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Photosynthetic response to salt of aquatic-living colonies of the terrestrial cyanobacterium *Nostoc flagelliforme*

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Abstract

Aquatic-living colonial filaments of the terrestrial cyanobacterium *Nostoc flagelliforme,* developed from single cells in laboratory under aquatic conditions, were cultured at different salt concentrations (0–400 mM), and their photosynthetic responses were investigated to see their physiological tolerance. Light-saturated photosynthesis, photosynthetic efficiency and dark respiration showed the highest values in treatments at 20 mM NaCl for 24 or 48 h incubation. Changes in salt level exerted little influence on light saturation point and light compensation point. Patterns of photosynthetic performance as a function of salt were the same after 48 h as those after 24 h treatment, with the largest values at 20 mM NaCl, though photochemical efficiency increased with increased NaCl concentrations in the colonies treated for 48 h. From an applied point of view, the laboratory-generated aquatic living colonies are able to tolerate salt stress when transferred from aquatic to terrestrial environments.

Abbreviations: P–I curve, photosynthesis–irradiance curve; PAR, photosynthetic active radiation; PFD, photon flux density; P_{max}, maximum photosynthetic rate; α, photosynthetic efficiency; I_c, light compensation point; I_k, light saturation point

Introduction

Nostoc sphaeroides, *N. commune* and *N. flagelliforme* are the *Nostoc* species used for food delicacies by the Chinese, of which *N. flagelliforme* is the most important due to its history in Chinese culture (Gao, 1998). It is terrestrial and distributed in northern and west-northern parts of China. Overexploitation has reduced its resources, and techniques for cultivation need to be developed to meet its market demand and to conserve its endangered resource and the environment. Researchers in China have tried to culture this organism for decades. When the terrestrial colonies collected from nature were cultured in aqueous medium, it hardly grew but disintegrated (Qian et al., 1989), though it can use bicarbonate in water as a photosynthetic inorganic carbon resource (Gao & Zou, 2001). Although enrichment of CO_2 and periodic watering did enhance its growth and photosynthesis when cultured in air (Gao & Yu, 2000; Qiu & Gao, 2002), cultivation technique for commercial production of *N. flagelliforme* has not been successfully developed.

A previous study (Gao & Ye, 2003) showed that terrestrial colonies disintegrated under aquatic conditions due to bacterial activities, but that aquatic-living colonies could be developed from single cells isolated from rehydrated natural colonies. Such an aquatic-formed and living colony is morphologically identical with its terrestrial counterpart, but does not disintegrate in water even in the presence of bacteria, and propagates fast in liquid medium, showing a great potential as "seed" for cultivation in terrestrial environments. However, they will inevitably undergo high salt stress, when shifted from the aquatic to terrestrial environment in application. The study reported here aimed to

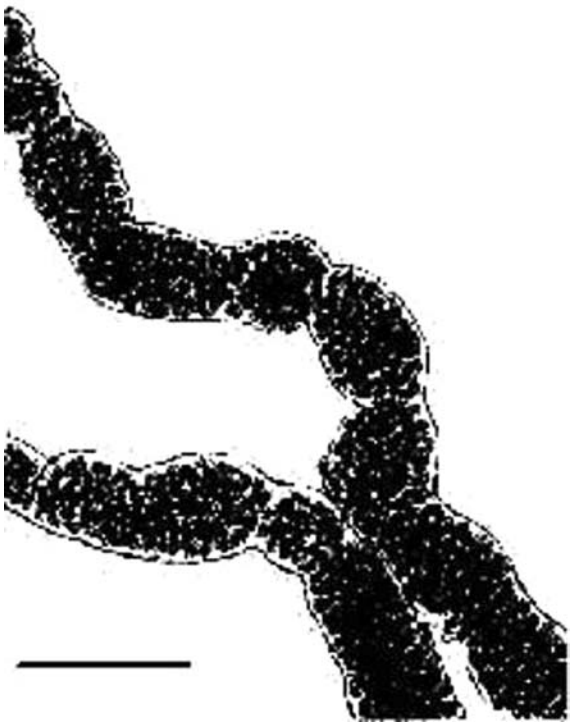

Figure 1. The stage of aquatic-living colony used in this experiment. Bar, 30 μm

investigate the photosynthetic responses of the aquatic colonies to salt.

