

# Complete genome sequence of *Anabaenopsis* sp. NIES-4685 (Nostocales, Cyanobacteriota) from Lake Abashiri, a brackish water lake in Japan

Yuuhiko Tanabe<sup>1)\*</sup>, Shuhei Ota<sup>1)</sup> and Shigekatsu Suzuki<sup>1,2)</sup>

<sup>1)</sup>Biodiversity Division, National Institute for Environmental Studies  
16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

<sup>2)</sup>Institute of Life and Environmental Sciences, University of Tsukuba  
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

A high-quality complete genome sequence of *Anabaenopsis* sp. NIES-4685, isolated from Lake Abashiri, a brackish water lake in Japan, was obtained using Oxford Nanopore sequencing technology. The genome assembly yielded a single 4.6 Mbp circular genome and a 12 kbp putative plasmid. Phylogenomic analyses indicated that *Anabaenopsis* sp. NIES-4685 clustered with other *Anabaenopsis* strains, confirming the morphology-based classification of this strain. The genome contains putative genes for osmolytes sucrose and trehalose syntheses, which is consistent with its brackish water origin and low salt tolerance. In agreement with its taxonomic features, it carries genes for nitrogen fixation (*nifDKH*) and gas vesicle synthesis (*gvp*). Additionally, genes encoding mycosporine-like amino acids, which function as UV protectants, were identified. Unexpectedly, no genes encoding non-ribosomal peptide synthetases or polyketide synthases, which are key enzymes in the biosynthesis of diverse secondary metabolites, including cyanotoxins, were identified. The strain and its complete genome data will be useful for further ecophysiological, toxicological, and taxonomic investigations of *Anabaenopsis* and related genera in the order Nostocales.

Key words: Cyanobacteria, *Anabaenopsis*, genome sequence, brackish water, ecophysiology

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## INTRODUCTION

The genus *Anabaenopsis* Miller 1923 belonging to the order Nostocales comprises filamentous bloom-forming cyanobacterium (Strunecký *et al.*, 2023). Although less common than other fresh and brackish waters bloom-forming cyanobacteria (e.g., *Microcystis*, *Planktothrix*, and *Dolichospermum*), *Anabaenopsis* blooms have been reported worldwide in tropical, subtropical, and temperate regions (Komárek, 2005). *Anabaenopsis* favors warm and brackish or alkaline waters (Komárek, 2005; de Souza Santos *et al.*, 2011), where it presumably outcompetes other cyanobacteria that cannot thrive in such environments, resulting in its dominance in cyanobacterial blooms. It prefers low salt concentrations as evidenced by its reported salt tolerance (Duval *et al.*, 2018), which is conferred by the syn-

thesis and accumulation of compatible solutes (osmolytes) (Hagemann, 2011).

Like other cyanobacteria belonging to Nostocales, *Anabaenopsis* is a diazotroph that fixes nitrogen using nitrogenases. Nitrogen fixation in *Anabaenopsis* is supposed to occur only in specialized cells termed heterocytes (also referred to as heterocysts), which lack photosynthetic activity, because nitrogenases are highly sensitive to oxygen (Muro-Pastor & Hess, 2012). A characteristic morphological feature of *Anabaenopsis* is the intercalary formation of two adjacent heterocytes, which arise from the division of a proto-heterocyte located centrally within a trichome (Komárek, 2005). Upon maturation of the heterocyte pair, the trichome breaks up at the heterocyte-heterocyte boundary, yielding two daughter trichomes, each bearing a heterocyte at both poles.

The genus *Anabaenopsis* was first described with its type species *Anabaenopsis elenkinii* in 1923 (Miller, 1923). Currently, 60 species (excluding synonyms) are listed in AlgaeBase (Guiry & Guiry,

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\*Corresponding author

E-mail: ytanabehiko@gmail.com

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2025), while 22 species names are recognized as valid because they appear in the List of Prokaryotic names with Standing in Nomenclature (Parte *et al.*, 2020). On the other hand, only three species are recognized in CyanoDB (Hauer & Komárek, 2022). This extreme numerical discrepancy of listed species among taxonomic databases reflects the “fuzzy” nature and unresolved taxonomic status of this genus. Indeed, recent studies indicated that *Anabaenopsis* species are genetically homogenous, making it difficult to differentiate between morpho-species by 16S rDNA molecular analysis (Delbaje *et al.*, 2021).

