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SHORT COMMUNICATION

Occurrence of mycosporine-like amino acids (MAAs) in the bloom-forming cyanobacterium *Microcystis aeruginosa*

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Here we report the finding of two mycosporine-like amino acids (shinorine and Porphyra-334) in both a culture of the cyanobacterium Microcystis aeruginosa isolated from Lake Taihu (China) and a natural phytoplankton sample collected from this lake which included Microcystis spp. Our results are the first to clearly document the occurrence of these UV-sunscreen compounds in a freshwater bloom-forming cyanobacterium.

Cyanobacteria often dominate the phytoplankton of shallow eutrophic waters. This dominance has been attributed to a number of adaptive characteristics of cyanobacteria including superior uptake kinetics for inorganic carbon, resistance to grazing by zooplankton, and suppression of the growth of other algae through the excretion of organic compounds (Dokulil and Teubner, 2000). Another mechanism leading to the dominance of cyanobacteria is the ability of certain species to regulate their position within the water column (Reynolds *et al.*, 1987). Depth is regulated in response to changing environmental conditions through physiological changes that alter cell buoyancy, a behavior that is commonly referred to as buoyancy regulation (Walsby, 1991; Wallace and Hamilton, 2000). Persistent positive buoyancy leads to the accumulation of cyanobacteria at the water surface (i.e. surface scums), and as a consequence light penetration is restricted, resulting in a competitive advantage for cyanobacterial species over nonbuoyant subsurface phytoplankton populations (Paerl, 1988). However, the organisms floating at the water surface are exposed directly to high solar irradiance levels including ultraviolet radiation (UVR, 290–400 nm). Therefore, bloom-forming cyanobacteria must have evolved adaptations to protect their photosynthetic apparatus and other labile cellular constituents against photooxidation and direct cell damage. For example, Paerl *et al.* (Paerl *et al.*, 1983) showed that carotenoids were important in protecting surface *Microcystis aeruginosa* populations against short wavelength radiation. This species is a common bloom-forming cyanobacterium in eutrophic waters ranging from temperate regions to the tropics (Paerl, 1988; Huszar *et al.*, 2000), and from lowlands to highlands (Liu, 1999; Chen *et al.*, 2003).

Mycosporine-like amino acids (MAAs), a family of UV-absorbing compounds, have recently received much attention as important photoprotective molecules against UVR in aquatic organisms (Karentz, 2001; Shick and Dunlap, 2002; Banaszak, 2003). MAAs are small (<400 Da) water-soluble compounds composed of either an aminocyclohexenone or aminocycloheximine ring with nitrogen or imino alcohol substituents (Karentz, 2001). The photoprotective effects of MAAs have been documented for the dinoflagellate *Gymnodinium sanguineum* (Neale *et al.*, 1998) and for other organisms (Banaszak, 2003).

The aim of the present study was to test whether MAAs are synthesized by *M. aeruginosa*. To achieve this objective,

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we assessed the occurrence of MAAs in a culture isolated from Lake Taihu (China). In addition, these results were compared with those from a sample of natural phytoplankton collected from the same lake, where *Microcystis* spp. were present. Lake Taihu is a large (2338 km^2) and shallow (maximum depth 2.6 m) eutrophic subtropical lake located in eastern China (30°55′–31°32′N, 119°52′–126°36′E). The culture was routinely grown in the sterile liquid BGA medium (Safferman and Morris, 1964) in Erlenmeyer flasks filled to 40% of their nominal volume and placed in a culture room at 20°C under continuous white fluorescent light (55.2 μmol m^{-2} s^{-1}, measured over the whole wavelength range including UV and PAR). The cultures used in the experiments were in the exponential growth phase. Samples of 2 mL were taken from the culture and cells were harvested by centrifugation (10 min, 5000 *g*).

