J. Gen. Appl. Microbiol.
doi 10.2323/jgam.2021.03.003

Short Communication

Characterization of mycosporine-like amino acids in the edible cyanobacterium *Nostoc commune* (Di Pi Cai) from China

Running title: **Nostoc-756 in Di Pi Cai**

(Received December 17, 2020; Accepted March 5, 2021; J-STAGE Advance publication date: August 4, 2021)

Yang Wei[1], Takumi Nishiuchi[1, 2], and Toshio Sakamoto[1, 3 *]

[1]Division of Biological Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan.

[2]Division of Functional Genomics, Advanced Science Research Center, Kanazawa University, Takara, Kanazawa 920-0934, Japan.

[3]School of Biological Science and Technology, College of Science and Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan.

***Address for correspondence:**
Dr. Toshio Sakamoto
School of Biological Science and Technology, College of Science and Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan.
Phone: +81-76-264-6227
Fax: +81-76-264-6215
E-mail: tsakamot@staff.kanazawa-u.ac.jp

The terrestrial cyanobacterium *Nostoc commune* has a cosmopolitan distribution. It is edible, and dry thalli are sold as a food in China under the name of Di Pi Cai. The pigment composition and the genotypes were characterized to identify the cyanobacterium Di Pi Cai from China as *N. commune*. Myxol glycosides and ketocarotenoids were detected, as expected in *Nostoc* sp., but β-carotene and hydroxylated carotenoids were not detected. Nostoc-756, mycosporine-2-(4-deoxygadusoyl-ornitine), was found to be a main mycosporine-like amino acid, which indicates that Di Pi Cai belongs to the *N. commune* chemotype C. However, the 16S rRNA gene and the *petH* gene encoding ferredoxin-$NADP^+$ oxidoreductase of Di Pi Cai did not exactly match those of genotype C found in Japan. These results suggest the unique molecular genetic features of Di Pi Cai and the global diversity of *N. commune.*

Keywords: carotenoid; mycosporine-like amino acid (MAA); scytonemin; terrestrial cyanobacterium

Nostoc commune is a terrestrial cyanobacterium that has a cosmopolitan distribution from the tropics to the polar regions of Earth (Potts, 2000). *N. commune* forms visually conspicuous colonies with massive extracellular polysaccharides and is unique due to its extreme desiccation tolerance. The desiccated colonies show undetectable photosynthetic and respiratory activities and are able to rapidly resume metabolism upon rehydration (Scherer et al., 1984; Tamaru et al., 2005; Sakamoto et al., 2009). Under a desiccated state, *N. commune* retains the ability to grow for over 100 years (Lipman, 1941; Cameron, 1962). This phenomenon is termed "anhydrobiosis", and the molecular mechanism of extreme desiccation tolerance by this anhydrobiotic cyanobacterium is thought to involve multiple processes that include extracellular polysaccharide production (Tamaru et al., 2005; Morsy et al., 2008; Sakamoto et al., 2011; Inoue-Sakamoto et al., 2018a), compatible solute accumulation (Yoshida and Sakamoto 2009; Sakamoto et al., 2009; Sakamoto et al., 2011), regulation of photosynthesis (Satoh et al., 2002; Fukuda et al., 2008) and protection from UV radiation (Matsui et al., 2011; 2012; Nazifi et al., 2013; 2015; Inoue-Sakamoto et al., 2018b; Wada et al., 2015).

Some of *Nostoc* such as *N. commune*, *Nostoc verrucosum* and *Nostoc flagelliforme* are known to be edible and have been consumed as local food for a long time (Potts, 2000). In China, the traditional eating of *N. flagelliforme*, which is called Fa Cai (hair vegetable) due to its unique hair-like morphology, is believed to bring good luck. However, *N. flagelliforme* occurs in specific semi-desert habitats and grows very slowly and all further collection, sale and exportation have been banned since 2000 (Roney et al., 2009). On the other hand, it is easy to collect large amounts of *N. commune* colonies which are found in habitats worldwide. Thus, the dry thalli of *N. commune* are sold as a food in China under the name of Di Pi Cai at a reasonable price. In this study, the carotenoid composition, mycosporine-like amino acid (MAA) chemotype and the genotype using 16S rRNA gene

and the *petH* gene encoding cyanobacterial ferredoxin-$NADP^+$ oxidoreductase (FNR) were characterized in Di Pi Cai from China to identify its chemo-genotype.