Materials and methods

Colonial filaments (Figure 1) of *N flagelliforme* (Berk. & Curtis) Bornet & Flahault growing in an aquatic environment were obtained by isolating cells from a natural colony. The colony had been rehydrated at 25 °C and 40 μmol m^{-2} s^{-1} for 9-10 h (Gao et al., 1998) and then cultured at the same temperature but slightly higher irradiance (60 μmol m^{-2} s^{-1}, as reported by Gao and Ye, 2003) before routine maintainance at 10–20 μmol m^{-2} s^{-1}. The filaments were cultured with HGZ medium ($NaNO_3$, 49.6 mg; $K_2HPO_4.3H_2O$, 5.2 mg; $MgSO_4.7H_2O$, 7.5 mg; $CaCl_2.2H_2O$, 3.6 mg; $Na_2SiO_3.9H_2O$, 5.8 mg; PIV metal solution, 0.3 mL; soil extract, 0.3 mL, distilled water, 99.4 mL. PIV metal solution: $FeCl_3.6H_2O$, 9.7 mg; $MnCl_2.4H_2O$, 4.1 mg; $ZnCl_2$, 0.5 mg; $CoCl_2.6H_2O$, 0.2 mg; $Na_2MoO_4.2H_2O$, 0.4 mg; EDTA, 75 mg; distilled water, 100 mL) (as listed in Song & Liu, 1996) at 60 μmol photon m^{-2} s^{-1} and 25 °C. Salt concentrations were established at 0 (without addition of NaCl to HGZ), 20, 40, 80, 100, 150, 200 and 400 mM NaCl. Filaments that have formed 3–5 days (Figure 1) were cultured in triplicate at each salt concentration. After 24 and 48 h incubations, colonial filaments were harvested and resuspended in fresh medium of the same NaCl concentration as in the treatment. Photosynthetic rates were then measured as O_2 evolution by using a Clark-type oxygen electrode (YSI Model 5300, Ohio, USA) at 25 °C and different levels of light (halogen lamp, PAR, 400–700 nm). Irradiance was adjusted by altering the distance between the light source and the reaction chamber. The dark respiratory rates were determined by covering the chamber with a black cloth. Photosynthetic efficiency (α) was estimated as the light-limited slopes of the P–I curves, light compensation point (I_c) and light saturation point (I_k) were estimated by R_d/α, and $(P_{max} + R_d)/\alpha$, respectively, according to Henley (1993). The ratio of variable to maximal chlorophyll *a* fluorescence (F_v/F_m) of the colonies, after being dark-adapted for 15 min, was determined with a Plant Efficiency Analyser (Hansatech Instrument Ltd, Kings Lynn, UK). It represents the photochemical efficiency of PSII or the maximal quantum yield of the photosynthetic apparatus (Krause & Weis, 1991).

Chlorophyll *a* was determined according to Inskeep and Bloom (1985) by extracting the samples in 100% *N*, *N*-dimethylformamide (>12 h, in dark) and measuring the absorbance with a spectrophotometer (Shimadzu UV-1206, Kyoto, Japan).

Data were analyzed by or One-Way ANOVA followed by a multiple comparison using least significant differences (LSD).