Like other freshwater bloom-forming cyanobacteria, strains affiliated with the order Nostocales are known to produce a variety of bioactive secondary metabolites (Dreher *et al.*, 2021). These include low molecular weight polyketides and polypeptides, mycosporine-like amino acids (MAAs), alkaloids, and polysaccharides (Jones *et al.*, 2021). However, such data are extremely limited, and only one strain producing microcystins, a type of cyclic heptapeptide with hepatotoxicity, has been reported (Mohamed & Al Shehri, 2009). As such, the distribution of secondary metabolite production across the *Anabaenopsis* lineage remains largely unknown.

Despite the frequent occurrence of *Anabaenopsis* blooms, particularly in brackish water worldwide, it is surprising that its ecology and taxonomy, as well as its toxicology, have been poorly investigated. This may be partly due to the fewer *Anabaenopsis* strains in culture collections than other bloom-forming cyanobacteria (Table 1). To advance research on the ecophysiology and taxonomy of *Anabaenopsis*

species, it is essential to identify more strains from diverse geographic regions and investigate their genomic information. To this end, we have sequenced and characterized the complete genome of *Anabaenopsis* sp. NIES-4685, which was recently isolated from Lake Abashiri, a brackish water lake in Japan. We discuss the ecophysiology of this diazotrophic bloom-forming cyanobacterium based on the functional annotation of its whole genome sequence.

## MATERIALS AND METHODS

### Strains, isolation, and cultivation

*Anabaenopsis* sp. ABLD-23 was isolated from a brackish water sample collected in Lake Abashiri (43.99351944 N, 144.22198611 E) on September 4, 2024. A single trichome was picked up using a micropipette-washing technique to obtain a bacteria-free culture. The axenicity of the culture was confirmed by staining with SYBR<sup>TM</sup> Green I Nucleic Acid Gel Stain (BMA, Rockland, USA). The established axenic strain ABLD-23 was cultured and maintained in a test tube containing a working volume of 10 mL of liquid CT medium (<https://mcc.nies.go.jp/medium/ja/ct.pdf>) at 20°C under 12:12 h (L:D) white fluorescence light at 10–15  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The strain was deposited in Microbial Culture Collection at National Institute for Environmental Studies (MCC-NIES), Japan, under the strain number NIES-4685. Hereafter, the strain is denoted as NIES-4685.

### Morphological observations

Trichomes were observed using a Nikon ECLIPSE

**Table 1 Freshwater bloom-forming cyanobacterial strains available in culture collections**

Genus	MCC-NIES	PCC <sup>a</sup>	CCAP <sup>b</sup>	NORCCA <sup>c</sup>	LEGE-CC <sup>d</sup>
<i>Anabaenopsis</i>	3	2	0	7	0
<i>Planktothrix</i>	29	8	15	199	11
<i>Microcystis</i>	372	13	12	75	40
<i>Dolichospermum</i>	161	0	7	58	12

<sup>a</sup> The Pasteur Culture Collection of Cyanobacteria (<https://webext.pasteur.fr/cyanobacteria/>)

<sup>b</sup> Culture Collection of Algae and Protozoa (<https://www.sams.ac.uk/facilities/ccap/>)

<sup>c</sup> The Norwegian Culture Collection of Algae (<https://norcca.scrol.net/>)

<sup>d</sup> Blue Biotechnology and Ecotoxicology Culture Collection (<https://lege.ciimar.up.pt/>)

Ni-U microscope (Nikon, Tokyo, Japan) equipped with differential interference contrast optics, and images were obtained using a DS-Fi3 digital camera (Nikon, Tokyo, Japan). A total of > 50 measurements of vegetative cells and heterocytes and 10 measurements of akinetes were made using the Fiji package (Schindelin *et al.*, 2012).