Food samples of *N. commune* (Di Pi Cai) were purchased through an internet shopping site (Table S1). After rehydration of Di Pi Cai, the wet thalli became elastic and jelly-like, and their appearance and smell were similar to those of living *N. commune* colonies isolated from the field. These features of Di Pi Cai suggest that it is a fresh food; however, no photosynthetic oxygen evolving activity was detected in the hydrated thalli of Di Pi Cai when measured using a Clark-type oxygen electrode. Two types of fragments in Di Pi Cai were observed microscopically: the typical trichomes of cells, as expected in *N. commune* colonies, were obvious in the extracellular matrix (Fig. S1A), or there were no trichomes but separated cells were observed instead (Fig. S1B). These results suggest that cells in Di Pi Cai were not sustaining biological activities and may have been damaged during the production process and/or storage.

Cyanobacteria synthesize unique ketocarotenoids and glycosyl carotenoids that are not found in higher plants (Takaichi and Mochimaru, 2007). We have reported that carotenoid patterns are useful as a chemotaxonomic marker for distinguishing *Nostoc* strains (Arima et al., 2012). Three groups with distinct patterns of carotenoids are related to the phylogenetic tree constructed on the basis of the 16S rRNA genes of *N. commune* and its neighboring *Nostoc* species. *N. commune* and *Nostoc punctiforme* are clustered in one monophyletic group and characterized by the occurrence of nostoxanthin, canthaxanthin and myxol glycosides (Arima et al., 2012).

The carotenoid composition of Di Pi Cai was analyzed according to Arima et al. (2012). Pigments were extracted from the dry thalli with acetone/methanol (7:2, v/v) using a mortar and pestle. After centrifugation, the extract was injected into an HPLC system with a pump (PU-2087 Plus quaternary gradient pump, Jasco, Tokyo) equipped with a

reversed-phase column (Cosmosil $5C_{18}$-MS-II, 4.6 × 150 mm, Nacalai Tesque, Inc., Kyoto). The UV/VIS spectrum was acquired with a detector (MD-2018 Plus photodiode array detector, Jasco). After elution with 9:1 methanol/water (v/v) for the initial 12 min, the mobile phase was changed to 100% methanol gradually over 1 min. After elution with 100% methanol for 15 min, the mobile phase was changed to 1:2 chloroform/methanol (v/v) gradually over 1 min. The third elution with 1:2 chloroform/methanol (v/v) continued for 11 min to elute β-carotene. The flow rate of the mobile phase was kept at 1.0 ml min^{-1}. Scytonemin (Matsui et al., 2012) and chlorophyll *a* were identified according to their characteristic absorption spectra. Carotenoids were assigned according to their retention times and characteristic absorption spectra as described by Takaichi et al. (2009). For quantitative analysis, the molar extinction coefficient at the maximum wavelengths of each carotenoid was assumed to be the same (140 mM^{-1} cm^{-1}). The molar extinction coefficient of chlorophyll *a* (66.7 mM^{-1} cm^{-1} at 665 nm) was used to calculate the molar ratio of total carotenoids to chlorophyll *a*.

A 2D chromatogram and the absorption maxima of the detected pigments are shown in Fig. S2. Table 1 shows the carotenoid composition of *N. commune* (Di Pi Cai). Hydroxymyxol glycoside, myxol glycosides, canthaxanthin, and echinenone were detected, but β-carotene and hydroxylated carotenoids such as nostoxanthin, calozanthin, and zeaxanthin were not detected (Table 1). The absence of β-carotene was unusual because it was expected to be a major carotenoid commonly present in the genus *Nostoc* (Arima et al., 2012). β-Carotene was not detected in *N. commune* samples that were stored at room temperature for over 8 years (Toshio Sakamoto, unpublished). Thus, β-carotene and hydroxylated carotenoids may be degraded during the production process of Di Pi Cai, although there was no evidence to rule out the possibility that the absence of β-carotene and hydroxylated carotenoids was a characteristic feature of this particular *Nostoc* species.

