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NOTE

RELATIONSHIP OF GROWTH AND PHOTOSYNTHESIS WITH COLONY SIZE IN AN EDIBLE CYANOBACTERIUM, GE-XIAN-MI *NOSTOC* (CYANOPHYCEAE)[1]

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Growth and photosynthesis of an edible cyanobacterium, Ge-Xian-Mi (*Nostoc*), were investigated with differently sized colonies. Both photosynthesis and growth were dependent on the colony size. Compared with larger ones, smaller colonies grew faster regardless of the levels of light and temperature for culture and showed higher values of maximal net photosynthetic rate, apparent quantum yield, light-saturating and compensating points, and dark respiration. The ratios of chl *a* content and mass to surface area of a colony increased and that of chl *a* to mass or mass to volume of a colony decreased with increased colonial sizes. A Ge-Xian-Mi colony appeared to increase its chl *a* content per surface area, enhancing the light-shading effect; however, at the same time it decreased its mass density on a volume basis, minimizing the enhanced effects of shading and diffusion barrier caused by the thickening outer layer with increasing colony size during growth.

Key index words: **colonial size; cyanobacterium; growth; *Nostoc*; photosynthesis**

Abbreviations: **RGR, relative growth rate; RWC, relative water content**

Nostoc species are filamentous cyanobacteria common in both terrestrial and aquatic habitats (Dodds et al. 1995). Some of the species are regionally being used as food delicacies and herbal ingredients (Gao 1998), and some are important in paddy rice fields because of their nitrogen fixation (Dodds et al. 1995). On the other hand, *Nostoc* can be an annoyance to humans (Baldwin and Whitton 1992), causing unpleasant odors in drinking water (Wnorowski 1992) or fouling buildings (Whitton 1987). As well as the great diversity of their relationships to human affairs and the variety of their habitats, species of this genus exhibit various colonial morphologies, such as filamentous, spherical, and mat shape. The smallest *Nostoc* colonies can be composed of a few trichomes, and the largest ones can reach almost 3 kg wet weight, as in *Nostoc pruniforme* (Dodds and Castenholz 1987). Thus, the volume of *Nostoc* colonies can range over at least a million-fold, and this span of sizes has significant influences on their physiological and ecological performances. Size-dependent differences in metabolic and growth rates have been documented in various groups of algae (Banse 1976, Gao and Hua 1997, Raven 1999a,b, Finkel and Irwin 2000, Finkel 2001). However, little is known about the relationships between colony morphology and metabolic rates in cyanobacteria (Whitton and Potts 2000).

Nostoc spp. collectively represent a unique biological resource, though there is no adequate species concept for this genus (Potts 2000). Ge-Xian-Mi has been regarded as *Nostoc sphaeroides* (Huang et al. 1998); however, its taxonomic identity remains controversial (Qiu et al. 2002). This *Nostoc* species has been used as a food delicacy for hundreds of years (Qiu et al. 2002) and is found in rice fields from December to May in Hubei, China. Colonies of Ge-Xian-Mi are dark green and pearl shaped, develop from hormogonia, and can reach 2.5 cm in diameter. Colonies of various sizes in this species may exhibit different metabolic and growth rates. However, nothing has been documented on this aspect in Ge-Xian-Mi and other *Nostoc* species. Here we investigate the photosynthetic and growth rates of Ge-Xian-Mi colonies of different sizes under different culture conditions.

Colonies of Ge-Xian-Mi (originally collected from Hefeng county in Hubei province) were obtained from the Institute of Hydrobiology, the Chinese Academy of Sciences (Wuhan). Hormogonia were induced according to Li (2000) at 25° C and 10 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$. Colonies of about 0.2 cm in diameter formed from the hormogonia were used at the initiation of cultures. The culture medium, BG_{110} (Stanier et al. 1971), was renewed every 7 days. All the cultures were carried out in 2-L serum bottles placed in a plant growth chamber (E_7, Conviron, Winnipeg, Manitoba, Canada) at 80 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at a 12:12-h light:dark cycle and were aerated with ambient air. Differently sized colonies were obtained for the experiments during the growth period when the colony diameter increased almost linearly (Fig 1). Photosynthetic responses to light (P-I) of the colonies were determined with a Clark-type oxygen electrode (YSI

