Antioxidative and Antiglycative Properties of Mycosporine-Like Amino Acids-Containing Aqueous Extracts Derived from Edible Terrestrial Cyanobacteria

Chananwat Korteerakul, Masaki Honda, Siripat Ngoennet, Takashi Hibino, Rungaroon Wadootee-Sirisattha, and Hakuto Kageyama

1 Department of Microbiology, Faculty of Science, Chulalongkorn University, Piyathai Road, Pathumwan, Bangkok 10330, Thailand
2 Department of Chemistry, Faculty of Science and Technology, Meijo University, 1–501 Shiogama-ku, Nagoya, Aichi 468–8502, Japan
3 Graduate School of Environmental and Human Sciences, Meijo University, 1–501 Shiogama-ku, Nagoya, Aichi 468–8502, Japan

(Received April 2, 2020)

Summary The terrestrial filamentous cyanobacterium, Nostoc commune, has been used as a food source in many countries, especially in Asia. In this study, N. commune-derived aqueous extracts were evaluated with regard to their antioxidative and antiglycative properties. The antioxidative activity was significantly higher in N. commune colonies isolated from the field than in extracts from colonies cultured in the laboratory. The antioxidative compound content of extracts, including phenolic compounds and phycoerythrobiliproteins, was correlated with their antioxidative power. In addition, two mycosporine-like amino acids (MAAs), specifically detected in colonies isolated from the field, were purified. In addition to assessing their antioxidative properties, the antiglycative activity of these MAAs was also assessed. Their inhibitory effects on glycation-dependent protein cross-linking might contribute to the antiglycative power of the extract prepared from field colonies. Taken together, the results from this study revealed that N. commune may have beneficial properties for functional food applications, both by preventing oxidative stress and suppressing the formation of advanced glycation end-products.

Key Words cyanobacteria, antioxidants, antiglycative compounds, mycosporine-like amino acids, polyphenol

Cyanobacteria have drawn much attention as a promising group of microorganisms that can produce useful compounds due to their photoautotrophic properties, high growth rates, and ease with which they can be genetically manipulated (1). Certain species of cyanobacteria have also been traditionally utilized as food. Among such cyanobacteria, the terrestrial filamentous cyanobacterium Nostoc commune (Japanese vernacular name, Ishikurage) has been widely used in Asia (2). In Japan, it is consumed as a vinegared food (3). N. commune is recognized for its remarkable ability to survive under various extreme environmental conditions, such as desiccation, frosts, and high temperatures. The ability of this cyanobacterium to survive long-term desiccation was demonstrated when dried N. commune colonies were successfully revived after more than 100 y (4). As well as N. commune, strains of the free-floating filamentous cyanobacterium, Spirulina, have been consumed as food for centuries in Africa and are now widely used as a food supplement worldwide (5). Another example is the freshwater unicellular cyanobacterium Aphanothece sacrum (Japanese vernacular name, Suizenji-nori), which is consumed in Japan (6). Thus, there is accumulating evidence to support the potential use of cyanobacteria as a foodstuff as well as a food supplement.

Several reports have demonstrated the antioxidative activities of extracts derived from cyanobacteria (7, 8). Antioxidants can reduce the harmful effects of free radicals and can thus help to prevent oxidative stress, and many people consume antioxidants in the form of commercial food additives to achieve this (7). Cyanobacteria are known to biosynthesize and accumulate various antioxidative molecules, such as phenolics, phycoerythrobiliproteins (PBPs), and mycosporine-like amino acids (MAAs). Furthermore, we recently reported that MAA molecules, including mycosporine-2-glucoside isolated from the halotolerant cyanobacterium Halothecesp. PCC7418, showed an inhibitory effect on protein glycation, in addition to their antioxidative properties (9). Protein glycation is known as the first step of the Maillard reaction and involves covalent bond formation between proteins and reducing sugars. Glycated products are further oxidized, dehydrated, and condensed to form cross-linked proteins, resulting in the formation of advanced glycation end-products (AGEs). AGEs are known
to be a risk factor for the occurrence of various diseases, such as diabetes, chronic kidney disease, Alzheimer’s disease, and metabolic syndrome in adults and children, and contribute to aging (10, 11). It has been known that protein glycation is frequently associated with oxidantive stress and brings about severe damages of proteins by abnormal post-translational modification, followed by the decrease of activities of proteosome and autophagy and the accumulation of AGEs in the body (12). Therefore, cyanobacteria that accumulate antioxidative and antiglycative compounds could also be considered to be promising functional foods based on their health benefits.