Except for the absence of β-carotene, the carotenoid composition of Di Pi Cai could belong to a previously described carotenoid pattern of *Nostoc* sp. (Arima et al., 2012). Nevertheless, it is noteworthy that the liposoluble pigments were almost intact and remained in the dry thalli of Di Pi Cai (Table 1; Fig. S2). Remarkable levels of scytonemin and chlorophyll *a* were detected as liposoluble pigments (Fig. S2), which indicates that Di Pi Cai is a scytonemin-producing cyanobacterium. Because *N. commune* is highly tolerant against desiccation, photosynthetic pigments, including chlorophyll *a* and carotenoids, must be sustained under desiccation conditions, although the mechanism by which these pigments are protected remains to be elucidated. Strong antioxidative compounds are assumed to be involved in the protection, and further study to identify such compounds is needed.

Mycosporine-like amino acids (MAAs) are water-soluble molecules that absorb UV-A and UV-B (Wada et al., 2015), and MAA compositions can be used for the classification of *N. commune* (Sakamoto et al., 2019). Although morphological features of the macroscopic appearance of colonies and microscopic patterns of cells are hardly distinguishable, the types of unique glycosylated MAAs can be used as markers to characterize *N. commune* in Japan (Sakamoto et al., 2019). *N. commune* is classified into four groups representing genetically different chemotypes, namely, the arabinose-bound porphyra-334 producer (chemotype A), the glycosylated nostoc-756 producer (chemotype B), the nostoc-756 producer (chemotype C) and the glycosylated palythine-threonine producer (chemotype D) (Sakamoto et al., 2019).

The MAA composition of Di Pi Cai was characterized according to Sakamoto et al. (2019). Pigments were extracted from the dry thalli with 100% methanol using a mortar and pestle. After centrifugation to remove the debris, 4 volumes of water were added to the methanol extract to yield 20% (v/v) methanol. After centrifugation, the MAA-containing solution was injected into an HPLC system with a pump (PU-2087 Plus, Jasco) equipped

with a reversed-phase column (InertSustain C18, 4.6 × 150 mm, GL Sciences, Inc., Tokyo). The mobile phase was 5% (v/v) methanol with 0.1% (v/v) acetic acid at a flow rate of 0.6 ml min^{-1}, and isocratic elution was implemented. The UV spectrum was recorded with a photodiode array detector (MD-2018 Plus, Jasco). For quantitative analysis, the molar extinction coefficient was assumed to be 58,800 M^{-1} cm^{-1} at 312 nm (Matsui et al., 2011).

Two MAAs with different retention times were detected, and both of the UV absorption spectra were highly similar to those of nostoc-756 and a 1050-Da MAA (nostoc-756 glycoside); a characteristic UV absorption spectrum with an absorption peak at 310 nm associated with another peak at 334 nm was observed (Fig. S4). The main MAA from Di Pi Cai accounted for approximately 96% of the total MAAs according to the HPLC chromatogram (Fig. S4), and its retention time was identical to that of the nostoc-756 standard. The main MAA was assumed to be nostoc-756 and characterized further for identification.

The MAAs were purified from Di Pi Cai to obtain enough sample for confirmation by MS analyses. The dry powder of Di Pi Cai (50 g) was suspended in 200 ml of 80% methanol and left at room temperature for 3 h. The 80% methanol extract was recovered with a Buchner funnel, and the residue was extracted again using 100 ml of 80% methanol. The second extract was recovered by filtration with coarse filter paper (Prowipe, Daio Paper Co., Tokyo). The extraction using 80% methanol was repeated 4 times in total, and these extracts were condensed using a rotary evaporator (EYELA N-1300). The dry materials were dissolved in water, and insoluble materials were removed by centrifugation at 22,300 × *g* for 5 min at 25°C and filtration with coarse filter paper (Prowipe). Equal volumes of methanol and chloroform were added to the water solution and mixed vigorously. After waiting to separate into two phases, the upper aqueous phase was recovered. The MAA-containing fraction was evaporated with a rotary evaporator and dissolved in