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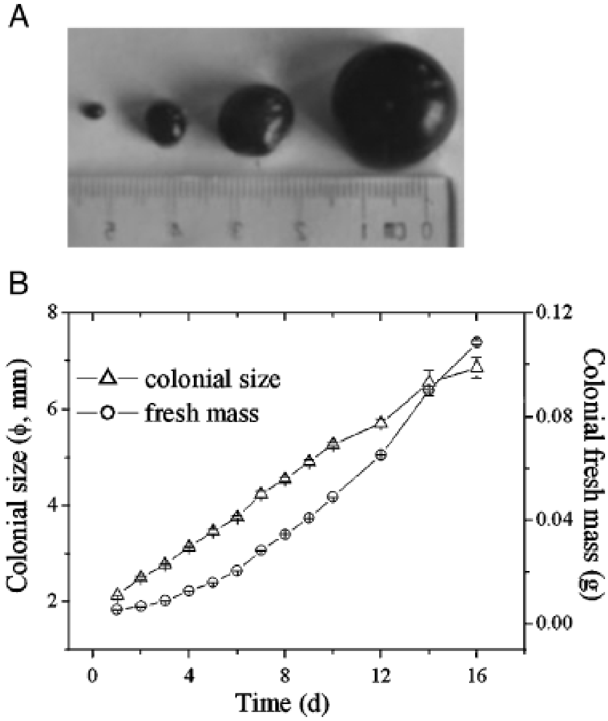

FIG. 1. Spherically shaped colonies (A) and changes in the colony diameter and fresh mass (B) of Ge-Xian-Mi while cultured at 80 μmol photons · $m^{-2} \cdot s^{-1}$ and 25° C. Data are means ± SD ($n = 30$).

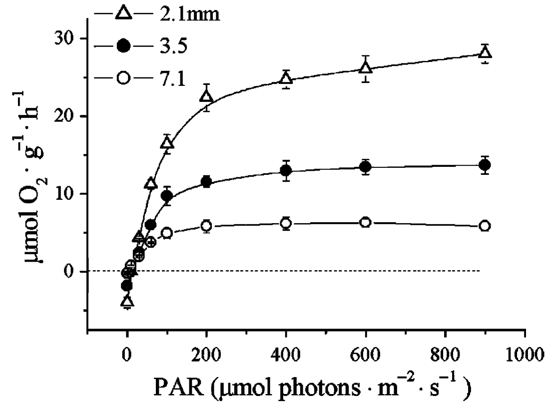

FIG. 2. Net photosynthesis of differently sized colonies of Ge-Xian-Mi as a function of light. Six to 45 colonies were used in triplicate measurements at each light level. Data are means ± SD (n = 6–45).

5300, YSI, Yellowsprings, OH, USA). Colonies of uniform size were placed so as not to shade each other in the electrode chamber. Various light (Halogen lamp) levels were obtained by adjusting the distance from the chamber. Irradiance was measured as PAR with a light sensor (SKP200, ELE International, Wales, UK). The medium in the chamber was renewed for each measurement. Parameters for P-I curves were analyzed according to Henley (1993).

Chl *a* was extracted in 100% methanol for 30 min at 60° C in the dark, and its content was determined according to Scherer and Zhong (1991). Cultures of the colonies for each size level were initiated with a constant biomass density of 1 g fresh mass · L^{-1}. The relative growth rate (RGR, % d^{-1}) was calculated as $RGR = (\ln W_t - \ln W_0)/T \times 100$, where W_0 represents the initial dry weight derived from the fresh weight and the ratios of dry to fresh weight and W_t, the dry weight after t number of days, during which period biomass increased linearly. Fresh weight was determined after water drops on the colonial surface were removed with tissue paper. Relative water content (RWC, %) of a colony was estimated by measuring its fresh weight (W_f), drying in an oven (80° C, 20 h), determining its dry weight (W_d), and calculating as follows: $RWC = (W_f - W_d)/W_f \times 100$. Diameters of the colonies were measured by using a vernier caliper to the nearest 0.1 mm. The surface area and volume of a colony were calculated assuming that it was spherical. All the data were analyzed by one-way analysis of variance.

