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Microcoleus pseudautumnalis sp. nov. (Cyanobacteria, Oscillatoriales) producing 2-methylisoborneol

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Abstract A new species, *Microcoleus pseudautumnalis*, producing both 2-methylisoborneol (2-MIB) and geosmin is described. We have conducted a systematic study of a bad-smelling, 2-MIB producing planktic *Pseudanabaena* species in Japan and described four new species (*P. foetida*, *P. subfoetida*, *P. cinerea*, and *P. yagii*). In the course of this study, we found another kind of filamentous cyanobacteria with a bad smell in a plankton sample collected from a pond in Japan. The morphology of *M. pseudautumnalis* resembles that of *M. autumnalis* (Trevisan ex Gomont) Strunecký, Komárek et Johansen (basionym: *Phormidium autumnale* Trevisan ex Gomont). The sheath is thin and always contains only one trichome. Trichomes are immotile, grayish-green, not constricted at the cross-walls, not attenuated or attenuated towards the ends with truncated or capitated apical cells, and sometimes with calyptrae that are relatively wider (6.9–7.6 µm) than those of *M. autumnalis*. The phylogeny of the 16S rRNA gene of *M. pseudautumnalis* revealed that it is in the clade of the genus *Microcoleus* and contains an 11-bp insert. *Microcoleus autumnalis s. str.* is said to lack this insert. *Microcoleus pseudautumnalis* has four kinds of 2-MIB genes, and the phylogeny of this taxon is different from those of *Pseudanabaena* sp. and *Planktothricoides raciborskii.*

Key words: 16S rRNA gene sequences, 2-MIB, 2-methylisoborneol, geosmin, *Microcoleus autumnalis*, *Microcoleus vaginatus*.

Introduction

Bad-smelling drinking water supplied from reservoirs and bad-smelling fish and shellfish cause genuine problems in Japan. The substance causing the odor was identified as 2-methylisoborneol (2-MIB) or geosmin (Yagi, 1983), and odor-producing cyanobacteria have been reported in several lakes, reservoirs, and ponds (cf. Morii *et al.*, 1982; Yamada *et al.*, 1985, 1986; Oikawa *et al.*, 2000, Oikawa and Ishibashi, 2004; Tsunoda *et al.*, 2014). The authors conducted a systematic study of the odor-producing filamentous cyanobacteria that included a detailed morphological description, ecological observation, and phylogenetic analysis, and we have clarified that thin filamentous planktic cyanobacteria producing 2-MIB belong to the genus *Pseudanabaena*. Four new *Pseudanabaena* species were described (*P. foetida* Niiyama, Tuji et Ichise, *P. subfoetida* Niiyama et Tuji, *P. cinerea* Tuji et Niiyama and *P. yagii* Tuji et Niiyama) (Niiyama *et al.*, 2016; Tuji and Niiyama, 2018).

In the course of the above-mentioned study, we observed another kind of cyanobacteria species producing 2-MIB, which showed clearly different morphological characteristics from *Pseudanabaena* but had some resemblance to *Phormidium* or *Microcoleus* species. In Japan, it has been reported that some benthic or periphytic cyanobacteria with a *Phormidium*-like morphology in streams or banks produce geosmin or

2-MIB. However, the classification of such organisms is unclear. This study focuses on the morphology and genetic characteristics of our newly found cyanobacteria that produces 2-MIB.

Materials and Methods

Sampling site and cultured strain

The sample was collected from Naka-numa Pond, Ryugasaki City, Ibaraki Pref., Japan in September 2016, using a plankton net. Naka-numa Pond is surrounded by paddy fields, and no rivers or streams flow in or out of this pond. Naka-numa Pond has an almost round shape with a diameter of about 100 m, and its maximum depth is about 14 m.

Isolation was done by the agar plate method (Tuji and Niiyama, 2014) with BG-11 medium (Waterbury and Stanier, 1981). Only one unialgal strain with a bad odor, Ak1609, was obtained. For the maintenance of the strain, 10 ml of modified C medium (Ichimura and Watanabe, 1977; Niiyama *et al.*, 2011) contained in a test tube was used. The culture was illuminated by cool-white fluorescent lamps, with a photon flux density of ca. 20 µmol/m^2/sec, a photoperiod of 8 hours light and 16 hours dark, and a temperature of 18℃. Morphological observation was performed for this cultured strain under a light microscope (BH-2, Olympus Corporation, Tokyo, Japan). Microphotographs were taken with a Canon digital camera EOS Kiss X5 (Canon Inc., Tokyo, Japan). The cultured strain is maintained in the Department of Botany, National Museum of Nature and Science. The specimens are housed in the herbarium of TNS (Department of Botany, National Museum of Nature and Science).