methanol. The methanol-soluble fraction was evaporated with a rotary evaporator and dissolved in water. After removing water-insoluble materials, the solvent was replaced to methanol and kept at -30°C. The methanol-insoluble materials were removed by centrifugation at 22,300 × g for 10 min. The supernatant was evaporated with a rotary evaporator and dissolved in ultrapure water. The aqueous solution was filtered through a centrifugal filter unit (Amicon Ultra-15 10K, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co., Cork, Ireland), and the filtrate was recovered. After centrifugation, the supernatant (0.1 ml) was injected into an HPLC system with a reversed-phase column (CAPCELL PAK C18 AQ, 10 × 250 mm, Shiseido Co., Ltd., Tokyo). The mobile phase was 10% (v/v) methanol with 0.1% (v/v) acetic acid at a flow rate of 3.0 ml min^{-1}. The absorbance at 330 nm was monitored with a UV-VIS detector (L-4200, Hitachi), and two fractions with different retention times were collected separately. Each recovered fraction was condensed with a rotary evaporator and injected into the same HPLC system again for chromatographic separation. The recovered peak fraction was condensed with a rotary evaporator, lyophilized, and dissolved in 2 ml of ultrapure water. The sample was further purified using an HPLC system with another reversed-phased column (Inertsil ODS-3, 4.6 × 250 mm, GL Sciences, Tokyo). The mobile phase was 7.5% (v/v) methanol with 0.1% (v/v) acetic acid, and the flow rate was kept at 1.0 ml min^{-1} for isocratic elution. The absorbance at 330 nm was monitored with a UV-VIS detector (L-4200, Hitachi). The recovered fraction was lyophilized, and the final product was dissolved in 2 ml of ultrapure water (Table 2).

MALDI-TOF MS analysis of the purified nostoc-756 was performed using a tandem mass spectrometer (4800 plus MALDI TOF/TOF™ analyzer; Applied Biosystems, Foster City, CA) using 2,5-dihydroxybenzoic acid (DHB) as a matrix. A molecular ion fragment with m/z 757 was detected. The accurate mass and the predicted elemental composition of the purified nostoc-756 was determined by FAB HR-MS analysis using a

mass spectrometer (JMS-700; JEOL Ltd., Tokyo) with glycerol as a matrix. A molecular ion fragment with m/z 757.3503 was detected, and its predicted molecular formula was $C_{34}H_{52}N_4O_{15}$ with an error of -0.6 ppm. For the purified 1050-Da MAA, a molecular ion fragment with m/z 1051 was detected by both MALDI-TOF and FAB MS analyses, although the result of FAB HR-MS analysis was not obtained because of the limitation of the sample. These results indicate that the main MAA in Di Pi Cai is nostoc-756, mycosporine-2-(4-deoxygadusoyl-ornitine) (Fig. S6A), and the second most abundant MAA is the 1050-Da MAA, notosc-756 glycoside (Fig. S6B). Because of the presence of nostoc-756, Di Pi Cai belongs to chemotype C of *N. commune* (Sakamoto et al., 2019). MAA compositions were compared among Di Pi Cai samples from different origins (Table S1) according to Sakamoto et al. (2019). UV absorption spectra and HPLC chromatograms of the MAA extracts were identical or highly similar to each other (Fig. S5), indicating that the chemotypes of these Di Pi Cai samples (Table S1) were identical and marked by the presence of nostoc-756 (Fig. S6A).

Four genotypes of *N. commune* have been identified on the basis of differences in the 16S rRNA gene and distributed throughout Japan without regional specificity (Arima et al., 2012; Sakamoto et al., 2019). Ferredoxin-$NADP^+$ oxidoreductase (FNR) catalyzes electron transfer from the reduced ferredoxin to $NADP^+$ to produce NADPH in the final step of photosynthetic electron transfer. The cyanobacterial FNR contains a unique N-terminal domain similar to the 9-kDa phycocyanin-associated linker polypeptide CpcD. Thus, the *petH* gene that encodes cyanobacterial FNR is thought to be a specific and characteristic marker gene to identify cyanobacterial species (Arima et al. 2012).

The genotypes of the Di Pi Cai samples (Table S1) were characterized by PCR-direct sequencing of the 16S rRNA gene and the *petH* gene encoding cyanobacterial FNR according to Arima et al. (2012). The nucleotide sequences of the 16S rRNA genes of

Di Pi Cai were highly similar to each other with >99% similarity (Table S2). Figure 1 shows the unrooted neighbor-joining tree of the 16S rRNA gene sequences comparing Di Pi Cai and four genotypes of *N. commune* found in Japan. These 16S rRNA gene sequences showed >95% similarity and Di Pi Cai formed a distinct cluster separated from the four genotypes of *N. commune* in Japan. Consistent with the sequence similarities of the 16S rRNA genes, the *petH* gene products of Di Pi Cai were identical or highly similar to each other (Table S3) but none of the *petH* genes exactly matched those of the genotypes found in Japan (Table S4). These results suggest that Di Pi Cai was genetically identified as a strain of *N. commune*; however, attributing Di Pi Cai to one of the four genotypes found in Japan was not possible.

