported to produce various toxins, including saxitoxins (Gkelis et al., 2015), microcystins (Shishido et al., 2013, 2019), anatoxins (Gugger et al., 2005; Faassen et al., 2012,; Bauer et al., 2020) and cylindrospermopsins (Gaget et al., 2017; Seifert et al., 2007). *Kamptonema* spp. PCC 6506 and PCC 6407 have been reported to produce cylindrospermopsin and anatoxin-a (Mazmouz et al., 2010). The cylindrospermopsin biosynthetic gene clusters from *Kamptonema* spp. UHCC 0994 and PCC 7926 are similar to those of other *Kamptonema* strains but vary in the composition and arrangement of the genes compared to the homologous loci from *Cylindrospermopsis* and *Aphanizomenon* (Fig. 5). The lack of *cyrN* and *cyrO* from *Kamptonema* genomes could indicate that the genes are non-essential for toxin synthesis. *cyrN* is an adenylsulfate kinase gene present in a different cluster in the genome of *Kamptonema* sp. PCC 6506 (Mazmouz et al., 2010) and absent from all other *Kamptonema* genomes, while *cyrO* is a transcriptional regulator (Mihali et al., 2008) also lacking from all *Kamptonema* genomes.

We detected a number of cylindrospermopsin chemical variants using UPLC-QTOF in intracellular and extracellular extracts of *Kamptonema* spp. UHCC 0994 and PCC 7926, as well as *Kamptonema* spp. PCC 6407 and PCC 6506 (Fig. 6). The synthesis of the cylindrospermopsin variants was relatively similar among all *Kamptonema* spp. analyzed in our laboratory conditions (Fig. 6). Different cylindrospermopsin variants, such as 7-deoxy-desulfo-cylindrospermopsin and 7-deoxy-cylindrospemopsin, are suggested to be precursors for the synthesis of cylindrospermopsin (Méjean and Ploux, 2021). Varying quantities of the cylindrospermopsin variants inside and outside various cells have previously been detected (Norris et al., 1999; Li et al., 2001; Seifert et al., 2007; Mazmouz et al., 2010; Cirés et al., 2011; Davis et al., 2014; Wimmer et al., 2014; Ballot et al., 2020; Gonzáles-Blanco et al., 2020). Cylindrospermopsins are believed to be actively exported outside the cells or to be a result of cell leakage in the stationary phase of growth or accumulation of cylindrospermopsins due to lack of degradation (Saker and Griffiths, 2000; Hawkins et al., 2001; Dyble et al., 2006; Wiedner et al., 2008; Preuβel et al., 2009; Cirés et al., 2011). Higher concentrations of cylindrospermopsins have been detected in the extracellular fraction *Kamptonema* sp. PCC 6506 (Mazmouz et al., 2010; Bormans et al., 2014). However, in our current analysis, the four *Kamptonema* grown in the laboratory in Helsinki for 17 days produced higher amounts of cylindrospermopsin and 7-epi-cylindrospermopsin inside the cells (Fig. 6). We grew *Kamptonema* sp. PCC 6506 under similar conditions and collected biomass and supernatants in exponential phase. The discrepancies between the cylindrospermopsin concentrations inside and outside the cells in our results and previous results remain to be further studied.

Gas vesicles are hollow gas-filled protein compartments that allow diverse groups of bacteria to regulate their buoyancy (Min et al., 2007). Members of the genus *Kamptonema* are found in aquatic systems, from puddles to thermal springs, in addition to being isolated from periphyton and greenhouses (Strunecký et al., 2014). Here, we report two strains of *Kamptonema* that were isolated from brackish water 43 years apart. The genomes of *Kamptonema* spp. UHCC 0994 and PCC 7926 encode genes for gas vesicle production in multiple regions of the genome, which are arranged in similar positions to those found in other *Kamptonema* spp. (Fig. 4). Differences in the *gvpA-gvpC* gene region have previously been described for *Microcystis* strains, which also indicated the possible use of this region to identify geographical isolates or ecotypes from this genus (Min et al., 2007). We observed this capacity for buoyancy in *Kamptonema* sp. UHCC 0994 when grown in culture (Fig. 1C). *Kamptonema* sp. PCC 7926 also to floats at the medium/air interface in culture 43 years after this strain was isolated (data not shown). Other benthic strains of cyanobacteria can float, as exemplified by mats of *Tychonema* sp. from the benthic environment that have accumulated on the banks of a reservoir and were observed to float on the water surface (Bauer et al., 2020). In addition, benthic *Planktothrix* were shown to be capable of producing gas vesicles and floating on the surface (Pancrace et al., 2017). *Kamptonema* may display a tychoplanktonic lifestyle in the Baltic Sea, which could explain how this strain was originally isolated from a water bloom (Vaara et al., 1979).