The natural phytoplankton sample was collected in September 2002 with a plastic bottle directly from the surface of Lake Taihu. In the laboratory, 100 mL of lake water was filtered onto a Whatman GF/F filter and then freeze-dried for subsequent laboratory analysis in Innsbruck, Austria. MAAs were extracted following the protocol recommended for phytoplankton by Tartarotti and Sommaruga (Tartarotti and Sommaruga, 2002). Shortly, the pellet of the *M. aeruginosa* culture and the freeze-dried phytoplankton sample were extracted three times consecutively with 25% aqueous methanol (v:v) at 45°C for 2 h. The methanol extracts of the cultures of *M. aeruginosa* were scanned between 250 and 700 nm in a single beam spectrophotometer (DU 70, Beckman).

For the High Performance Liquid Chromatography (HPLC) analysis, the methanol extracts were evaporated to dryness at 45°C and the residue was resuspended in 500 μL of 25% aqueous methanol (v:v). Then, 20 μL aliquots were injected in a Phenosphere 5 μm pore size C8 column (250 × 4.6 mm, Phenomenex) protected with a RP-8 (Brownlee) guard column, for isocratic reverse-phase HPLC analysis. The mobile phase consisted of 0.1% acetic acid in 25% aqueous methanol (v:v) at a flow rate of 0.79 mL min^{-1}. The MAAs in the eluate were detected by in-line diode array spectroscopy (Dionex, UVD340S). MAAs were identified by comparison co-chromatography with standards as described in Tartarotti and Sommaruga (Tartarotti and Sommaruga, 2002).

The absorption spectrum of the aqueous methanol extract from the culture (Figure 1) showed five major peaks: 665 nm (Chl *a*), 618 nm (phycocyanin), 472 nm (carotenoids), 437 nm (Chl *a*), and 334 nm (UV-absorbing compounds). HPLC chromatograms of the culture revealed the occurrence of two MAAs identified as shinorine and Porphyra-334 (Figure 2). Based on the calculation of

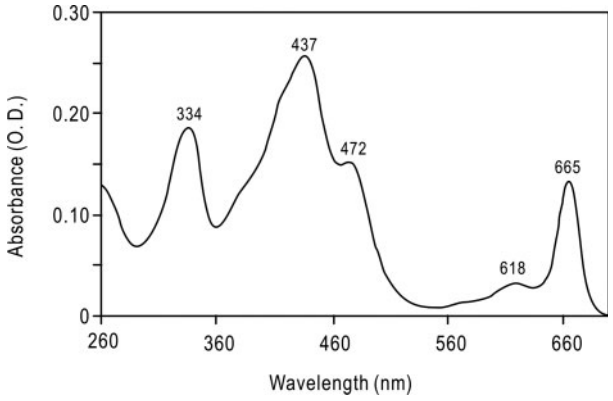

Fig. 1. Absorption spectrum of *Microcystis aeruginosa* culture. Values indicate the wavelength maximum for each peak: 334 nm (UV-absorbing compounds), 437 nm (Chl *a*), 472 nm (carotenoids), 618 nm (phycocyanin), and 665 nm (Chl *a*).

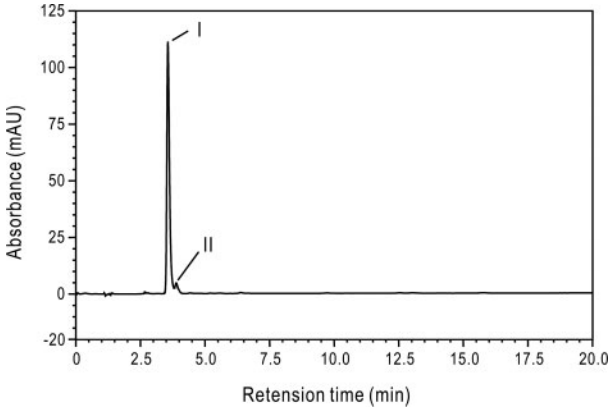

Fig. 2. HPLC chromatogram (detection at 334 nm) of a methanol extract from a *Microcystis aeruginosa* culture. Fractions I and II correspond to shinorine and Porphyra-334, respectively.

peak areas, shinorine accounted for >95% of the total MAAs. The HPLC chromatogram of the natural phytoplankton sample from Lake Taihu showed a similar picture to that of the *Microcystis* culture with the same two dominant compounds found (Figure 3). However, there were some other unknown UV-absorbing compounds with maximum absorption at 336 nm (Fraction III), 326 nm (Fraction IV), and 304 nm (Fraction V).