### Genome DNA extraction, NGS, and functional annotations

Liquid cultures ( $2 \times 10$  mL) of the strain were used for genome DNA extraction. Cells were harvested by centrifugation at  $3,000 \times g$ , and cell pellets were frozen using liquid nitrogen. Genome DNA extraction and purification were performed using a DNeasy PowerSoil Pro Kit (Quiagen, Hilden, Germany) and Genomic DNA Clean & Concentrator<sup>TM</sup>-10 (Zymo Research, Irvine, CA). Whole genome sequencing was carried out using Oxford Nanopore sequencing technology. DNA libraries were constructed using a Native Barcoding Kit 24 V14 (SQK-NBD114.24; Oxford Nanopore Technologies, Oxford, UK) following the manufacturer's protocol. Libraries were pooled and sequenced using Flongle (Oxford Nanopore Technologies). The raw reads were basecalled using Dorado version 0.9.0 (<https://github.com/nanoporetech/dorado>) using the "super accuracy mode". The obtained reads were assembled using Flye version 2.4.9 (Kolmogorov *et al.*, 2019) and polished using medaka version 2.0.1 (<https://github.com/nanoporetech/medaka>). The obtained circular genome and a plasmid were annotated using dFAST (Tanizawa *et al.*, 2018). Genome quality was assessed using CheckM based on the Nostocales marker set (Parks *et al.*, 2015). Average nucleotide identity values (ANI) were calculated based on MUMmer using JspeciesWS (Richter *et al.*, 2016). Average amino acid identity (AAI) values were calculated using CompareM (<https://github.com/dparks1134/CompareM>). Genes for secondary metabolites were inspected using the antiSMASH bacterial version web server version 8.0 (Blin *et al.*, 2025). The whole genome and plasmid sequence data of NIES-4685 were deposited in DDBJ under accession numbers

AP043602 and AP043603, respectively.