Photosynthetic responses to light of differently sized colonies showed that higher values were associated with smaller colonies for maximal net photosynthesis (P_m), light saturating point (I_K), apparent photosynthetic efficiency (α), and dark respiration (R_d) and compensation irradiance (I_c) (Fig. 2, Table 1). Pm, I_k, α, and R_d decreased, respectively, by 49%, 42%, 12%, and 45% when the colony diameter increased from 2.1 to 3.5 mm and further decreased when it increased to 7.1 mm. The differences in the photosynthetic parameters among the colonial sizes were significant ($P < 0.05$). The compensation point (I_c) was not significantly ($P > 0.05$) different between diameters of 2.1- and 3.5-mm colonies but significantly ($P < 0.05$) declined when the diameter increased to 7.1 mm.

RGR of Ge-Xian-Mi increased with increased light levels and saturated at about 100 μmol photons ·

TABLE 1. Maximal net photosynthetic rate, P_m (μmol $O_2 \cdot g^{-1} \cdot h^{-1}$), apparent quantum yield, α (μmol $O_2 \cdot g^{-1} \cdot h^{-1}$)/(μmol photons · $m^{-2} \cdot s^{-1}$), light-saturating, I_K, and compensation points, I_c (μmol photons · $m^{-2} \cdot s^{-1}$), and dark respiration, R_d (μmol $O_2 \cdot g^{-1} \cdot h^{-1}$) derived from the P-I curves of different colony sizes in Ge-Xian-Mi.

Colonial size (diameter, mm)	P_m	α	I_K	R_d	I_c
2.1	28.9 ± 1.1	0.24 ± 0.03	132.6 ± 2.1	2.9 ± 0.9	12.3 ± 1.0
3.5	14.8 ± 0.4	0.14 ± 0.01	117.2 ± 0.8	1.6 ± 0.4	11.7 ± 0.4
7.1	6.2 ± 0.2	0.07 ± 0.05	86.7 ± 0.3	0.1 ± 0.1	6.3 ± 0.5

Values are means ± SD (n = 6–45).

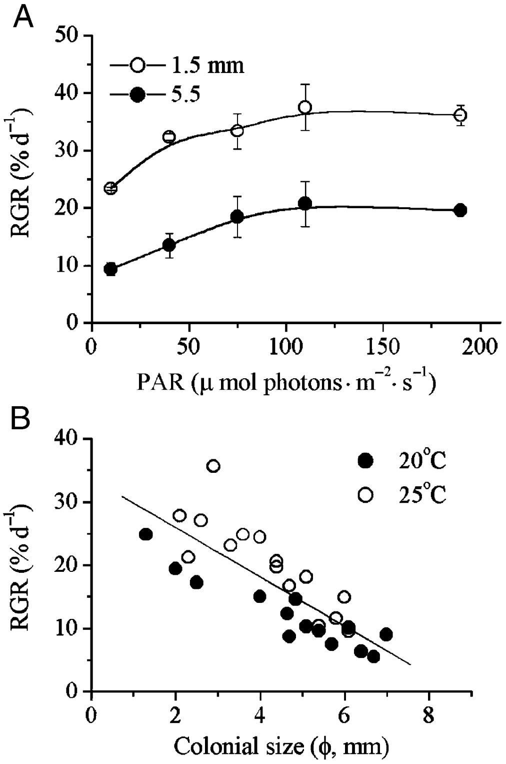

FIG. 3. RGR of Ge-Xian-Mi of 1.5- and 5.5-mm-dimater colonies as a function of PAR (A), and the relationship of RGR with colonial sizes (B). Data in A are means ± SD (n = 30).

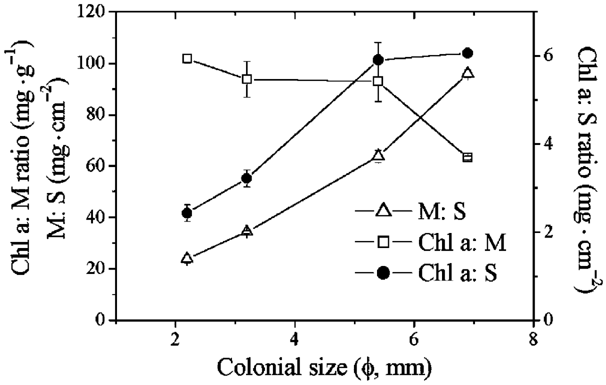

FIG. 4. Ratios of chl *a* content to colony surface area (chl *a*:S) and to fresh mass (chl *a*:M) and that of M:S. Data are means ± SD (n = 3–45).

m$^{-2}\cdot$s^{-1} for both diameters of 5.5- and 1.5-mm colonies (Fig. 3A). Maximal RGR of 1.5-mm-diameter colonies was about two times that of 5.5-mm-diameter colonies (Fig. 3A). The RGR decreased linearly with the increasing colony size (Fig. 3B), regardless of temperatures in culture, by about 76% when the colonial diameter increased from 2 to 7 mm.