SPME-GC/MS analysis

The odor-producing substance in the culture medium of Ak1609 was analyzed using the gas chromatography/mass spectrometry combined with solid phase micro extraction (SPME-GC/MS) method (JWWA, 2011) at Hiroshima Environment and Health Association.

Genomic DNA extraction, polymerase chain reaction (PCR) amplification, sequencing and assembling

A 1.5-ml volume of fresh cultured material was centrifuged at 10,000 rpm for 5 min at room temperature. The supernatant was removed, and the cell pellets were kept in a freezer at −20℃ until extraction. Total genomic DNA was extracted using an extraction kit (DNeasy Plant Mini Kit, Qiagen) in accordance with the manufacturer's instructions. The region between the 16S rRNA gene and the internal transcribed spacer (ITS) was amplified using four primers sets, set G (PLG1.3 and pits-CyanR), set J (PS-16S-27f and pits-CyanR), set K (PS-16S-27f and 16S-1492R), and set L (PLG1.3 and 16S-1492R) (Table 1). The region between rbcL and rbcX was amplified using primer set M (cx-b and cw-b). The region of the gene cluster for 2-MIB was amplified using primer sets N (1609-mtf-r1 and MIB-cnbA-852f), O (1609-mtf-r2 and MIB-cnbA-736fb), P (PS-cnbA-start and 1609-mtf-r1), Q PS-cnbA-start and 1609-mtf-r2, R (MIB-SAM-F2 and 1609-mtf-r1), S (MIB-SAMF2 and 1609-mtf-r2), T (MIB-SAMF2 and MIB-SAMR1), and U (MIB-CnbA-852f and MIB-SAMR1). These primers for MIB genes were designed using Wang *et al.* (2011) and modified during this study.

PCR was performed on a thermal cycler (Veriti Thermal Cycler, Thermo Fisher Scientific) using 0.25 µl polymerase (Phusion Hot Start Flex DNA Polymerase, BioLabs), 5 µl GC Buffer in the polymerase kit, 2 µl 2.5 mM dNTPS, 14.25 µl sterile deionized water, 0.25 µl each of 10 pM concentrations of the forward and reverse primers, and 1 µl of DNA template. The temperature cycling program used the following conditions: 16S rRNA to ITS regions; 98℃ for 30 sec; 35 cycles of 98℃ for 10 sec, 54℃ for 15 sec, and 72℃ for 60 sec, and the final elongation step was 72℃ for 7 min; rbcLX regions; 98℃ for 30 sec; 10 cycles of 98℃ for 10 sec, 60℃ to 50℃ (−1.0℃ per cycle) for 15 sec, 72℃ for 60 sec; 35 cycles of 98℃ for 10 sec, 50℃ for 15 sec, and 72℃ for 60 sec, and the final elongation step was

Table 1. Primers for PCR and sequences used in this study.

Target	Primer name		Reference
16S rRNA-ITS	16S-27f	AGAGTTTGATCMTGGCTCAG	Lane 1991
	PLG1.3 (CYA108F)	ACGGGTGAGTAACRCGTRA	Urbach *et al.* 1992
	pits-CyanR	CTCTGTGTGCCAAGGTATC	Ernst *et al.* 2003
	F3 L	GTCCCGCAACGAGCGCAAC	Hiraishi *et al.* 1994
	16S-1492r	GGTTACCTTGTTACGACTT	Turner *et al.* 1999
rbcL–rbcX	cx-b	GGGGCARGTARGAAAGGRTTTCGTA	Tuji & Niiyama 2018
	cw-b	CGTAGCTTCYGGTGGTATCCACGT	Tuji & Niiyama 2018
MIB genes	MIB-SAMF2	GAVTTCCTSVTGGRCCACCTCG	Wang *et al.* 2011
	cnba-852f	GMRYTGCGBGARCGYCARGARYACGA	This study
	MIB-cnba-736fb	TTGTCGAYTACGARACCTCKCCRCG	This study
	PS-mtf-start	ATGTCAACGCCCCAAAMTATCACTGC	This study
	MIB-mtf-278f	GGCGGTTCYGGTCGCGGCGG	This study
	MIB-mtf-446r	CGCATGGCTCCCGTCTCGAAGCC	This study
	PS-mtf-end	TTACCGAATGATGCGGTCAGCAACG	This study
	PS-mic-start	ATGAAAGATACCAACYTGGATAATAC	This study
	MIB-mic-400f	GACCCAKMTCGGCTGTTGAT	This study
	PS-mic-end	TTAGGCTAGTGATTGTGAATCTGGC	This study
	PS-cnbB-start	ATGACCCAAGACTTTAACTCCCATGG	This study
	MIB-cnbB-681f	CGCCCGCCAAAAGCCCAAGATA	This study
	MIB-cnbB-890r	CGCTCGCGCAACTCATGCACAGTC	This study
	1609-cnbB-1300fb	TATCCTTGTCCCCGACGCCGTCGGCATTC	This study
	PS-cnbB-end	CTACCGCCCGATCTCGACATCCTCG	This study
	MIB-SAMR1	TCSACGTACATGSTSGACTCGT	Wang *et al.* 2011
	1609-mtf-r1	TGGTGGTAATAGCCGTCAACTTGGCCGAGC	This study
	1609-mtf-r2	AGTTGGCAACCGACTTCTGATATTCGCTGCG	This study