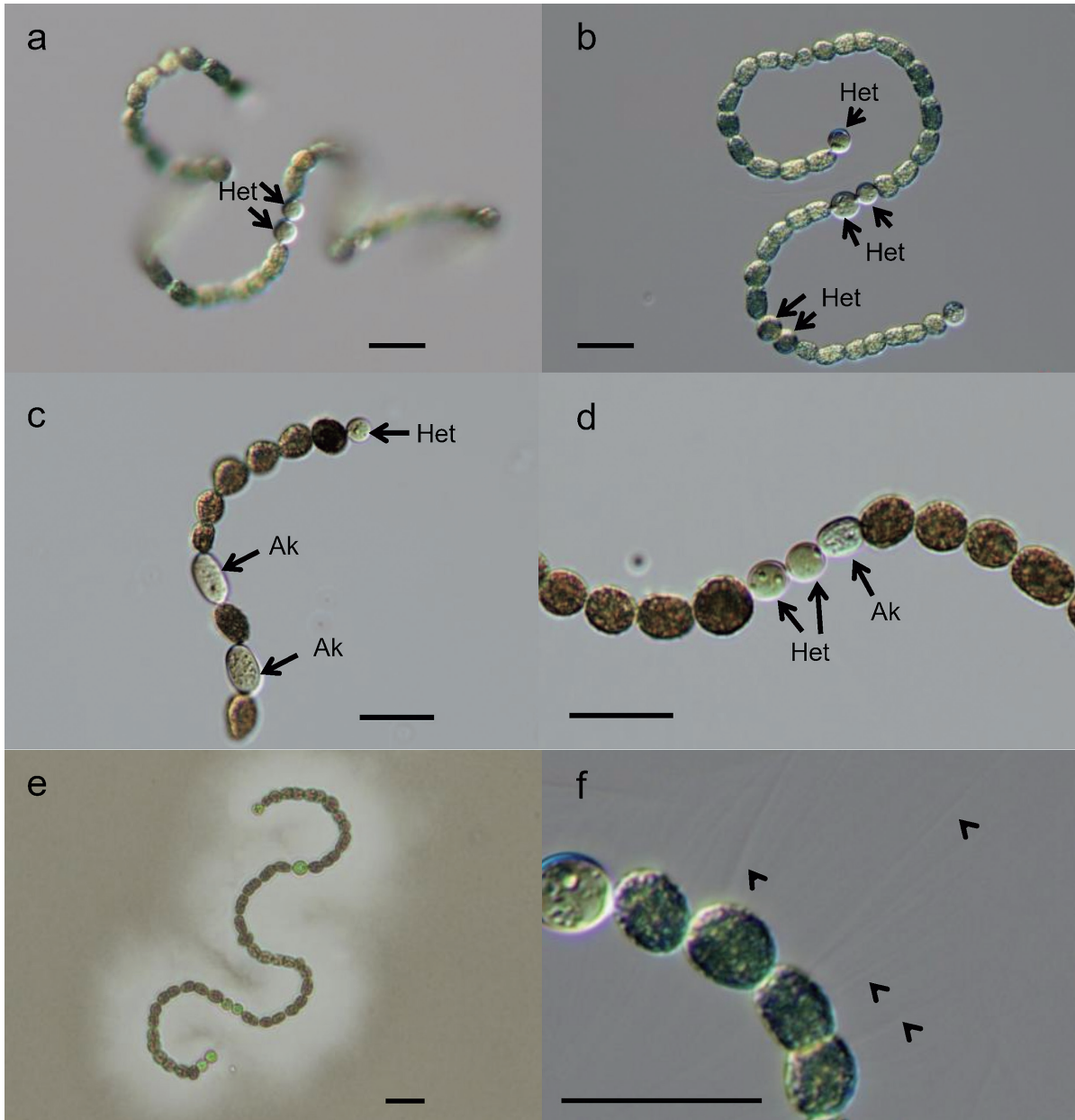
### Phylogenomic analyses

RefSeq genome data of broad representative cyanobacteria were retrieved from GenBank with reference to Strunecký *et al.* (2023). Because cyanobacterial strains have often been misidentified because of their simple morphology, as many type strains as possible were included. Preliminary analysis using *Gloeobacter violaceus* PCC 7421<sup>T</sup> (GCF\_000011385.1) as an outgroup yielded a phylogeny with low statistical support due to the small length of the amino acid alignment. Thus, *Gloeomargarita lithophora* Alchichica-D10<sup>T</sup> was used instead as an outgroup. Maximum likelihood (ML) phylogenomic trees were reconstructed with RAxML (Stamatakis, 2014) via PhyloPhlAn version 3.1.68 (Asnicar *et al.*, 2020), employing the PROTGAMMA model and an amino acid alignment of 400 universal marker genes proposed in PhyloPhlAn (Segata *et al.*, 2013) with the "--diversity medium" option. ML bootstrap analyses were performed using 100 random replicates.

## RESULTS AND DISCUSSION

### Morphological observation

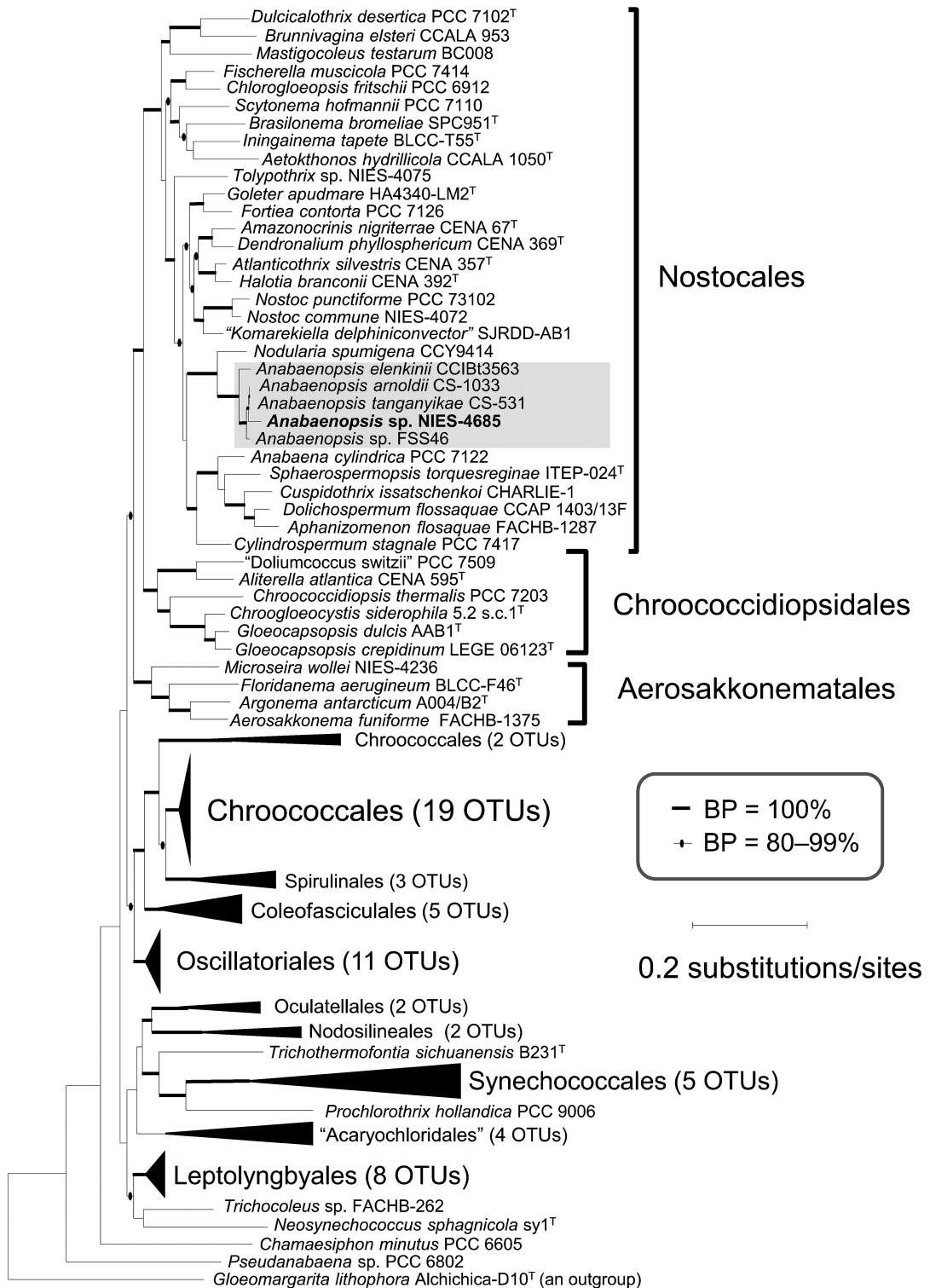
Our irregular monitoring of cyanobacterial blooms in Lake Abashiri since 2016 revealed blooms containing *Anabaenopsis* from early Summer to Autumn. This motivated us to isolate the bloom-forming *Anabaenopsis* strains. Here, we characterize one such strain, *Anabaenopsis* sp. NIES-4685 (= ABLD-23), an axenic strain isolated from the lake in 2024. A microphotograph revealed irregular trichome coiling (Fig. 1a). The vegetative cells were barrel-shaped, occasionally biconvex, and measured  $5.7\text{--}10.7 \times 4.8\text{--}13.8 \mu\text{m}$ . Heterocytes were spherical and measured  $5.2\text{--}8.6 \mu\text{m}$  in diameter. The strain showed paired heterocyte development in the middle of the trichome (Fig. 1a, b), a diagnostic morphological feature of *Anabaenopsis*, confirming that it belonged to this genus. This was supported by its placement in a statistically supported clade composed solely of *Anabaenopsis* strains within the Nostocales in the phylogenomic tree (Fig. 2). Akinetes (thick-walled dormant cells) were cylindri-