Our results clearly show that *M. aeruginosa*, a common bloom-forming cyanobacterium isolated from Lake Taihu, is able to synthesize MAAs. However, this finding is not surprising as MAAs have been found before in cultured and natural populations of terrestrial and aquatic benthic cyanobacteria (Garcia-Pichel *et al.*, 1993; Kinzie *et al.*, 1998; Sinha *et al.*, 1999; Sommaruga and Garcia-Pichel, 1999; Xiong *et al.*, 1999; Gröniger *et al.*, 2000) and are also known to be in high concentrations in other bloom-forming phytoplankton species such as marine

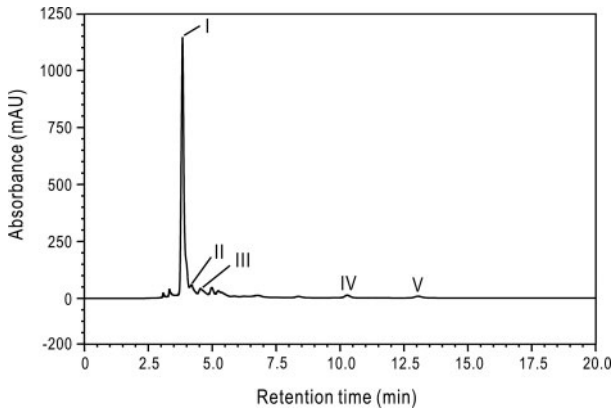

Fig. 3. HPLC chromatogram (detection at 334 nm) of a methanol extract from phytoplankton of Lake Taihu. Fractions I and II correspond to shinorine and Porphyra-334, respectively, whereas fractions III, IV, and V were unknown compounds with an absorption maximum at 336 nm, 326 nm, and 304 nm, respectively. Other minor peaks in the chromatogram did have an absorption maximum <300 nm.

dinoflagellates (Carreto *et al.*, 1990). Cyanobacteria with high concentrations of MAAs are ~20% more resistant to UV radiation centered at 320 nm than those with no or low concentrations (Garcia-Pichel *et al.*, 1993).

M. aeruginosa is able to migrate vertically by changing its buoyancy in response to environmental changes, and its vertical displacement speed could exceed 3 m h^{-1} (Granf, 1975). In eutrophic lakes such as Lake Taihu, UVR is rapidly attenuated in the water column. Thus, *M. aeruginosa* experiences a large range of UV irradiance levels during intense mixing periods. Recent laboratory experiments (Z. Liu, unpublished data) showed that *M. aeruginosa* synthesizes MAAs continuously even in the absence of UVR. Thus, it appears that the most efficient protection of *M. aeruginosa* against UV damage in such lakes could be provided by a constant synthesis of MAAs rather than by UV induction as has been demonstrated for many other species (Sinha *et al.*, 1999; Xiong *et al.*, 1999). Finally, our results suggest that in addition to carotenoids (Paerl *et al.*, 1983), direct absorption of UVR by MAAs could explain the ability of *M. aeruginosa* to develop and maintain surface blooms. Further studies are needed to investigate the factors controlling the efficiency of MAAs in protecting *M. aeruginosa* against UV damage in such wind-exposed shallow lakes.

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REFERENCES

Banaszak, A. T. (2003) Photoprotective physiological and biochemical response of aquatic organisms. In Helbling, E. W. and Zagarese, H. (eds), *UV Effects in Aquatic Organisms and Ecosystems*. Comprehensive Series in Photochemistry and Photobiology, Vol 1, Royal Society of Chemistry, Cambridge, pp. 329–356.

Carreto, J. I., Carignan, M. O., Daleo, G. and De Marco, S. G. (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? *J. Plankton Res.*, **12**, 909–921.

Chen, Y., Qin, B., Teubner, K. and Dokulil, M. (2003) Long-term dynamics of phytoplankton assemblages: *Microcystis*-domination in Lake Taihu, a large shallow lake in China. *J. Plankton Res.*, **25**, 445–453.