A high level of cylindrospermopsin production can be observed in benthic mats, but the lack of information on the gas vesicles per unit biomass of toxin prevents hazard assessment for these samples (Scarlett et al., 2020). We did not detect cylindrospermopsins in the mat material from where the toxic *Kamptonema* sp. UHCC 0994 was isolated (data not shown). This toxic cyanobacterium was a minor component of the mixed algal assemblage in microbial mat (data not shown). However, cylindrospermopsin-producing *Kamptonema* may dominate the community composition in some microbial mats in the Baltic Sea. Microbial mats can accumulated on shorelines of lakes or rivers and have led to dog deaths (Edwards et al., 1992; Wood et al., 2007). Previous studies have indicated that abiotic factors influence the accumulation and release of cylindrospermopsins (Bormans et al., 2014). Lower concentrations of phosphorus and nitrogen have been related to a higher accumulation of cylindrospermopsins in cyanobacterial cells (Bar Yosef et al., 2010, Cirés et al., 2011, Saker and Neilan, 2001). Lower concentrations of nutrients in the Baltic Sea could trigger an increase in the detection of toxins such as cylindrospermopsins, and there is a constant need to monitor such changes in this environment. The World Health Organization suggests that safe levels of cylindrospermopsin are from

0.7 to 3 μg/L in drinking water and 6 μg/L in water for recreational use (WHO, 2020). Benthic cyanobacteria are linked to animal poisoning events (Catherine et al., 2013). However, benthic environments are not well surveyed for the production of cyanobacterial toxins and microbial diversity of benthic mats of cyanobacteria is poorly understood.

5. Conclusion

Kamptonema sp. UHCC 0994 and PCC 7926 were isolated 43 years apart from a benthic mat and surface water, respectively, from the Baltic Sea. We sequenced their genomes, to gain a better understanding of their biology, which are the first representatives of this genus in this brackish water environment. Gas vesicle genes are encoded by all *Kamptonema* genomes examined, which might explain why *Kamptonema* sp. PCC 7926 was collected from surface waters on the Helsinki coast. The isolated cultures of *Kamptonema* spp. UHCC 0994 and PCC 7926 to float on the surface in culture. It remains to be investigated whether *Kamptonema* have a planktic phase during their life cycle or whether the detachment of benthic mats results could result in their occurrence in planktic environments. Noteworthy, these benthic strains are also capable of cylindrospermopsin production. This preliminary work suggests that benthic mats of cyanobacteria in the Baltic Sea could also be a source of environmental toxins. Further research including for example measurement of cell-specific content of toxins and frequency of detachment of benthic cells from the mats are necessary to evaluate the necessity to include these strains in monitoring guidelines.

Authors' contributions

T.K.S.: writing the manuscript, molecular and bioinformatic analyses; E. D.: bioinformatic analyses; M.W.: mass spectrometric analyses; I.V.: molecular analyses; J.J.: mass spectrometric analyses; M.G.: strains, genome and writing contribution; M.F.F.: text revision; D.P.F.: sampling, data analyses and writing the manuscript.

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Ethics approval and consent to participate

No such approval was necessary.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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List of abbreviations

cyn	cylindrospermopsin biosynthetic gene cluster
CYN	Cylindrospermopsin
EC	extracellular
EIC	extracted ion chromatograms
gvp	vesicle protein gene cluster
IC	intracellular
KEGG	Kyoto Encyclopaedia of Genes and Genomes
MAG	metagenome-assembled genome
MIBiG	Minimum Information about a Biosynthetic Gene Cluster
NCBI	National Center for Biotechnology Information
NRPS	non-ribosomal peptide synthetase
PCC	Pasteur Culture collection of Cyanobacteria
PKS	polyketide synthase
RiPPs	ribosomal synthesized and post-translationally modified peptides
UHCC	University of Helsinki Culture Collection
UPLC-QTOF	ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxicon.2023.107205.

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