**Fig. 1** Micrographs of *Anabaenopsis* sp. NIES-4685. **a**) A differential interference contrast (DIC) image shows an irregular spiral shape and heterocytes. **b**) A DIC image shows heterocytes located in the middle of the trichome and at both poles. **c, d**) DIC images show akinetes. **e**) India ink treatment indicates dense cloud-like extracellular matrix surrounding the trichome. **f**) A DIC image shows vertical spine-like extensions (arrowheads) from the trichome, adopting a caterpillar-like appearance. Scale bar, 20  $\mu\text{m}$ . Abbreviations: Ak, akinetes; Het, heterocytes.

cal in shape ( $6.1\text{--}10.3 \times 7.6\text{--}19.4 \mu\text{m}$ ) (Fig. 1c, d) and were observed infrequently, but their prevalence increased under higher light irradiation ( $> 25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Akinetes were observed developing adjacent to both vegetative cells (Fig. 1c) and het-

erocytes (Fig. 1d). Notably, the strain possessed cloud-like extracellular substances, possibly corresponding to polysaccharide derivatives (Fig. 1e). A closer examination revealed a spine-like structure emerging from the trichome (Fig. 1f). It is unclear



**Fig. 2** Phylogenomic tree of cyanobacteria focused on Nostocales. ML bootstrap values below 80% are not shown. The *Anabaenopsis* clade is highlighted in gray. Orders distantly related to Nostocales are compressed. Taxa not validly published are indicated with double quotation marks.

whether this structure is unique to this strain. Further study of available *Anabaenopsis* strains will be required to confirm this. As mentioned in the Introduction, identification of *Anabaenopsis* species is not straightforward, and this was also the case for NIES-4685. The morphological characteristics of NIES-4685 did not match with those of any other previously described species of *Anabaenopsis* (Komárek, 2005), suggesting that it might be a new species. However, comparative genome analyses (also see below section) ruled out this possibility; the ANI values between NIES-4685 and two strains of morphologically different *Anabaenopsis* species *A. arnoldii* CS-1033 and *A. tanganyikae* CS-531 was  $\approx$  98% (Table 2), which is higher than the 95% threshold used to differentiate bacterial species (Jain *et al.*, 2018). This result, however, should be treated with caution because none of the strains included in this study are nomenclatural type strains of the genus *Anabaenopsis*, leaving open the possibility of misidentification. This further reinforces the highly problematic nature of traditional morphology-based classification of species in *Anabaenopsis*. At this stage, therefore, we consider it premature to propose that this strain represents a new species.