The main objective of this work was to evaluate the capabilities of edible terrestrial cyanobacterium N. commune-derived aqueous extracts with regard to their beneficial health properties. We found a correlation between the presence of bioactive compounds and antioxidative properties in N. commune extracts. Moreover, two MAAs purified from N. commune exhibited antiglycative activity in vitro. The functional characteristics reported here provide evidence that N. commune could represent a promising natural resource, especially in view of its potential health benefits.

**MATERIALS AND METHODS**

**Field collection and culture conditions for cyanobacteria.**

Nostoc species from the field (“field strain”) were collected near the mouth of the River Sannou in Mihama town, Aichi, Japan (N 34.785188, E 136.855795) on 1 May 2019. Wet colonies were stored at 4˚C for 5 d. Then, the colonies were washed excessively with tap water on 6 May 2019. After that, washed colonies were air-dried at room temperature for 4 d, and stored at −30˚C until being subjected to the extraction process. Some parts of the colonies of the field strain were picked out prior to storage and cultured in the laboratory on 1.5% BG11 and BG11 0 media were prepared according to a standard protocol. Field collection and culture conditions for cyanobacteria.

**Genotyping of Nostoc spp.**

The genotype of Nostoc spp. was investigated by direct sequencing of the 16S rRNA gene, using the primer pair Nostoc_16S_F, 5’-AGTGGCGGAGCGGTAGTAA-3’ and Nostoc_16S_R, 5’-CAGTCCAATAGTCCGACCTG-3’. PCR amplification was carried out using KOD-Plus-Neo (Toyobo, Japan). PCR products were sub-cloned into pCR2.1 (Invitrogen, MA, USA) and sequenced. Nucleotide sequencing data were compared with a database library of known 16S rRNA gene sequences of N. commune stored in GenBank (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), using multiple sequence alignment.

**Preparation of aqueous extracts of N. commune.**

Air-dried N. commune colonies were disrupted using a Crush Millser (Iwatani, Tokyo, Japan), with a standard treatment for 30 s. Then, distilled water was added to give a concentration of 10 mg dry weight (DW) of biomass/mL. The suspensions were further sonicated using an Ultra Sonic Disrupter, Model UR-200P (Tomy Seiko, Tokyo, Japan). During sonication, the cells were disrupted for a total of 120 s (repeated time-on/time-off for 30 s each time), with the output power set to 10. After centrifugation at 2,330 × g for 10 min at 4˚C, the supernatants were transferred to fresh tubes, and the protein concentration was determined using the Bradford method. MAA content in the extracts was estimated spectrophotometrically using an extinction coefficient of 120 Lg⁻¹ cm⁻¹ (14).

ABTS [2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay. The ABTS assay was performed as previously described, with minor modifications (15). Briefly, the ABTS radical solution was generated by mixing 7 mM ABTS stock solution in water with 2.45 mM potassium persulfate in water. This mixture was then incubated in the dark for 16 h at 25˚C. Prior to the assay, the ABTS solution was diluted in water to generate an absorbance of approximately 0.5 at 405 nm. Test samples (15 μL) or Trolox standards were added to 135 μL of diluted ABTS solution and mixed. After incubation for 15 min at 25˚C, the absorbance at 405 nm was measured. Quantification was performed based on the Trolox standard curve. The antioxidative power values were expressed in Trolox equivalents (TEs); TEs μg/mgDW or TEs μg/mOD314 for N. commune extracts or purified MAAs, respectively.