We characterized the edible cyanobacterium Di Pi Cai from China using molecular genetic markers according to Arima et al. (2012). This is the first report to identify the cyanobacterium Di Pi Cai as *Nostoc commune*. Although there is no experimental evidence to rule out the possibility that the food samples of Di Pi Cai consist of several cyanobacterial species, it is certain that *N. commune* is a dominant species in the thalli of Di Pi Cai. The presence of nostoc-756 indicates that Di Pi Cai belongs to chemotype C of *N. commune*, but its nucleotide sequence of the 16S rRNA gene and the deduced amino-acid sequence of the *petH* gene were different from those of the four genotypes found in Japan (Fig. 1 and Table S4). The MAA composition of Di Pi Cai samples with different sampling localities (Table S1) were identical or highly similar to each other (Fig. S5), consistent with their low genetic diversity (Table S3 and S4). To elucidate the global diversity of *N. commune*, worldwide studies are needed to compare the relationship between MAA chemotypes and *N. commune* genotypes. The pigments, including scytonemin, chlorophyll *a*, nostoc-756, carotenoid glycosides and ketocarotenoids, remained stable in the dry thalli of Di Pi Cai, although they were not biologically active. This feature may be relevant to the value of Di Pi Cai as food,

and to the mechanism of anhydrobiosis in *N. commune.* The dry thalli of Di Pi Cai contain indigestible polysaccharides as dietary fiber and nostoc-756 that functions as a radical scavenger *in vitro* (Sakamoto et al., 2019). Thus, the edible *N. commune* can be thought of as a healthy food and a potential resource for dietary supplements or functional food development.

Acknowledgments

We would like to thank Ms. Mari Ikurumi (Research Institute for Instrumental Analysis, Advanced Science Research Center, Kanazawa University) for the FAB-MS and FAB-HR-MS analyses. This work was supported by the Japan Society for the Promotion of Science (KAKENHI 20K05724).

Supporting Information

Table S1. List of *Nostoc commune* (Di Pi Cai) samples.
Table S2. A comparison of the 16S rRNA genes of *Nostoc commune* (Di Pi Cai).
Table S3. A comparison of the *petH* gene products of *Nostoc commune* (Di Pi Cai).
Table S4. Differences of the *petH* gene products in *Nostoc commune*.
Fig. S1. Microscopic observation of the edible cyanobacterium *Nostoc commune* (Di Pi Cai).
Fig. S2. Pigment analysis of the edible cyanobacterium *Nostoc commune* (Di Pi Cai).
Fig. S3. Chemical structures of scytonemin and carotenoids in the edible cyanobacterium *Nostoc commune* (Di Pi Cai).
Fig. S4. Analysis of mycosporine-like amino acids (MAAs) in the edible cyanobacterium *Nostoc commune* (Di Pi Cai).
Fig. S5. HPLC chromatograms of MAA-containing extracts from the edible cyanobacterium *Nostoc commune* (Di Pi Cai).
Fig. S6. Chemical structures of the mycosporine-like amino acids in the edible cyanobacterium *Nostoc commune* (Di Pi Cai).

References

Arima, H., Horiguchi, N., Takaichi, S., Kofuji, R., Ishida, K., Wada, K., and Sakamoto, T. (2012) Molecular genetic and chemotaxonomic characterization of the terrestrial cyanobacterium *Nostoc commune* and its neighboring species. *FEMS Microbiol. Ecol.*, **79**, 35-45. DOI: 10.1111/j.1574-6941.2011.01195.x

Cameron, R. E. (1962) Species of *Nostoc* vaucher occurring in the Sonoran Desert in Arizona. *Trans. Amer. Microsc. Soc.,* **81**, 379-384. DOI: 10.2307/3223790