The ratios of the fresh mass and chl *a* content to surface area of a colony significantly ($P<0.01$) increased with greater colony diameter (Fig. 4). When the colonial diameter increased from 2 to 5.5 mm, ratios chl *a* to surface and fresh mass to surface increased by 1.4 and 1.7 times, respectively. With increasing size of a colony, the chl *a*-to-fresh mass ratio decreased slightly at diameters less than 5.5 mm and declined sharply at a diameter of 7 mm. RWC increased, whereas the ratio of dry mass to volume of colonies decreased with increasing colonial sizes (Fig. 5).

Photosynthesis and growth rates of Ge-Xian-Mi were negatively related with the sizes of its colonies, that is, smaller colonies had higher photosynthetic rates and grew faster. This relationship was also evident when the rates were expressed on a basis of dry mass or chl (Li and Gao 2004). Lower values of P_m, I_k, α, and R_d with larger colonies indicated an acclimation to the changing microenvironment within the Ge-Xian-Mi colonies. An increase in colony diameter decreases the probability of individual chl *a* molecules absorbing photons in a given irradiance field, assuming a constant concentration of chl *a* and a constant spherical geometry (Enríquez et al. 1994, Raven 1999a,b). The inner cells of the colony tend to be shaded by the thicker outer layers, thus receiving less light when the colony grows larger. The larger Ge-Xian-Mi colonies increased chl *a* content per colonial surface area (Fig. 3), decreased biomass density per unit colony volume (Fig. 5), and decreased the light-compensation point of photosynthesis. However, these acclimations do not compensate for the light attenuation through the outer layer. About 90% of the incoming light was absorbed within the 100-μm surface layer of a *Nostoc* colony (Dodds et al. 1995). Cells within a larger colony of Ge-Xian-Mi could experience light levels and wavebands different from those within a smaller one because of the shading and absorption by the outer layer. The mass-to-surface ratio increased as a Ge-Xian-Mi colony grew larger (Fig. 3), indicating that higher shading levels were associated with larger colonies. Decreased dry mass-to-volume ratios of Ge-Xian-Mi with increased colonial size implies that greater buoyancy was associated with larger colonies. Such buoyancy may enable the larger colonies to float or to be stirred up and raise its light availability when water circulates or flows in accordance with wind

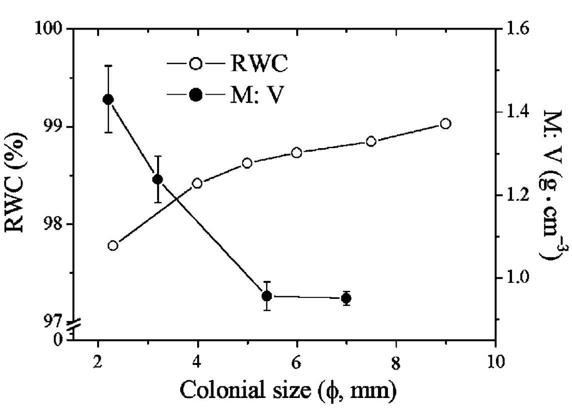

FIG. 5. RWC (%) and the ratio of dry mass to colony volume (M:V) as a function of colony size (n = 10–30).

dynamics, as reported for another cyanobacterial colony (Garcia-Pichel et al. 2002).

An increase in colony size also decreases the capacity of solute influx or efflux on a volume basis as a result of a thicker diffusion boundary (Raven and Kübler 2002). The outer layer of a Ge-Xian-Mi colony could be a barrier for its acquisition of inorganic carbon during photosynthesis (Li and Gao 2004) and may hold back outward diffusion of O_2, giving rise to a higher O_2 pressure in larger colonies, which consequently inhibits its net photosynthesis during daytime and enhances its dark respiration at night when the concentration of O_2 in the colony is higher than that of the medium. The decreased rates of photosynthesis and growth with increased size of Ge-Xian-Mi colonies could also be attributed to the exchanges of substances required (nutrients) or produced (excreted organic matters) during its metabolic activities.

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