72°C for 7 min; MIB genes region; 98°C for 30 sec; 35 cycles of 98°C for 10 sec, 55°C for 15 sec, 72°C for 180 sec, and the final elongation step was 72°C for 7 min.

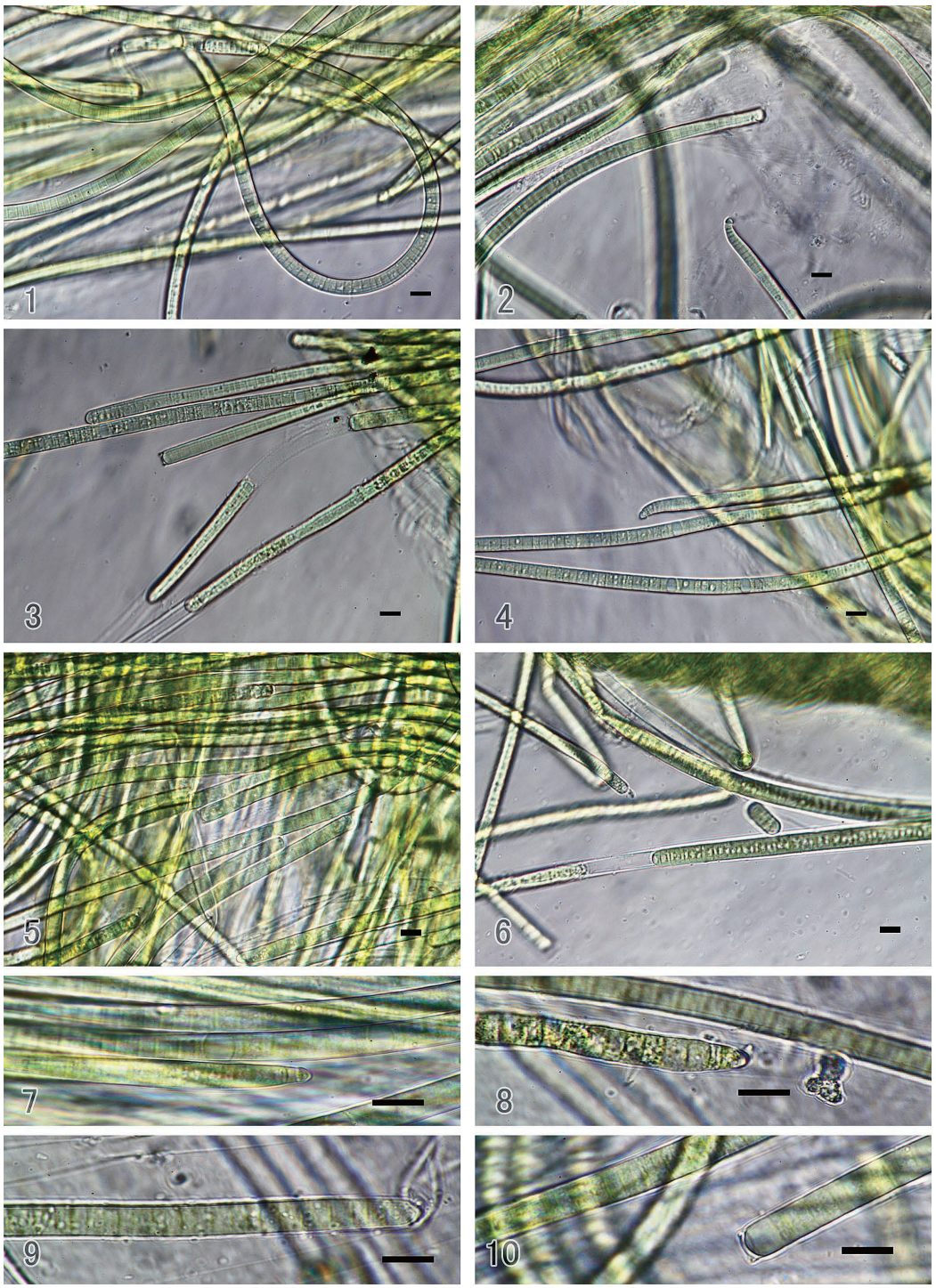
The concentrations of the amplified products were verified on a 1% agarose gel. Direct sequencing of the PCR products was undertaken using the primers presented in Table 1 with Big Dye Terminator Chemistry and an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). The obtained sequences were assembled using Chromas PRO (Technelysium Pty Ltd, Tewantin, Australia). The assembled results were checked manually after automatic assembling by Chromas Pro.