Fukuda, S., Yamakawa, R., Hirai, M., Kashino, Y., Koike, H., and Satoh, K. (2008) Mechanisms to avoid photoinhibition in a desiccation-tolerant cyanobacterium, *Nostoc commune*. *Plant Cell Physiol.,* **49**, 488-492. DOI: 10.1093/pcp/pcn018

Inoue-Sakamoto, K., Tanji, Y., Yamaba, M., Natsume, T., Masaura, T., Asano, T., Nishiuchi, T., and Sakamoto, T. (2018a) Characterization of extracellular matrix components from the desiccation-tolerant cyanobacterium *Nostoc commune*. *J. Gen. Appl. Microbiol*., **64**, 15-25. DOI: 10.2323/jgam.2017.03.001

Inoue-Sakamoto, K., Nazifi, E., Tsuji, C., Asano, T., Nishiuchi, T., Matsugo, S., Ishihara, K., Kanesaki, Y., Yoshikawa, H., and Sakamoto, T. (2018b) Characterization of mycosporine-like amino acids in the cyanobacterium *Nostoc verrucosum*. *J. Gen. Appl. Microbiol*., **64**, 203-211. DOI: 10.2323/jgam.2017.12.003

Lipman, C. B. (1941) The successful revival of *Nostoc commune* from a herbarium specimen eighty-seven years old. *Bull. Torr. Bot. Club,* **68**, 664-666. DOI: 10.2307/2481755

Matsui, K., Nazifi, E., Kunita, S., Wada, N., Matsugo, S., and Sakamoto, T. (2011) Novel glycosylated mycosporine-like amino acids with radical scavenging activity from the cyanobacterium *Nostoc commune*. *J. Photochem. Photobiol. B: Biology*, **105**, 81-89. DOI: 10.1016/j.jphotobiol.2011.07.003

Matsui, K., Nazifi, E., Hirai, Y., Wada, N., Matsugo, S., and Sakamoto, T. (2012) The cyanobacterial UV-absorbing pigment scytonemin displays radical-scavenging activity. *J. Gen. Appl. Microbiol*., **58**, 137-144. DOI: 10.2323/jgam.58.137

Morsy, F. M., Kuzuha, S., Takani, Y., and Sakamoto, T. (2008) Novel thermostable glycosidases in the extracellular matrix of the terrestrial cyanobacterium *Nostoc commune*. *J. Gen. Appl. Microbiol.,* **54**, 243-252. DOI: 10.2323/jgam.54.243

Nazifi, E., Wada, N., Yamaba, M., Asano, T., Nishiuchi, T., Matsugo, S., and Sakamoto, T. (2013) Glycosylated porphyra-334 and palythine-threonine from the terrestrial

cyanobacterium *Nostoc commune*. *Mar. Drugs*, **11**, 3124-3154. DOI: 10.3390/md11093124

Nazifi, E., Wada, N., Asano, T., Nishiuchi, T., Iwamuro, Y., Chinaka, S., Matsugo, S., and Sakamoto, T. (2015) Characterization of the chemical diversity of glycosylated mycosporine-like amino acids in the terrestrial cyanobacterium *Nostoc commune*. *J. Photochem. Photobiol. B: Biology*, **142**, 154-168. DOI: 10.1016/j.jphotobiol.2014.12.008

Potts, M. (2000) *Nostoc*. In The Ecology of Cyanobacteria, ed. by Whitton, B. A. and Potts, M., Kluwer Academic Publishers, Netherlands, pp. 465-504. DOI: 10.1007/0-306-46855-7_17

Roney, B. R., Renhui, L., Banack, S. A., Murch, S., Honegger, R., and Cox, P. A. (2009) Consumption of *fa cai Nostoc* soup: A potential for BMAA exposure from *Nostoc* cyanobacteria in China? *Amyotroph. Lateral Scler*., **10:sup2,** 44-49. DOI: 10.3109/17482960903273031

Sakamoto, T., Yoshida, T., Arima, H., Hatanaka, Y., Takani, Y., and Tamaru, Y. (2009) Accumulation of trehalose in response to desiccation and salt stress in the terrestrial cyanobacterium *Nostoc commune. Phycol. Res.,* **57**, 66-73. DOI: 10.1111/j.1440-1835.2008.00522.x