Results

Light-saturated photosynthesis (P_{max}) was the highest in the colonies treated with 20 mM NaCl either in 24 or 48 h treatments, and higher salt concentrations resulted in lower values of P_{max} (Figure 2, Table 1). However, it was only significantly reduced at salt levels higher than 150 mM NaCl. The P_{max} values at 200 and 400 mM NaCl were lowed by 53 and 54%, respectively in 24 h incubation, and reduced by 68 and 76% in 48 h treatments (Figure 2, Table 1). The incubations for 48 h resulted in further suppressed values in P_{max} (Figure 2, Table 1), but did not lead to altered patterns of the change compared with 24-h treatment. Most of the photosynthetic parameters were not significantly different between 24 and 48-h treatments (Table 1). The P_{max} at 20 mM NaCl in 48-h was about

Table 1. Photosynthetic parameters of the *N flagelliforme* aquatic-living colonies grown at different levels of salt for 24 and 48 h. P_{max}, the maximum photosynthetic rate (μmol O_2 mg chla^{-1} h^{-1}); R_d, the dark respiratory rate (μmol O_2 mg chla^{-1} h^{-1}); α, photosynthetic efficiency (μmol O_2 mg chla^{-1} h^{-1}/μmol m^{-2} s^{-1}); I_c, light compensation point (μmol m^{-2} s^{-1}); I_k, light saturation point (μmol m^{-2} s^{-1}). Within each column of the data, values with different superscript are significantly different at $p = 0.05$. Mean ± S.D. ($n = 3$); data in ( ) are for 48 h incubation.

NaCl (mM)	P_{max}	R_d	α	I_c	I_k
0	185.3 ± 22.3^{abc}	64.4 ± 7.9^{abcd}	2.5 ± 0.3^{ab}	25.5 ± 3.9^{a}	99.1 ± 12.7^{a}
	(138.6 ± 29.4^{abc})	(44.6 ± 20.1^{abcd})	(1.5 ± 0.6^{bc})	(30.5 ± 2.1^{a})	(125.5 ± 34.9^{a})
20	198.1 ± 2.5^{a}	76.8 ± 11.9^{abc}	3.1 ± 0.2^{a}	24.8 ± 2.2^{a}	88.9 ± 16.4^{a}
	(147.1 ± 12.1^{bd})	(62.6 ± 2.4^{abcd})	(2.2 ± 0.1^{b})	(28.6 ± 2.9^{a})	(95.7 ± 26.3^{a})
40	159.1 ± 14.1^{abc}	73.4 ± 2.3^{b}	2.9 ± 0.3^{ab}	24.7 ± 3.5^{a}	78.3 ± 12.1^{a}
	(143.3 ± 5.6^{ab})	(50.4 ± 4.4^{cd})	(1.7 ± 0.2^{bcd})	(30.1 ± 5.5^{a})	(115.3 ± 11.2^{a})
80	144.5 ± 37.9^{acde}	49.6 ± 16.3^{abcd}	2.4 ± 0.3^{ab}	20.7 ± 4.1^{a}	81.2 ± 26.8^{a}
	(107.9 ± 2.3^{d})	(44.4 ± 15.6^{abcd})	(1.8 ± 0.2^{bcd})	(23.6 ± 6.2^{a})	(69.3 ± 36.5^{a})
100	134.4 ± 2.4^{bc}	52.4 ± 8.9^{abcd}	2.2 ± 0.4^{ab}	23.5 ± 5.1^{a}	83.6 ± 14.1^{a}
	(96.3 ± 37.8^{abcd})	(43.2 ± 3.7^{abcd})	(2.1 ± 0.2^{bcd})	(21.3 ± 6.8^{a})	(73.4 ± 31.6^{a})
150	131.7 ± 33.2^{abcde}	57.7 ± 5.9^{abcd}	2.1 ± 0.1^{abcd}	27.5 ± 5.3^{a}	90.2 ± 20.2^{a}
	(85.9 ± 33.7^{bcde})	(40.6 ± 21.8^{abcd})	(1.8 ± 0.3^{bcd})	(22.1 ± 6.6)	(74.4 ± 30.3^{a})
200	92.6 ± 24.6^{bcde}	45.4 ± 9.8^{d}	1.7 ± 0.8^{abcd}	27.7 ± 7.4^{a}	84.2 ± 26.1^{a}
	(46.9 ± 8.9^{e})	(43.1 ± 6.1^{d})	(1.5 ± 0.3^{cd})	(28.7 ± 3.2^{a})	(60.1 ± 20.8^{a})
400	90.8 ± 31.5^{bcde}	26.8 ± 2.3^{d}	1.4 ± 0.1^{d}	19.2 ± 7.6^{a}	84.1 ± 24.9^{a}
	(40.1 ± 12.3^{e})	(24.4 ± 1.8^{d})	(1.1 ± 0.3^{d})	(23.7 ± 1.9^{a})	(62.4 ± 12.9^{a})