Phylogenetic reconstruction

Phylogenetic and molecular evolutionary analyses for the sequence of Ak1609 and similar sequences retrieved from National Center for Biotechnology information (NCBI) were conducted using the MEGA 7 computer program (Kumar *et al.*, 2016). The alignments were checked manually. A maximum likelihood (ML) tree was calculated using MEGA software with the best fit model determined by Akaike Information Criterion (AIC) corrected scores, and the substitution nucleotide matrix parameters were calculated by the software. A tree using 1000 bootstrap replicates was generated. Each codon position was partitioned and analyzed for 16S rRNA. Neighbor-joining (NJ) trees with 1000 bootstrap replicates were also calculated using MEGA software. All positions containing gaps and missing data were partially deleted (site coverage cutoff 50%). *Planktothricoides raciborskii* (strain: NIES-207) was used for the outgroup.

Results and Discussion

The morphology of the cultured strain, Ak1609, resembles that of *Microcoleus autumnalis* (Trevisan ex Gomont) Strunecký, Komárek et Johansen (≡ *Phormidium autumnale* Trevisan ex Gomont) (Figs. 1–10, Table 2). Ak1609 and



Figs. 1–10. Light microscopy photographs of *Microcoleus pseudautumnalis sp. nov.* Scale bars = 10 µm.

Table 2. Comparison of morphological, ecological and genetic characteristics of *Microcoleus pseudautumnalis*, *M. autumnalis*, and *M. vaginatus*. Taxon names are identical with thorse shown in Fig. 11.

Taxa	trichome(s) in sheath	cell width µm	cell length µm	cell L/W	habitat	motility	11 bp insert	bad smell	reference
M. pseudautumnalis	1	6.9–7.6(8.3)	2.0–5.0	0.5–2.5	planktic	−	+	+	this study
M. autumnalis	1	4–7(9)	2–5(7)	0.5 or more	on wet soil, mud, walls, rocks in rivers			−	Whitton 2002 (as "*Phormidium autumnale*")
M. autumnalis	1	(3.5)4–7	2–4(5)	0.5	periphytic on submersed substrate, in streams	+		−	Komárek & Anagnostidis 2005 (as "*P. autumnale*")
M. autumnalis	1	4–6		0.3–1	epipelic	+	−	−	Hašler *et al.* 2012 (as "*P. autumnale*")
M. autumnalis	1	4.3–8.1	1.3–3.8		periphytic	+	−	−	Strunecký *et al.* 2013
M. vaginatus	many	(2.5)3–7	2–5(6.7)		subaerophytic	+		−	Komárek & Anagnostidis 2005
M. vaginatus	many	3.5–7		0.5–1.5	on moist soil	+		−	Whitton 2002
M. vaginatus	many	(4)5–7		0.3–1	epipelic	+	+	−	Hašler *et al.* 2012
M. vaginatus	many	1.5–9.8	1.3–8.5		on various kinds of soils or walls, stream periphyton	+	+	−	Strunecký *et al.* 2013
M. vaginatus	many	(3.8)4.5–5.5	2–5(6.7)		on arid soils	+ +	+	−	Boyer *et al.* 2002