Dokulil, M. and Teubner, K. (2000) Cyanobacterial dominance in lakes. *Hydrobiologia*, **438**, 1–12.

Garcia-Pichel, F., Wingard, C. E. and Castenholz, R. W. (1993) Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. *Appl. Environ. Microbiol.*, **59**, 170–176.

Granf, G. G. (1975) Photosynthetic production and irradiance-photosynthesis relationships of the phytoplankton from a shallow equatorial lake (Lake George, Uganda). *Oecologia*, **18**, 165–183.

Gröniger, A., Sinha, R. P., Klisch, M. and Häder, D.-P. (2000) Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – a database. *J. Photochem. Photobiol. B: Biol.*, **58**, 115–122.

Huszar, V. L. M., Silva, L. H. S., Marinho, M., Domingos, P. and Sant'Anna, C. L. (2000) Cyanoprokaryote assemblages in eight productive tropical Brazilian waters. *Hydrobiologia*, **424**, 67–77.

Karentz, D. (2001) Chemical defenses of marine organisms against solar radiation exposure: UV-absorbing mycosporine-like amino acids and scytonemin. In McClintock, J. B. and Baker, B. J. (eds), *Marine Chemical Ecology*. CRC Press, Boca Raton, Florida, pp. 481–520.

Kinzie, R. A. III., Banaszak, A. T. and Lesser, M. P. (1998) Effects of ultraviolet radiation on primary productivity in a high altitude tropical lake. *Hydrobiologia*, **385**, 23–32.

Liu, N. (1999) The characteristics and forming mechanisms of algal blooms in Lake Dianchi. *Environ. Sci. Res.*, **12**, 36–37 (in Chinese).

Neale, P. J., Banaszak, A. T. and Jarriel, C. R. (1998) Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): Mycosporine-like amino acids protect against inhibition of photosynthesis. *J. Phycol.*, **34**, 928–938.

Paerl, H. W. (1988) Growth and reproductive strategies of freshwater blue-green algae (cyanobacteria). In Sandgren, C. D. (ed.), *Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, Cambridge, pp. 261–315.

Paerl, H. W., Tucker, J. and Bland, P. T. (1983) Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aeruginosa*) surface blooms. *Limnol. Oceanogr.*, **28**, 847–857.

Reynolds, C. S., Oliver, R. L. and Walsby, A. E. (1987) Cyanobacterial dominance: the role of buoyancy regulation in the billowing environment. *N. Z. J. Mar. Freshwater Res.*, **21**, 379–390.

Safferman, R. S. and Morris, M. E. (1964) Growth characteristics of the blue-green algal virus LPP-1. *J. Bacteriol.*, **88**, 771–775.

Shick, J. M. and Dunlap, W. C. (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.*, **64**, 223–262.

Sinha, R. P., Klisch, M. and Häder, D.-P. (1999) Induction of a mycosporine-like amino acid (MAA) in the rice-field cyanobacterium *Anabaena* sp. by UV irradiation. *J. Photochem. Photobiol. B: Biol.*, **52**, 59–64.

Sommaruga, R. and Garcia-Pichel, F. (1999) UV-absorbing mycosporine-like compounds in planktonic and benthic organisms from a high-mountain lake. *Arch. Hydrobiol.*, **144**, 255–269.

Tartarotti, B. and Sommaruga, R. (2002) The effect of different methanol concentrations and temperatures on the extraction of mycosporine-like amino acids (MAAs) in algae and zooplankton. *Arch. Hydrobiol.*, **154**, 691–703.

Wallace, B. B. and Hamilton, D. P. (2000) Simulation of water-bloom formation in the cyanobacterium *Microcystis aeruginosa*. *J. Plankton Res.*, **22**, 1127–1138.

Walsby, A. E. (1991) The mechanical properties of the *Microcystis* gas vesicle. *J. Gen. Microbiol.*, **137**, 2401–2408.

Xiong, F., Kopechy, J. and Nedbal, L. (1999) The occurrence of UV-B absorbing mycosporine-like amino acids in freshwater and terrestrial microalgae (Chlorophyta). *Aquat. Bot.*, **63**, 37–49.