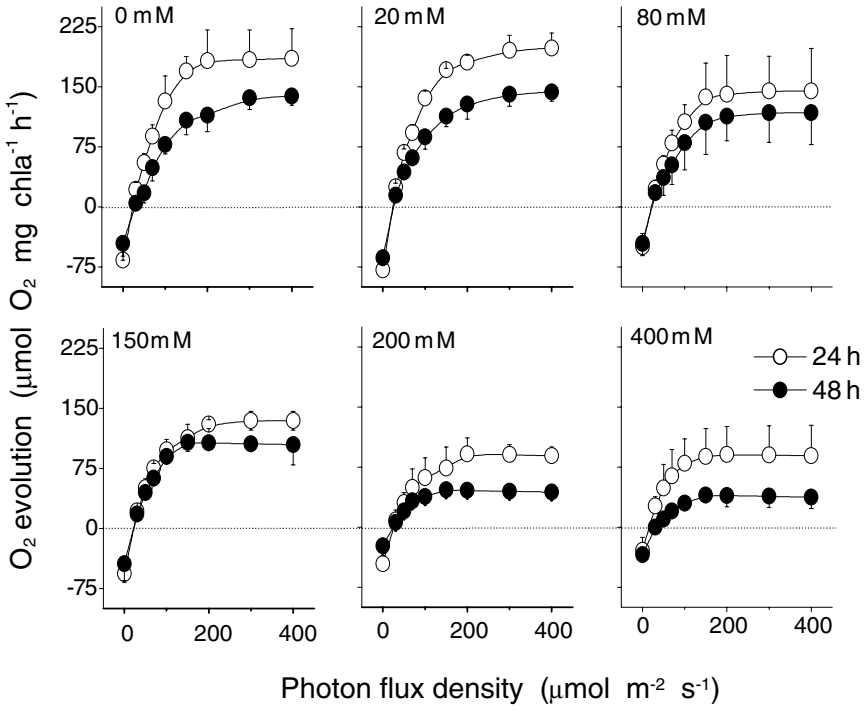

Figure 2. Influence of salt concentration for 24 or 48 h on photosynthetic O_2 evolution of aquatic-living colonies of *N flagelliforme* as a function of irradiance at 25 °C. Mean ± S.D. ($n = 3$).

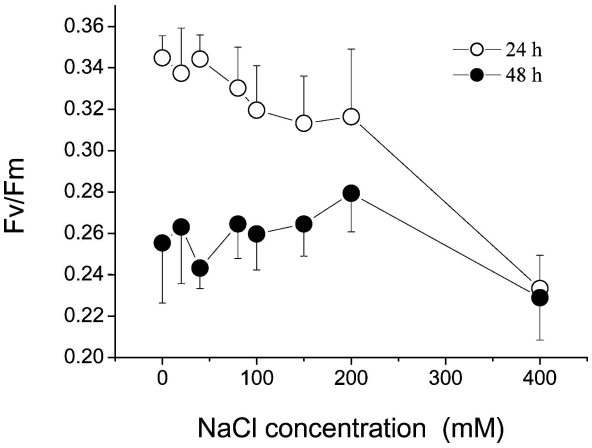

Figure 3. Influence of salt concentration for 24 or 48 h on PSII photochemistry efficiency (F_v/F_m) of the *N flagelliforme* aquatic-living colonies as a function of salt concentrations. Mean ± S.D. ($n = 3$).