Table 3. The similarity (%) and the number of base differences per partial 16S rRNA sequence (1036 positons) from 26 strains between sequences are shown. All positions containing gaps and missing data were eliminated.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	*Microcoleus pseudautumnalis* Ak1609	—	99.90	99.81	99.81	99.81	99.81	99.81	99.81	99.71	99.71	99.71	99.71	99.71	99.52	99.52	99.52	99.52	99.42	99.32	99.23	99.13	98.94	98.75	98.65	98.26	91.99
2	*Microcoleus vaginatus* ISBAL M6	1	—	99.90	99.90	99.71	99.71	99.90	99.71	99.81	99.61	99.71	99.81	99.81	99.61	99.61	99.61	99.42	99.32	99.23	99.32	99.23	99.03	98.65	98.75	98.17	91.99
3	*Microcoleus vaginatus* SRS1-KK2	2	1	—	100.00	99.81	99.81	100.00	99.81	99.90	99.71	99.61	99.90	99.90	99.52	99.52	99.71	99.52	99.23	99.32	99.23	99.32	99.13	98.55	98.65	98.07	92.08
4	*Microcoleus* cf. *vaginatus* Ru-6-12	2	1	0	—	99.81	99.81	100.00	99.81	99.90	99.71	99.61	99.90	99.90	99.52	99.52	99.71	99.52	99.23	99.32	99.23	99.32	99.13	98.55	98.65	98.07	92.08
5	*Microcoleus vaginatus* El 1	2	3	2	2	—	100.00	99.81	100.00	99.71	99.90	99.52	99.90	99.71	99.32	99.32	99.71	99.71	99.23	99.52	99.03	99.32	99.13	98.55	98.46	98.07	92.18
6	*Microcoleus vaginatus* E17	2	3	2	2	0	—	99.81	100.00	99.71	99.90	99.52	99.90	99.71	99.32	99.32	99.71	99.71	99.23	99.52	99.03	99.32	99.13	98.55	98.46	98.07	92.18
7	*Microcoleus vaginatus* ISBAL M14	2	1	0	0	2	2	—	99.81	99.90	99.71	99.61	99.90	99.90	99.52	99.52	99.71	99.52	99.23	99.32	99.23	99.32	99.13	98.55	98.65	98.07	92.08
8	*Microcoleus vaginatus* ISBAL M22	2	3	2	2	0	0	2	—	99.71	99.90	99.52	99.90	99.71	99.32	99.32	99.71	99.71	99.23	99.52	99.03	99.32	99.13	98.55	98.46	98.07	92.18
9	*Microcoleus vaginatus* ISBAL M2	3	2	1	1	3	3	1	3	—	99.61	99.52	99.81	99.81	99.42	99.61	99.61	99.42	99.13	99.23	99.13	99.23	99.23	98.65	98.55	98.17	91.99
10	*Microcoleus vaginatus* KZ-23-1	3	4	3	3	1	1	3	1	4	—	99.61	99.81	99.61	99.23	99.23	99.61	99.61	99.13	99.42	99.13	99.23	99.03	98.46	98.36	98.07	92.18
11	*Microcoleus vaginatus* K25 08	3	3	4	4	5	5	4	5	5	4	—	99.52	99.52	99.32	99.32	99.32	99.23	99.13	99.03	99.23	98.94	98.75	98.46	98.46	98.07	92.08
12	*Phormidium autumnale* sv30	3	2	1	1	1	1	1	1	2	2	5	—	99.81	99.42	99.42	99.81	99.61	99.13	99.42	99.13	99.42	99.23	98.46	98.55	97.97	92.18
13	*Phormidium* cf. *autumnale* CCALA 145	3	2	1	1	3	3	1	3	2	4	5	2	—	99.42	99.42	99.61	99.42	99.13	99.23	99.13	99.23	99.03	98.46	98.55	97.97	91.99
14	*Microcoleus vaginatus* SNM1-KK1	5	4	5	5	7	7	5	7	6	8	7	6	6	—	99.23	99.23	99.03	98.94	98.84	98.94	98.84	98.65	98.26	98.36	97.78	91.60
15	*Microcoleus* sp. PET 11 7	5	4	5	5	7	7	5	7	4	8	7	6	6	8	—	99.23	99.03	99.13	98.84	99.32	99.03	99.03	98.65	98.55	98.17	91.99
16	*Microcoleus vaginatus* ISBAL M10	5	4	3	3	3	3	3	3	4	4	7	2	4	8	8	—	99.42	99.32	99.23	98.94	99.61	99.42	98.65	98.75	98.17	91.99
17	*Oscillatoria amoena* CCAP 1459/39	5	6	5	5	3	3	5	3	6	4	8	4	6	10	10	6	—	98.94	99.81	99.32	99.03	98.84	98.46	98.75	98.36	91.99
18	*Microcoleus* sp. MUM 11 5	6	7	8	8	8	8	8	8	9	9	9	9	9	11	9	7	11	—	98.75	98.65	99.52	99.32	99.13	99.03	98.65	91.80
19	*Phormidium autumnale* JR12	7	8	7	7	5	5	7	5	8	6	10	6	8	12	12	8	2	13	—	99.13	98.84	98.65	98.46	98.94	98.55	92.18
20	*Phormidium autumnale* CCALA 154	8	7	8	8	10	10	8	10	9	9	8	9	9	11	7	11	7	14	9	—	98.55	98.46	98.17	98.75	98.26	91.99
21	*Phormidium* sp. isolate: 2008	9	8	7	7	7	7	7	7	8	8	11	6	8	12	10	4	10	5	12	15	—	99.61	99.03	98.94	98.55	91.99
22	*Phormidium autumnale* LCR Cyant3a	11	10	9	9	9	9	9	9	8	10	13	8	10	14	10	6	12	7	14	16	4	—	98.84	99.13	98.75	92.18
23	*Phormidium autumnale* Arct-Ph5	13	14	15	15	15	15	15	15	14	16	16	16	16	18	14	14	16	9	16	19	10	12	—	98.75	98.75	91.31
24	*Phormidium autumnale* LCR-CYANT11	14	13	14	14	16	16	14	16	15	17	16	15	15	17	15	13	13	10	11	13	11	9	13	—	99.42	91.99
25	*Phormidium autumnale* SAG 78.79	18	19	20	20	20	20	20	20	19	20	20	21	21	23	19	19	17	14	15	18	15	13	13	6	—	91.80
26	*Planktothricoides raciborskii* NIES-207	83	83	82	82	81	81	82	81	83	81	82	81	83	87	83	83	83	85	81	83	83	81	90	83	85	—