**Quantification of total phenolic content (TPC) and total flavonoid content (TFC).** The total phenolic content (TPC) was quantified using a previously described colorimetric method (8). Quantification was performed based upon the gallic acid standard curve. The TPC values were expressed in gallic acid equivalents (GAEs), μg/mgDW. The total flavonoid content (TFC) was quantified using a previously described colorimetric method (8). Quantification was performed based on the quercetin standard curve. The TFC values were expressed in quercetin equivalents (QEs), μg/mgDW.

**Quantification of phycobiliproteins (PBPs).** Absorption spectra of N. commune-derived aqueous extracts were measured using a BioSpec-nano spectrophotometer (Shimadzu, Kyoto, Japan). The quantities of phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) were calculated from the absorbances at 562, 615, and 652 nm, respectively, using the following equations (16, 17):

\[
\text{APC} = \frac{(A_{652} - 0.208 \times A_{615})}{5.09} \quad \text{(mg/mL)}
\]

\[
\text{PC} = \frac{(A_{615} - 0.474 \times A_{652})}{5.34} \quad \text{(mg/mL)}
\]

\[
\text{PE} = \frac{(A_{632} - 2.41 \times PC - 0.849 \times APC)}{9.62} \quad \text{(mg/mL)}
\]

**Purification of MAAs from the field strain of N. commune.**

One gram of air-dried N. commune colonies was mixed with 50 mL distilled water and disrupted by Crush Millser treatment for a total of 4 min (repeated time-on/time-off for 30 s each). After centrifugation at 22,900...
Antioxidative and Antiglycative Properties of Edible Cyanobacteria

Table 1. Antioxidative power and phenolic content (TPC and TFC) of N. commune-derived aqueous extracts.

| Colony   | Antioxidative power (ABTS assay, TEs µg/mgDW) | Phenolic content (TPC, GAEs µg/mgDW) | TFC (QEs µg/mgDW) |
|----------|----------------------------------------------|-------------------------------------|-------------------|
| FD       | 11.18±0.56                                   | 5.70±0.13                           | 28.27±1.51        |
| Lab_−N   | 6.43±0.44                                    | 1.58±0.13                           | 7.15±0.97         |
| Lab_+N   | 7.31±0.29                                    | 2.18±0.11                           | 4.54±0.76         |

Table 2. Protein content and PBP content of N. commune-derived aqueous extracts.

| Colony   | Protein content (µg/mgDW) | PBP content |
|----------|--------------------------|-------------|
|          | APC (µg/mgDW)            | PC (µg/mgDW) | PE (µg/mgDW) | APC+PC+PE (µg/mgDW) |
| FD       | 41.50±1.37               | 3.25±0.08    | 2.07±0.01    | 11.29±0.23          |
| Lab_−N   | 18.63±0.69               | 4.03±0.14    | 1.13±0.03    | 7.12±0.11           |
| Lab_+N   | 11.23±0.69               | 1.05±0.05    | 0.38±0.01    | 2.51±0.07           |

×g for 10 min at 4°C, the supernatants were transferred to fresh tubes. Then, the supernatants were passed through Amicon Ultra Ultracentrifug 3K centrifugal filters (Merck Millipore, Darmstadt, Germany). The resulting flow-through fractions were lyophilized then dissolved in 5 mL 1% acetic acid. Following this, two MAAs (RT7 and RT12) were purified using a previously described three-step liquid chromatographic purification process (15). High performance liquid chromatography (HPLC) analysis to detect MAAs was then conducted, as previously described (15).

Liquid chromatography–mass spectrometry (LC-MS) analysis. Liquid chromatography–mass spectrometry (LC-MS) analysis was performed at the Global Facility Center, Hokkaido University, using an Accela LC System (Thermo Scientific, Massachusetts, USA) and LTQ Orbitrap Discovery (Thermo Scientific). Separation was conducted using InertSustain AQ-C18 (1.9 µm; 150 by 2.1 mm). The mobile phase was run at a flow rate of 0.1 mL/min using 0.1% formic acid. The MS analysis was performed in positive electrospray ionization (ESI) mode with a scan range of m/z 200–1,500.