Sakamoto, T., Kumihashi, K., Kunita, S., Masaura, T., Inoue-Sakamoto, K., and Yamaguchi, M. (2011) The extracellular-matrix-retaining cyanobacterium *Nostoc verrucosum* accumulates trehalose, but is sensitive to desiccation. *FEMS Microbiol. Ecol.*, **77**, 385-394. DOI: 10.1111/j.1574-6941.2011.01114.x

Sakamoto, T., Hashimoto, A., Yamaba, M., Wada, N., Yoshida, T., Inoue-Sakamoto, K., Nishiuchi, T., and Matsugo, S. (2019) Four chemotypes of the terrestrial cyanobacterium *Nostoc commune* characterized by differences in the mycosporine-like amino acids. *Phycol. Res*., **67**, 3-11. DOI: 10.1111/pre.12333

Satoh, K., Hirai, M., Nishio, J., Yamaji, T., Kashino, Y., and Koike, H. (2002) Recovery of photosynthetic systems during rewetting is quite rapid in a terrestrial cyanobacterium, *Nostoc commune*. *Plant Cell Physiol.,* **43**, 170-176. DOI: 10.1093/pcp/pcf020

Scherer, S., Ernst, A., Chen, T.-W., and Böger, P. (1984) Rewetting of drought-resistant blue-green algae: Time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. *Oecologia,* **62**, 418-423. DOI: 10.1007/BF00384277

Takaichi, S. and Mochimaru, M. (2007) Carotenoids and carotenogenesis in cyanobacteria:

unique ketocarotenoids and carotenoid glycosides. *Cell. Mol. Life Sci.,* **64**: 2607-2619. DOI: 10.1007/s00018-007-7190-z

Takaichi, S., Maoka, T., and Mochimaru, M. (2009) Unique carotenoids in the terrestrial cyanobacterium *Nostoc commune* NIES-24: 2-Hydroxymyxol 2'-fucoside, nostoxanthin and canthaxanthin. *Curr. Microbiol.* **59**, 413-419. DOI: 10.1007/s00284-009-9453-4

Tamaru, Y., Takani, Y., Yoshida, T., and Sakamoto, T. (2005) Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Appl. Environ. Microbiol.,* **71**, 7327-7333. DOI: 10.1128/AEM.71.11.7327-7333.2005

Wada, N., Sakamoto, T., and Matsugo, S. (2015) Mycosporine-like amino acids and their derivatives as natural antioxidants. *Antioxidants,* **4**, 603-646. DOI: 10.3390/antiox4030603

Yoshida, T. and Sakamoto, T. (2009) Water-stress induced trehalose accumulation and control of trehalase in the cyanobacterium *Nostoc punctiforme* IAM M-15. *J. Gen. Appl. Microbiol.,* **55**, 135-145. DOI: 10.2323/jgam.55.135

Figure legend

Fig. 1. Phylogenetic analysis of Di Pi Cai (CHN1-6) from China and four genotypes of *Nostoc commune* in Japan.

The unrooted neighbor-joining tree of the 16S rRNA gene sequences was generated by ClustalW version 2.1 at DNA Data Bank of Japan (DDBJ). The nucleotide sequences are deposited in GenBank/EMBL/DDBJ under the following accession numbers: LC549649 (CHN1), LC595227 (CHN2), LC596954 (CHN3-6), AB088375 (strain KU002, NIES-2538, A type), AB933330 (strain KU006, NIES-3989, B type), AB113666 (B type), AB986249 (strain KU007, NIES-3990, C type), LC013478 (strain KU008, NIES-3991, D type), AB933329 (D type), and AB428654 (D type). Four chemotypes of *N. commune* have been identified on the basis of differences in the mycosporine-like amino acid (MAA) composition (Sakamoto et al., 2019).

Table 1. Carotenoid composition of *Nostoc commune* (Di Pi Cai).

Myxol glycosides		Ketocarotenoids			
Hydroxymyxol glycoside	Myxol glycoside	Canthaxanthin	Echinenone	β-carotene	[car]:[Chl *a*] Molar ratio
		(mol%)			
5.0 ± 1.2	20.4 ± 3.3	49.1 ± 7.2	25.3 ± 4.8	N.D.	0.52 ± 0.04

Pigments were extracted using 7:2 acetone/methanol (v/v) from the dry thalli of Di Pi Cai (CHN1), and carotenoid composition was analyzed using 2D-LC. A typical 2D chromatogram is shown in Fig. S2. The chemical structures of the carotenoids are shown in Fig. S3.