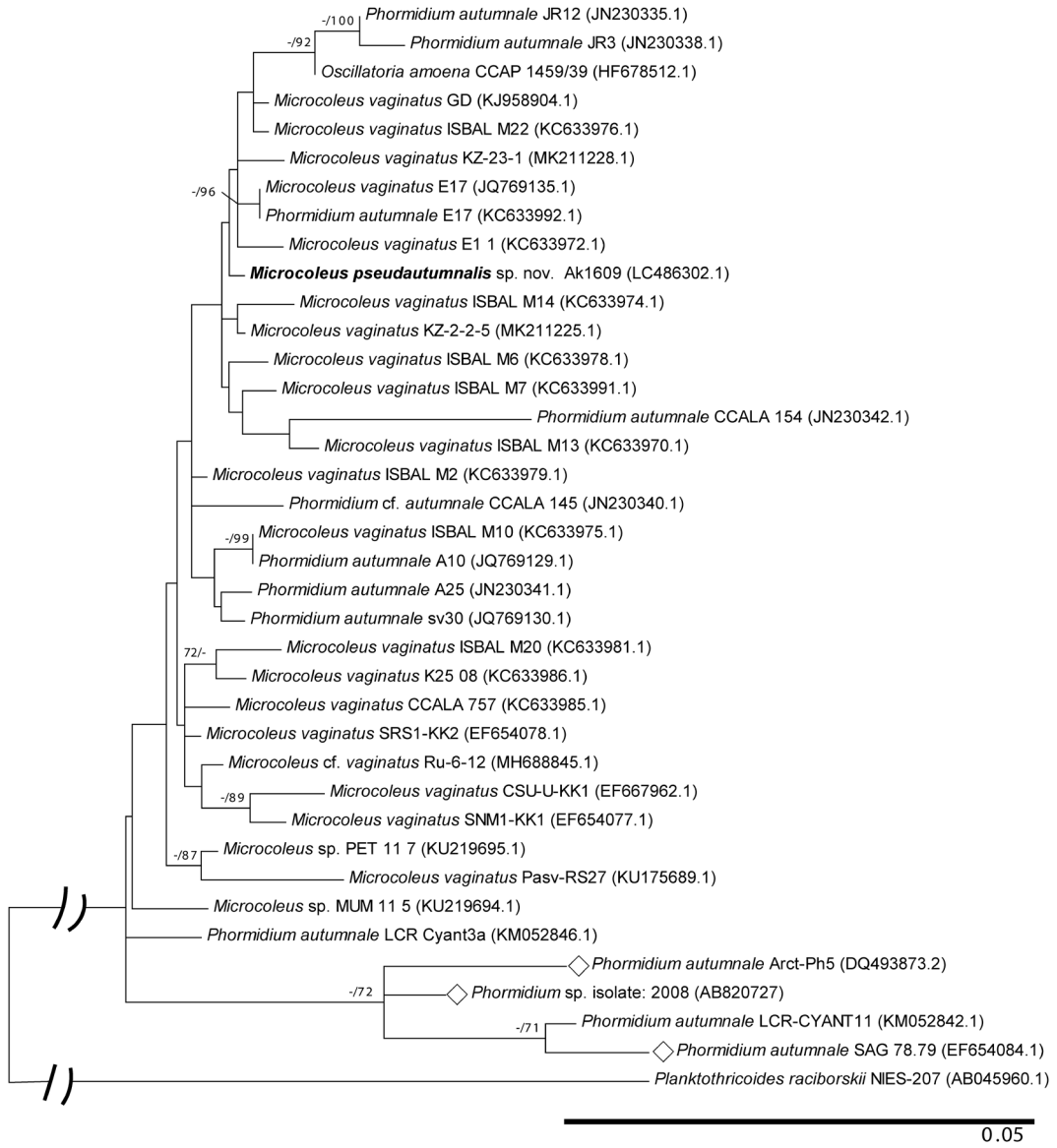


Fig. 11. Maximum likelihood tree based on 16S rRNA gene sequences. The general time reversible with gamma distribution and invariable sites model (GTR + G + I) was used. Sequences retrieved from NCBI are used for phylogenetic analysis, and the OTUs are shown with their registered name, culture number and accession number. Numbers at nodes indicate NJ (Neighbor Joining)/ML bootstrap support values (only values higher than 70 are shown). ◇: lacking11-bp insert.

its cultured solution have a strong bad smell. Both 2-MIB and geosmin were detected from the cultured solution by the SPME-GC/MS. Ak1609 is in the *Microcoleus* clade including *M. autumnalis* and *M. vaginatus* for the phylogenetic tree of the 16S rRNA gene (Fig. 11, Table 3). However, it is immotile and planktic and produces 2-MIB and geosmin, and these characteristics differ from those of *M. autumnalis* and *M. vaginatus*. Thus, we propose a new species, *M. pseudautumnalis*, as follows.

Microcoleus pseudautumnalis Niiyama et Tuji, *sp. nov.*

Description: Filaments mostly straight, without any type of branching, combine to make a black colored thin membranous colony in a test tube. Sheath thin, firm, colorless, always containing one trichome. Trichomes immotile, with or without sheath, grayish-green to pale yellowish-green colored, 6.9–7.6 µm in width, not constricted at the cross-walls, somewhat granulated at the cross-walls, not attenuated at the ends with rounded apical cells or attenuated towards the curved ends with truncated or capitated apical cells, sometimes with calyptra. Cells shorter or longer than wide, cell width to length ratio 0.5–2.5, without gas vesicles. Thallus has the extreme musty odor that comes from 2-MIB and geosmin.