### Genome analyses of *Anabaenopsis* sp. NIES-4685

Whole genome sequencing of NIES-4685 using the Oxford Nanopore platform yielded a circular chromosome of 4.6 Mbp and a putative plasmid of 12 kbp. The basic genome properties of NIES-4685 relative to those of currently available *Anabaenopsis* refseq genomes are summarized in Table S1. CheckM analysis of strain NIES-4685 indicated 96.26% completeness with 0.77% contamination. Because the obtained genome was a single circular

sequence, the estimated contamination value indicates the acquisition of genes by horizontal gene transfer (HGT) rather than sequence contamination. In addition, the NIES-4685 genome is the longest ever reported and contains the highest number of genes among all *Anabaenopsis* strains. Given that this is the only circular genome of *Anabaenopsis* species reported to date, it most likely constitutes the most complete and highest-quality genome currently available for the genus *Anabaenopsis*. The identified plasmid harbors only four coding DNA sequences (CDS), none of which shows homology to known proteins; thus, the function of this plasmid remains unclear.

### Genes related to ecophysiology of *Anabaenopsis* sp. NIES-4685

The salinity of the location where *Anabaenopsis* sp. NIES-4685 was isolated was 4.6 psu, a condition under which freshwater cyanobacteria cannot survive without accumulating compatible solutes (osmolytes) (Hagemann, 2011). Annotation of the DNA sequences in the whole genome using BLAST identified genes involved in the syntheses of two compatible solutes, sucrose and trehalose (Table 3), both of which are widely distributed in fresh and brackish water cyanobacterial lineages (Hagemann, 2011). The NIES-4685 genome contains three genes (*treY*, *treZ*, and *treS*) encoding enzymes belonging to two trehalose synthesis pathways. *treY* and *treZ* belong to the TreY-TreZ pathway of trehalose synthesis, in which trehalose is produced via the degradation of ( $\alpha$  1,4) glucose polymers (Klähn & Hagemann, 2011). *treS* belongs to the TreS synthesis pathway and encodes trehalose synthase, an enzyme that catalyzes the conversion of maltose to trehalose (Klähn &

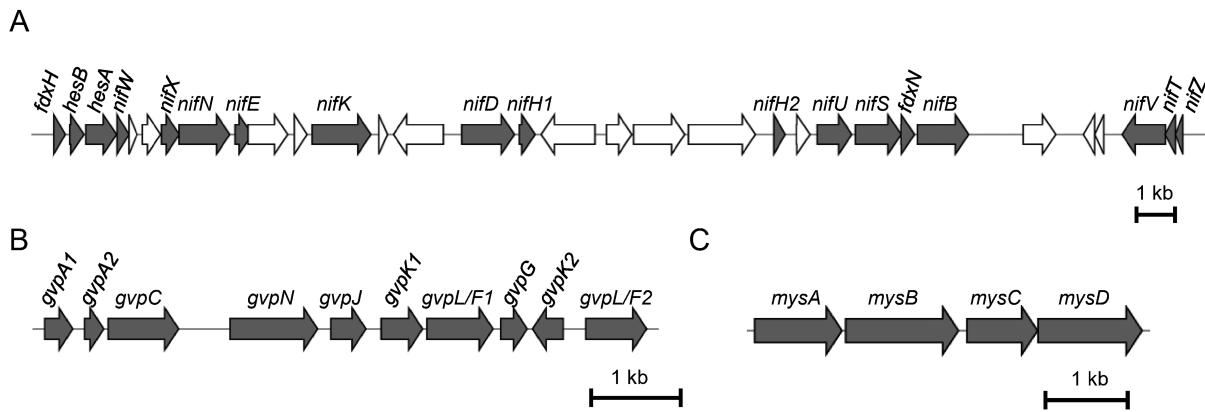
**Table 2 ANI and AAI values<sup>a</sup> among *Anabaenopsis* spp.**

	<i>A. arnoldii</i> CS-1033	<i>A. elenkinii</i> CCIBt3563	<i>A. tanganyikae</i> CS-531	<i>A. sp.</i> NIES-4685
<i>A. arnoldii</i> CS-1033		92.1	<b>99.6</b>	<b>98.6</b>
<i>A. elenkinii</i> CCIBt3563	91.8		92.1	92.1
<i>A. tanganyikae</i> CS-531	<b>99.6</b>	91.8		<b>98.6</b>
<i>A. sp.</i> NIES-4685	<b>98.7</b>	91.9	<b>98.7</b>	

<sup>a</sup> Values above the proposed species boundary (> 95%) are indicated in bold.