Protein cross-linking assay. The protein cross-linking assay was performed according to a previously reported procedure (9). The reaction mixtures (10 µL each), which contained MAA or N. commune-derived aqueous extract, 2.5 µg hen egg white lysozyme (HEWL), and 4 mM D-ribose, were prepared in 0.1 M phosphate buffer (pH 7.4). These mixtures were then incubated at 37°C for 144 h in a thermal cycler. Samples were then subjected to 15% SDS-polyacrylamide gel electrophoresis. The intensity of protein bands stained with Coomassie brilliant blue (CBB) was measured using ImageJ software for the preparation of graphs.

Quantification of reducing sugars. Dinitrosalicylic acid (DNS) solution was generated by mixing 75 mL of 2.1 M sodium potassium tartrate solution in water and 30 mL of 0.25 M DNS in 2 M NaOH. Mixtures (75 µL of sample and 75 µL of DNS solution) were prepared, then the reaction mixtures were placed in a boiling water for 10 min. After boiling, the samples were cooled to room temperature, and the absorbance at 540 nm was measured. Quantification was performed based on the glucose standard curve. The reducing sugar content value was expressed in glucose equivalents (Glues) µg/mgDW.

**RESULTS AND DISCUSSION**

**Genotype of Nostoc spp. isolated from the field**

To evaluate the useful properties of their aqueous extracts, Nostoc spp. colonies were collected from the field in Japan. In the environment, this strain formed spherical and sheet-like colonies (Fig. S1, Supplemental Online Material). Following direct PCR amplification of their 16S rRNA gene, it was found that these field colonies of N. commune contained at least two genotypes. One was identical to the sequence of N. commune strain HK-02 (accession number: AB694927). Another was very similar to N. commune strain KU002, with an identity of 99.3%, and was named N. commune strain Mihama1. Nucleotide sequence data for the 16S rRNA gene fragment of this strain is available in the GenBank database, under accession number MN725779. We tried to culture these two strains in the laboratory, but only strain HK-02 could grow under our culture conditions.

N. commune species are known to be filamentous cyanobacteria that can catalyze nitrogen fixation, which takes place in differentiated heterocyst cells, under nitrogen-deficient growth conditions (18). Conversely, nitrogen-rich environments suppress the formation of heterocysts, in which case vegetative cells occupy the filaments. Photographs taken under bright-field microscopy
showed the presence of heterocysts in the colonies collected from the field (Fig. S2A, Supplemental Online Material), suggesting that these colonies were growing under nitrogen-deficient conditions. We washed the colonies excessively with tap water to remove impurities before drying (see “Materials and Methods”). However, it was possible that this field colonies contained any microorganisms other than *N. commune* although the bright-field microscopic analysis could not detect them. In this study, we also used laboratory colonies to evaluate *N. commune* aqueous extracts. The laboratory strain was cultured under both nitrogen-deficient and nitrogen-rich conditions. As shown in Fig. S2B and C (Supplemental Online Material), heterocyst formation was observed only under nitrogen-deficient conditions. Hereafter, we refer to the laboratory colonies cultured under nitrogen-rich and nitrogen-deficient conditions as Lab_—N and Lab_+_N, respectively, whereas the field colonies are referred to as FD.

**Antioxidative activity of *N. commune* aqueous extracts**

First, we examined the antioxidative activity of *N. commune*-derived aqueous extracts using the ABTS assay. As their growth conditions were quite different, it might be difficult to compare the activity between the field and laboratory colonies. In particular, water content in the colonies could be one of the most changeable factors influenced by growth environment. In this study, we therefore used dry biomass weight of each *N. commune* colony for normalizing. As described in the “Materials and Methods” section, the concentration of extracts was normalized prior to the extraction process, with the dry biomass weight suspended in water (10 mgDW/mL). The extract from FD showed the highest antioxidative power, followed by Lab_—N and Lab_+_N (Table 1). The TE value of the FD extract was 1.7- and 1.5-times higher than that of the Lab_—N and the Lab_+_N extract, respectively.