Holotype: A formalin-fixed specimen, TNS-AL-61750 in TNS (Department of Botany, National Museum of Nature and Science), from cultured strain Ak1609 maintained in TNS.

Isotype: A formalin-fixed specimen, TNS-AL61748 in TNS.

Type locality: Naka-numa pond, Ibaraki Pref., Japan.

Habitat: Plankton in ponds.

Table 2 shows a comparison of the morphological, ecological, and genetic traits and the presence or absence of a bad smell for *M. autumnalis* (*P. autumnale*), *M. vaginatus*, and the new species reported in this study, *M. pseudautumnalis*. *M. pseudautumnalis* always has one trichome in the sheath, just like *M. autumnalis*. *Microcoleus pseudautumanalis* has very closed sequence to *M. autumnalis* and *M. vaginatus* in 16S rRNA (table 3). It is noted that *M. vaginatus* has 11 base pairs inserted into the 16S rRNA gene (Boyer *et al.*, 2002; Siegesmund *et al.* 2008; Hašler *et al.*, 2012; Strunecký *et al.*, 2013). On the other hand, *M. autumnalis* (*P. autumnale s. str.*) lacks this 11-bp insert (Hašler *et al.*, 2012; Strunecký *et al.*, 2013) and forms a monophyletic group in the genus *Microcoleus* (Strunecký *et al.*, 2013). *Microcoleus pseudautumnalis* is in the same clade as *M. vaginatus* based on an analysis of the 16S rRNA gene and has the 11-bp insert as does *M. vaginatus* (Fig. 12). *Microcoleus autumnalis* is said to be an epipelic or epiphytic species in aquatic habitats, and *M. vaginatus* is thought to be a soil or aquatic species, and both are motile, especially *M. vaginatus* (Boyer *et al.* 2002). However, *M. pseudautumnalis* is an immotile and planctic species. No studies have been reported on *Microcoleus* species producing 2-MIB.