Average ± SD (N=5) are shown.

N.D., not detected.

Table 2. Purification of MAAs from the edible *Nostoc commune* (Di Pi Cai).

Step	Solvent	Volume (ml)	Concentration (mg ml^{-1})	Amount (mg)	Yield (%)
80%-methanol extract	80%-methanol	450	0.19[a]	87.3	100.0
Dissolved in water	Water	50	1.34[a]	67.0	76.7
Condensed solution in methanol	Methanol	50	1.11[a]	55.3	63.3
Condensed solution in water	Water	15	3.30[a]	49.6	56.7
Filtrate through a centrifugal filter	Water	10	3.35[a]	33.5	38.4
Peak 1 separated by HPLC	Water	2	0.54[b]	1.1	1.2
Peak 2 separated by HPLC	Water	2	4.72[b]	9.4	10.8
Peak 1 lyophilized (1050-Da MAA)				1.2[c]	1.4
Peak 2 lyophilized (nostoc-756)				5.6[c]	6.4

The dry powder (50 g) of Di Pi Cai (CHN1) containing nostoc-756 was used as starting materials, and the MAAs were extracted and separated as described in the text. The chemical structures of the MAAs are shown in Fig. S6.

[a]MAA concentration was calculated by absorption coefficient of 77.8 l g^{-1} cm^{-1} at 312 nm (Sakamoto et al., 2019).

[b]MAA concentration was calculated by absorption coefficient of 56.0 l g^{-1} cm^{-1} at 312 nm (Matsui et al., 2011).

[c]The weight was measured with an electronic balance.

1

2

Fig. 1.

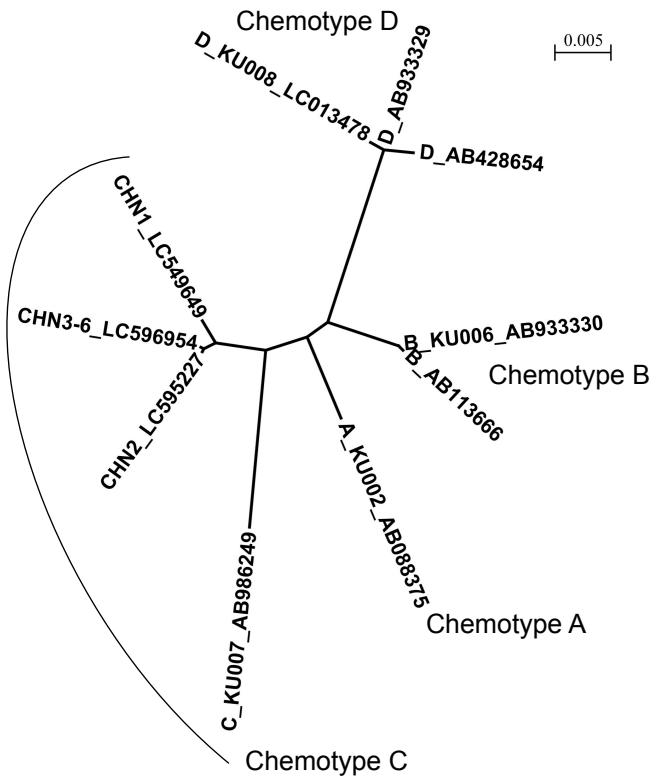

Fig. 1. Phylogenetic analysis of Di Pi Cai (CHN1-6) from China and four genotypes of *Nostoc commune* in Japan.

The unrooted neighbor-joining tree of the 16S rRNA gene sequences was generated by ClustalW version 2.1 at DNA Data Bank of Japan (DDBJ). The nucleotide sequences are deposited in GenBank/EMBL/DDBJ under the following accession numbers: LC549649 (CHN1), LC595227 (CHN2), LC596954 (CHN3-6), AB088375 (strain KU002, NIES-2538, A type), AB933330 (strain KU006, NIES-3989, B type), AB113666 (B type), AB986249 (strain KU007, NIES-3990, C type), LC013478 (strain KU008, NIES-3991, D type), AB933329 (D type), and AB428654 (D type). Four chemotypes of *N. commune* have been identified on the basis of differences in the mycosporine-like amino acid (MAA) composition (Sakamoto et al., 2019).