To date, Ninomiya and colleagues have reported the detection of antioxidative activity in an ethanol extract of *N. commune* harvested from the field in China, using a β-carotene oxidation assay (19), whereas Jerez-Martel and colleagues could not detect 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity with either methanol or aqueous extracts of *N. commune* cultured in the laboratory (20). Thus, differences in genotype, culture conditions, and the assay used might influence the detection of the antioxidative properties of *N. commune* extracts.

**Antioxidative compounds in *N. commune*-derived aqueous extracts**

Different antioxidative activity was detected in each *N. commune*-derived aqueous extract; this prompted us to investigate which potential antioxidative compounds were present in the extracts. A variety of polyphenols, mainly comprising flavonoids and phenolic acids, are known to be powerful antioxidants (21), therefore we investigated TPC and TFC in the extracts (Table 1). We found that the FD extract exhibited higher TPC and TFC than the extracts from the laboratory colonies. Of the laboratory colonies, the Lab_—N extract had higher TFC, whereas TPC was higher in the Lab_+_N extract. In

---

**Table 3. MAA content of *N. commune*-derived aqueous extracts.**

| Colony      | MAA content (µg/mgDW) |
|-------------|-----------------------|
| FD          | 3.87 ± 0.25           |
| Lab_—N     | 0.73 ± 0.04           |
| Lab_+_N    | 0.49 ± 0.01           |

---

**Fig. 1.** High performance liquid chromatography (HPLC) chromatogram of *N. commune*-derived aqueous extracts and absorption spectra of purified MAAs. (A) An HPLC chromatogram. The solid, dotted, and dashed lines indicate chromatograms of FD, Lab_—N, and Lab_+_N extracts, respectively. (B) and (C) UV-absorption spectra of the purified MAAs, RT7 (B) and RT12 (C).
addition, the PBP content, including APC, PC, and PE, was also determined (Table 2). PBPs are known to be powerful antioxidants (7). Although the Lab_2–N extract showed the largest PC content, it was found that the FD extract contained the largest amount of APC and PC, similar to the results found for phenolic content. This higher antioxidant content of the field-isolated samples compared with that of the laboratory-cultured samples correlated closely with the pattern of antioxidative power (Table 1). Abiotic environmental stresses in the field, such as drought and exposure to UV, might be linked to the accumulation of antioxidants in FD. It is noteworthy that the total protein content was also significantly higher in FD extracts than in the Lab_1–N and Lab_2–N extracts (Table 2). These results indicate that culture conditions could be an important factor for optimizing the nutritional value of this edible cyanobacterium. Needless to say, differences in the properties of strains are also thought to be essential factors that should be considered.

MAAs in N. commune extracts

MAAs are an important group of natural sunscreen compounds that help protect organisms from UV radiation; they are characterized by absorption maxima from 310 to 362 nm, with a large molar extinction coefficient (22). It is known that N. commune can accumulate MAAs. Several studies have shown that MAAs derived from N. commune possess radical scavenging activity (14, 23, 24). As shown in Table 3, among the N. commune colonies used in this study, the highest estimated MAA content was found in FD (5.3- and 7.9-times the MAAs content of Lab_2–N and Lab_2–N extracts, respectively). In fact, HPLC analysis revealed that two putative MAA peaks were detected in FD extract only by monitoring at 330 nm (Fig. 1A). The putative MAA molecules responsible for these two peaks, which were eluted at retention times of around 7 and 12 min, were named RT7 and RT12, respectively. Absorption spectra of these purified molecules revealed both had absorption maxima at 334 nm (Fig. 1B and C). Furthermore, LC-MS analysis established the molecular masses of RT7 and RT12 to be 464 and 478, respectively, according to the MH+ peak at m/z (Fig. S3, Supplemental Online Material). Very recently, Sakamoto and colleagues showed that four chemotypes of N. commune could be characterized by differences in their respective MAAs (24). Their study showed that one of the four chemotypes, N. commune strain KU002, mainly accumulated two MAAs, pentose-bound shinorine (464 Da) and arabinose-bound porphyra-334 (478 Da). Taking into account the very high similarity between strains KU002 and Mihama1, it is highly possible that RT7 and RT12 were pentose-bound shinorine and arabinose-bound porphyra-334, respectively.