The 4507-bp sequence of the gene cluster for 2-MIB including the *cnb*A, *mtf*, *mic*, and *cnb*B genes was obtained from *M. pseudautumnalis* (cultured strain Ak1609: accession no.: LC486303). These genes and their order are the same as *Pseudanabaena* sp. dqh15 (HQ830028) and *Planktothricoides raciborskii* CHAB3331 (HQ830029) presented in Wang *et al.* (2011). The gene cluster sequences excluding ITS regions (4079 bp) were compared. *Microcoleus pseudautumnalis* is closer to *Pl. raciborskii* (216 bp differences) than *Pseudanabaena* sp. dqh15 (429 bp differences). *Pseudanabaena* sp. dqh15 and *Pl. raciborskii* had 508 bp differences. These large differences between *M. pseudautumnalis* and other 2-MIB-producing taxa suggest that these 2-MIB-producing genes evolved separately and do not exhibit lateral gene transfer, which is found in many genes producing secondary metabolites such as geosmin (Hayashi *et al.*, 2019).

Tsunoda *et al.* (2014) reported a 2-MIB producing peryphytic *Phormidium* sp. from the Tama River in Japan, and they proposed that this species may be *P. autumunale* or *P. favosum* based on their microscopic observation. Because the 16S rRNA sequence of the 2-MIB-producing cultured strain presented in Tsunoda *et al.* (2014) (accession no.: AB820727) lacks the 11-bp insert (see Fig. 12) and is close to *P. autumnale* Arct-Ph5 in the phylogenetic tree (Fig. 11), it is different from *M. pseudautumnalis* and should be *M. autumnalis* (*P. autumnale s. str.*) (Hašler *et al.*, 2012; Strunecký *et al.*, 2013).

Microcoleus pseudautumnalis produces not only 2-MIB but also geosmin. Although many musty-odor-producing cyanobacteria strains are

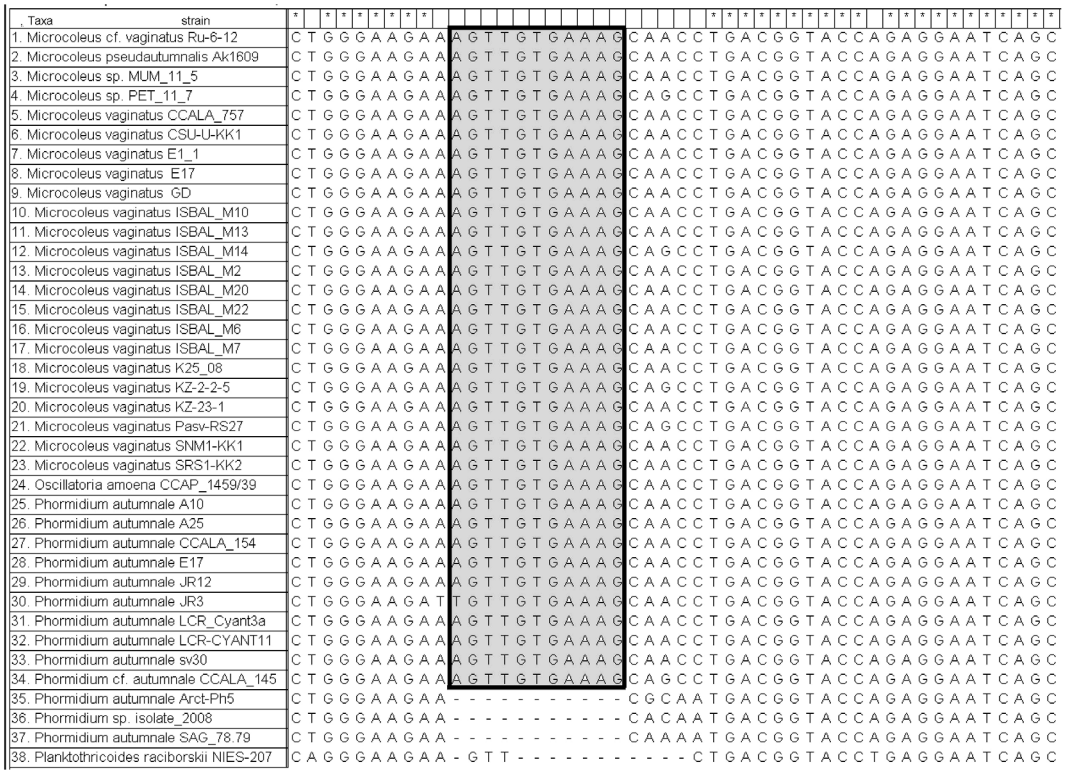


Fig. 12. Variable positions of the rRNA regions between *Phormidium autumnale* (= *Microcoleus autumnalis*), *M. pseudautumnalis* and *M. vaginatus* showing 11-bp insert presented in Hašler *et al.* (2012) and Strunecký *et al.* (2013).

reported in Japan, each cultured strain produces only one kind of odor-producing substance (Yagi, 1983; Oikawa and Ishibashi, 2004). The present study is the first to report the production of both 2-MIB and geosmin by one strain of cyanobacteria.

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