26% lower compared with that in 24-h. In 24-h incubations, dark respiration (R_d) was the highest at 20 mM NaCl, being lowered by 41% at 200 mM NaCl, and by 62% at 400 mM NaCl, however, in 48 h treatments the effect of salinity on R_d was insignificant. The salinity treatments exerted little influence on the photosynthetic efficiency (α), which was only significantly suppressed at salt higher than 200 mM NaCl in both 24 and 48-h treatments. Salt hardly affected light compensation point (I_c) and light saturation point (I_k) either in 24 or 48-h incubation. A remarkable difference was found in photochemical efficiency between 24 and 48-h treatments(Figure 3). In 24-h treatments, when the salt increased from 0 to 200 mM NaCl, photochemical efficiency decreased slightly, then dropped by 32% at 400 mM NaCl (Figure 3). In 48-h treatment, the photochemical efficiency increased slightly from 0 to 200 mM NaCl with increased salt to 200 mM NaCl, then decreased by about 20% at 400 mM NaCl.

Discussion

The study showed that aquatic-living colonies of the terrestrial *Nostoc flagelliforme* can tolerate a wide ranges of salt, with the highest photosynthetic performance at 20 mM NaCl. The enhanced P_{max} under low levels of salt reflected an increased demand for carbon by the salt acclimation, as suggested in planktonic cyanobacteria (Moisander et al., 2002). Salt had little influence on light saturation point (I_k) and light compensation point (I_c), indicating that light requirements were insensitive to salt changes. The terrestrial-lived colonies collected from nature exhibited the highest P_{max} at 150 mM NaCl and linearly decreased R_d with increased concentrations of NaCl (Shi et al., 1992). The NaCl concentration for the P_{max} of the terrestrial colonies was much higher than that for the aquatic-living colonies found in the present study. Such a discrepancy may be due to the following reasons: (1) the terrestrial colonies were tested immediately without previous incubations in salt solutions (Shi et al., 1992); (2) its sheath could be thicker than that of the aquatic-living colonies; (3) physiological functions of cells or/and sheath in tolerating salt-stress are different between the two types of colonies. In nature, the jelly sheath of *N. flagelliforme* plays roles in heat insulation or preservation and water holding. Thicker sheath in cyanobacteria has been suggested to associate with more powerful resistance against environmental stresses (Rath & Adhikary, 1994). Although the relationship between photosynthetic activity and sheath layer thickness and functions has not been tested between the two types of colonies, the sheath of the aquatic colony must function in a way different from that of the terrestrial one, because it is bacteria-resistant and does not disintegrate in water. Different patterns of the responses to salt between the photosynthetic oxygen evolution and the photochemical efficiency imply that dark-reaction process rather than PSII photochemistry was affected by salt in the aquatic-living colonies. The increased values of PSII photochemistry efficiency after 48 h adaptation may be attributed to the gathered osmolytes under salt-stressed conditions, such as sucrose, trehalose, glucosyglycerol and betaine (Joset et al., 1996; Kaku et al., 2000). That PSII photochemistry efficiency dropped at 400 mM NaCl could be related to damaged photosynthetic apparatus due to hypersalt-caused inactivity of photosynthetic pertinent enzymes and disorganization of PSII (Shen et al., 1992).

The aquatic-living colonial filaments of *N. flagelliforme* appeared to be able to tolerate a wide range of salt under aquatic conditions. This indicates that it may resist to the salt stress due to desiccation when transferred from the aquatic to its mother terrestrial environment. However, shifting to the terrestrial environment must cause morphological, physiological and biochemical changes in the colony. Its adaptive strategies must be known before the aquatic-living colony can be applied as a "seed" for cultivation or restoration of the endangered resources of *N. flagelliforme* in China.

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