**Table 3** Genes for compatible solute syntheses in *Anabaenopsis* sp. NIES-4685

Gene	Locus tag
<i>treY</i> (malto-oligosyltrehalose synthase)	ABLANap23_04230
<i>treZ</i> (malto-oligosyltrehalose trehalohydrolase)	ABLANap23_04210
<i>treS</i> (trehalose synthase)	ABLANap23_02920
<i>susA</i> (sucrose synthase)	ABLANap23_30030, ABLAnap23_35150
<i>sppA</i> (sucrose-phosphate phosphatase)	ABLANap23_00420
<i>spsA</i> (unidomainal sucrose-phosphate synthetase)	ABLANap23_13570
<i>bsps</i> (bidomainal sucrose-phosphate synthetase)	ABLANap23_02610
<i>inv</i> (invertase)	ABLANap23_18990

**Fig. 3** Gene organization within the genome of *Anabaenopsis* sp. NIES-4685. A) *nif* cluster. B) *gvp* cluster. C) the gene cluster for MAA synthesis (*mys*). Locus tags of genes are listed in Tables S3 to S5.

Hagemann, 2011). The NIES-4685 genome contains two genes for sucrose synthesis, *spsA* and *sppA*. The products of *spsA* and *sppA* catalyze the synthesis of sucrose from UDP-glucose and fructose-6-phosphate via sucrose-6-phosphate in a two-step reaction (Hagemann, 2011). In addition to canonical *spsA*, NIES-4685 possesses a gene encoding bidomainal Sps, which is a fused protein of SpsA and SppA that synthesizes sucrose as a single enzyme (Kolman *et al.*, 2015). NIES-4685 possesses two copies of *susA*, the product of which catalyzes the bidirectional reaction between fructose and U(A)DP-glucose to form sucrose and *vice versa*, at least *in vitro* (Hagemann, 2011). However, *in vivo* experiments have suggested that SusA is involved mainly in the degradation of sucrose in response to decreased salinity (Hagemann, 2011). Interestingly, unlike other cyanobacteria where sucrose-related genes are typically clustered (e.g., *Microcystis*, Tanabe *et al.*,

2018), these genes are dispersed throughout the NIES-4685 genome. In addition, NIES-4685 harbors an additional gene involved in sucrose degradation, *inv*, which encodes invertase, an enzyme that catalyzes the hydrolysis of sucrose to glucose and fructose (Santos-Merino *et al.*, 2023). The presence of these compatible solute synthesis and breakdown genes corroborates the brackish water origin of NIES-4685. Previous studies have suggested that sucrose and trehalose confer tolerance to salinity levels corresponding to those of 50–100% seawater (Hagemann, 2011). Indeed, a preliminary experiment showed that NIES-4685 could grow at a salinity of 15 psu (50% sea water) but not at a salinity of 30 psu (100% sea water) (Supplementary Fig. S1).

Genome mining of NIES-4685 identified a nitrogen fixation gene (*nif*) cluster (Fig. 3A, Supplementary Table S3) that included *nifDKH*, a gene encoding Mo nitrogenase that is essential for nitrogen fixation

(Bothe *et al.*, 2010). In addition, the *nif* cluster includes *nifB-fdxN-nifSU*, *nifENXW*, and *nifVZTI*, the products of which are important for nitrogenase enzyme assembly (Esteves-Ferreira *et al.*, 2017). The gene organization within the *nif* cluster of NIES-4685 is the same as that found in the *nif* cluster of most typical cyanobacteria (type A, Chen *et al.*, 2022). The evolutionary history of *nif* cluster of Nostocales is unclear due to low statistical resolution, which is exemplified by the NifD phylogeny (Supplementary Fig. S2). The sequence of *nifD* as well as those of other *nif* DNA sequences are highly similar between *Anabaenopsis* species, suggesting a vertical transmission of *nif* genes at least in the *Anabaenopsis* lineage. The NIES-4685 genome also contains genes encoding HetR (ABLANap23\_08160), a protein that is essential for heterocyte development (Muro-Pastor & Hess, 2012). This is consistent with the presence and possible role of heterocytes in diazotrophy of *Anabaenopsis*.