Antioxidative and antiglycative properties of purified MAAs

Next, the antioxidative power of the purified MAAs was evaluated using the ABTS assay. As shown in Table 4, RT7 showed ABTS radical scavenging activity. To the best of our knowledge, if RT7 were pentose-bound shinorine (see the previous section), this is the first report that this MAA possesses radical scavenging activity. On the other hand, the antioxidative capacity of RT12 was very low (Table 4). RT12 may be arabinose-bound porphyra-334; it has been reported that this MAA is a slow-acting radical scavenger (14). However, when the incubation time with ABTS radicals was pro-

Table 4. Antioxidative power of purified N. commune MAAs.

| MAA      | Antioxidative power (TEs μg/mOD334) |
|----------|-----------------------------------|
| RT7      | 4.80±0.13                         |
| RT12     | 0.27±0.01                         |

1 1 mL of MAA aqueous solution, which showed an optical density of 1.0 at 334 nm, was defined as mOD334.

Fig. 2. Inhibition of glycation-dependent protein cross-linking. (A) Inhibitory effects of RT7 and RT12 on HEWL cross-linking are shown. The migration of the molecular weight marker is indicated by the arrowheads on the left. HEWL monomers, dimers, and trimers are denoted by the arrows on the right. The units of MAAs used were defined as mOD334/mL. A 1 mL sample of MAA aqueous solution, which showed an optical density of 1.0 at 334 nm, was defined as mOD334. (B) Relative intensity of HEWL dimer formation. Two independent experiments obtained essentially same patterns, and the levels of the band intensities are indicated by circles. The MAAs supplemented in the reaction mixtures are indicated by open (RT7) and filled (RT12) circles. The solid and dashed lines represent plots of average values. An asterisk indicates significant difference (p<0.05, Student’s t test) between RT7 and RT12.

Antioxidative and Antiglycative Properties of Edible Cyanobacteria

343
longed from 15 min to 1 h, similar antioxidative levels were obtained (data not shown). To further clarify the identity of RT12, it would be useful to conduct other assays of its antioxidative properties in the future.

In addition to their antioxidative properties, the anti-glycative activities of these MAAs were investigated. Figure 2 shows the effects of RT7 and RT12 on HEWL cross-linking. It was found that the intensities of bands corresponding to HEWL dimers (approximately 29 kDa) and trimers (approximately 43 kDa) observed in the absence of MAAs were decreased following the addition of MAAs, in a concentration-dependent manner. The formation of HEWL dimers was inhibited by approximately 50% and 20% by the supply of RT7 and RT12 at 50 mOD334/mL, respectively (Fig. 2B). Thus, both RT7 and RT12 could potentially be inhibitors of protein glycation.

Antiglycative properties of N. commune extracts
Since RT7 and RT12 showed antiglycative activity, we also examined the effect of N. commune extracts on protein glycation (Fig. 3). Unexpectedly, although the extract of FD, which contained RT7 and RT12, slightly inhibited protein cross-linking when supplemented with biomass of 0.5 and 1.0 mgDW/mL, respectively (Fig. 2B), this extract could potentially be inhibitors of protein glycation.