*Anabaenopsis* is a gas-vacuolated cyanobacterium, which possess gas vesicles that regulate buoyancy, enabling vertical migration between the nutrient-rich bottom layer and the water surface, where sunlight is abundant (Reynolds *et al.*, 1987). Cyanobacterial gas vesicles are composed of the products of *gvpA* and *gvpC*, both of which were identified together with those involved in the regulation of gas vesicle expression (Fig. 3B). At the water surface, planktonic cyanobacteria are exposed to sunlight and, consequently, are subject to UV-induced stress. To avoid UV damage, some cyanobacteria accumulate UV-absorbing compounds, such as MAAs, which have been suggested for use in sunscreens (Oren & Gunde-Cimerman, 2007). *Anabaenopsis* is likely to use MAAs for protection against UV light because we identified putative genes for MAA synthesis (*mysABCD*) in NIES-4685 (Fig. 3C). The product of the *mys* in NIES-4685 cannot be identified due to low DNA sequence similarity with known *mys*. On the other hand, antiSMASH analyses found no non-ribosomal peptide synthetases (NRPS) or polyketide synthases (PKS) synthesis genes within the genome of NIES-4685. Given the production of diverse bioactive compounds by NRPS

and PKS in freshwater bloom-forming cyanobacteria in general (Baunach *et al.*, 2024), the complete absence of PKS/NRPS in NIES-4685 was unexpected. This suggests that NIES-4685 is non-toxic, and thus, this strain and the bloom thereof is unlikely to pose risks to human health in Lake Abashiri. Whether *Anabaenopsis* strains are generally non-toxic is an open question and will require further investigation.

## FUTURE PERSPECTIVES

The genome data generated in this study provides an insight into the ecophysiology of *Anabaenopsis*, a poorly characterized bloom-forming genus, both from the genome and ecological perspective. We expect that acquiring more genome data for species in this genus will contribute to future studies examining the ecophysiological diversity, secondary metabolites, and taxonomy of *Anabaenopsis*. It should be noted that NIES-4685 is one of very few axenic *Anabaenopsis* strains available from microalgal culture collections worldwide. The high-quality complete genome of this strain obtained in this study has the potential to make NIES-4685 a model strain for investigations into the various biological features of diazotrophic bloom-forming cyanobacteria.

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## SUPPLEMENTARY INFORMATION

Supplementary information related to this article is available in the online version on J-STAGE.

<https://www.jstage.jst.go.jp/browse/microresys/list>

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日本の汽水湖（網走湖）から分離した *Anabaenopsis* sp. NIES-4685 の完全長ゲノムの報告

田辺雄彦<sup>1)</sup>, 大田修平<sup>1)</sup>, 鈴木重勝<sup>1,2)</sup>

<sup>1)</sup> 国立環境研究所生物多様性領域, <sup>2)</sup> 筑波大学生命環境系

日本の汽水湖である網走湖から分離した *Anabaenopsis* sp. NIES-4685 の高品質完全長ゲノムを Oxford Nanopore シークエンス技術によって取得した。得られた配列は 4.6 Mbp の 1 本の環状ゲノムと 12 kbp の環状プラスミド様配列であった。ゲノム系統解析の結果、*Anabaenopsis* sp. NIES-4685 は他の同属株と単系統群を形成し、この株の属同定の正しさを確認できた。本株のゲノムには抗浸透圧物質であるスクロースとトレハロースの合成に関与する遺伝子が含まれていたが、この結果は本株が汽水産であること、塩分耐性をもつことと一致する。本ゲノムからは、窒素固定に関与する遺伝子 (*nifDKH*) やガス胞形成に関与する遺伝子 (*gvp*) も見つかかり、本属を定義する特徴と一致していた。また、本ゲノムからは UV 体制を付与するマイコスポリン様アミノ酸合成に関与すると考えられる遺伝子も見つかった。意外なことに、本ゲノムからは、シアノトキシン等の多様な二次代謝産物を含むポリケタイドや非リボソーム型ポリペプチドを合成する酵素群は全く見つからなかった。今回報告する株とそのゲノムデータは、今後の *Anabaenopsis* およびネンジュモ目の類縁種の生理生態学、毒性学、分類学の研究に有用であると考えられる。