Table 5. Reducing sugar content of N. commune-derived aqueous extracts.

| Colony | Reducing sugar content (GluEs mg/mgDW) |
|--------|---------------------------------------|
| FD     | 20.58±0.66                             |
| Lab_.N | 4.79±0.17                              |
| Lab_+N | 4.16±0.22                              |

5.0 mgDW/mL (Fig. 3B, C, and D). These results prompted us to investigate the reducing sugar content in N. commune extracts. As shown in Table 5, the DNS assay demonstrated that the total reducing sugar content was significantly higher in FD extract than in Lab_.N and Lab_+N extracts. Thus, this larger quantity of reducing sugars might counteract the activity of antiglycative compounds, including RT7 and RT12, in FD extracts. These findings suggest that the intake of appropriate quantities of N. commune could reduce glycative stress and inhibit AGE formation in the human body.

CONCLUSION
In conclusion, the edible cyanobacterium, N. commune, could be regarded as a potential functional food,
which may help to prevent diabetes or other AGE-related diseases caused by oxidative and glycate stress. In this study, the antioxidative power and antiglycate ability of edible N. commune extract was examined using colonies obtained from the field (FD) and the laboratory (Lab. -N and Lab. +N). FD showed higher values for both antioxidative and antiglycative activity. This higher activity of FD extract could be attributable to abundant bioactive compounds with these properties, such as polyphenols, PBPs, and MAAs. Thus, the environment they are cultured in could critically affect the quantity of useful compounds present in N. commune cells. In addition to growth condition, the optimization of extraction method and selection of appropriate solvents could also significantly affect the efficiency of extraction of beneficial compounds. Besides, from the standpoint of food processing, the optimization of storage and pre-treatment procedures of N. commune colonies would be important for long term maintenance of their useful capabilities. With regard to MAAs, two were purified from FD (RT7 and RT12) and evaluated for both their antioxidative and antiglycative abilities. RT7 exhibited both activities, while RT12 showed only antiglycative activity. More detailed investigation of these MAAs would be an interesting avenue to pursue, since MAAs are known to be useful, multifunctional compounds.

Authorship
Research conception and design: HK and RWS; experiments and statistical analysis of the data: CK, MH, SN, TH, RWS, and HK; interpretation of the data: CK, MH, SN, TH, RWS, and HK; writing of the manuscript: HK and RWS.

Disclosure of state of COI
The authors declare no conflict of interest.

Acknowledgments
We thank our laboratory members for their helpful discussions. This work was supported in part by a grant from Koyanagi Foundation (19060001) to HK.

Supporting information
Supplemental online material is available on J-STAGE.

REFERENCES
1) Xie M, Wang W, Zhang W, Chen L, Lu X. 2017. Versatility of hydrocarbon production in cyanobacteria. Appl Microbiol Biotechnol 101: 905–919.
2) Panjir N, Mishra S, Yadav AN, Verma P. 2018. Functional food from cyanobacteria: An emerging source for functional food products of pharmaceutical importance. In: Microbial Functional Foods and Nutraceuticals, 1st ed (Gupta VK, Treichel H, Shapaval V, de Oliveira LA, Tuohy MG, eds), p 21–37. Wiley & Sons, Hoboken.
3) Watanabe F, Tanioka Y, Miyamoto E, Fujita T, Takenaka H, Nakano Y. 2007. Purification and characterization of corrinoid-compounds from the dried powder of an edible cyanobacterium, Nostoc commune (Ishikurage). J Nutr Sci Vitaminol 53: 183–186.
4) Cameron RE. 1962. Species of Nostoc vaquer occurring in the Sonoran Desert in Arizona. Trans Am Microsc Soc 81: 379–384.
5) Deng R, Chow T. 2010. Hypolipidemic, antioxidant, and anti-inflammatory activities of microalgae Spirulina. Cardiiovasc Ther 28: e33–e35.
6) Fujishiro T, Ogawa T, Matsuoka M, Nagashama K, Takeshima Y, Hagiwara H. 2004. Establishment of a pure culture of the lighthouse unculturable unicellular cyanobacterium Aphanotheca sacrum, and phylogenetic position of the organism. Appl Environ Microbiol 70: 3338–3345.
7) Hossain MF, Ratnayake RR, Meera-jini K, Wanasinha Kumara KL. 2016. Antioxidant properties in some selected cyanobacteria isolated from fresh water bodies of Sri Lanka. Food Sci Nutr 4: 753–758.
8) Patipong T, Hibino T, Waditee-Sirisattha R, Kageyama H. 2019. Induction of antioxidative activity and antioxidant molecules in the halotolerant cyanobacterium Halothecesp. PCC7418 by temperature shift. Nat Prod Commun 14: 1–6.
9) Tarasuntisuk S, Patipong T, Hibino T, Waditee-Sirisattha R, Kageyama H. 2018. Inhibitory effects of mycosporine-2-glycine isolated from a halotolerant cyanobacterium on protein glycation and collagenase activity. Lett Appl Microbiol 67: 314–320.
10) Gkogkolou P, Böh M. 2012. Advanced glycation end products: Key players in skin aging. Dermatoendocrinol 4: 259–270.
11) Prasad C, Davis KE, Imrhan V, Juma S, Vijayagopal P. 2017. Advanced glycation end products and risks for chronic diseases: Intervention through lifestyle modification. Am J Lifestyle Med 13: 384–404.
12) Inagi R. 2014. Glycate stress and glyoxalase in kidney disease and aging. Biochem Soc Trans 42: 457–460.
13) Kageyama H, Waditee-Sirisattha R, Sirisattha S, Tanaka Y, Mahakkhan T, Takabe T. 2015. Extraction and quantification of alkanes in cyanobacteria. Bio Protoc 5: e1684.
14) Matsui K, Nazil E, Kunita S, Wana N, Matsugo S, Sakamoto T. 2011. Novel glycosylated mycosporine-like amino acids with radical scavenging activity from the cyanobacterium Nostoc commune. J Photochem Photobiol B Biol 105: 81–89.
15) Ngoennet S, Nishikawa Y, Hibino T, Waditee-Sirisattha R, Kageyama H. 2018. A method for the isolation and characterization of mycosporine-like amino acids from cyanobacteria. Methods Protoc 1: 46.
16) Bennett A, Bogorad L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. J Cell Biol 58: 419–435.
17) Bryant DA, Guglielmi G, de Marsac NT, Castet AM, Cohen-Bazire G. 1979. The structure of cyanobacterial phycobilisomes: a model. Arch Microbiol 123: 113–127.
18) Meeks JC, Elhai J. 2002. Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66: 94–121.
19) Ninomiya M, Satoh H, Yamauchi Y, Takenaka H, Koketsu M. 2011. Antioxidative activity and chemical constituents of edible terrestrial alga Nostoc commune. Vauch. Biosci Biotech Biochem 71: 2175–2177.
20) Jerez-Martel I, García-Poza S, Rodríguez-Martel G, Rico M, Alfonso-Olives C, Gómez-Pinchetti JL. 2017. Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. J Food Qual-
ity 2924508.
21) Afroz R, Tanvir EM, Hossain MF, Gan SH, Parvez M, Aminul Islam M, Khalil MI. 2014. Protective effect of Sundarban Honey against acetaminophen-induced acute hepatonephrotoxicity in rats. *Evid Based Complementary Altern Med* **2014**: 143782.

22) Danlup WC, Shick JM. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. *J Phycol* **34**: 418–430.

23) Nazifi E, Wada N, Asano T, Nishituchi T, Iwamuro Y, Chinaka S, Matsugo S, Sakamoto T. 2015. Characterization of the chemical diversity of glycosylated mycosporine-like amino acids in the terrestrial cyanobacterium *Nostoc commune*. *J Photochem Photobiol B: Biol* **142**: 154–168.

24) Sakamoto T, Hashimoto A, Yamaba M, Wada N, Yoshida T, Inoue-Sakamoto K, Nishituchi T, 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.

25) Kageyama H, Waditee-Sirisattha R. 2019. Antioxidative, anti-inflammatory, and anti-aging properties of mycosporine-like amino acids: molecular and cellular mechanisms in protection of skin aging. *Mar Drugs* **17**: 222.