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PHOTOSYNTHETIC BICARBONATE UTILIZATION BY A TERRESTRIAL CYANOBACTERIUM, *NOSTOC FLAGELLIFORME* (CYANOPHYCEAE)[1]

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The photosynthetic characteristics of the terrestrial cyanobacterium, *Nostoc flagelliforme*, after complete recovery by rewetting, was investigated to see whether it could use bicarbonate as the external inorganic carbon source when submerged. The photosynthesis–pH relationship and high pH compensation point suggested that the terrestrial alga could use bicarbonate to photosynthesize when submerged. The photosynthetic oxygen evolution rates were significantly inhibited in Na^+-free and $Na^+ + Li^+$ media but were not affected by the absence of Cl^-, implying that the bicarbonate uptake was associated with Na^+/HCO_3^- symport rather than Cl^-/HCO_3^- exchange system.

Key index words: **bicarbonate; blue-green alga; cyanobacterium; *Nostoc flagelliforme*; photosynthesis**

Abbreviations: **DIC, dissolved inorganic carbon**

Nostoc flagelliforme is a terrestrial macroscopic cyanobacterium distributed in the northern and west-northern parts of China. It has been used as a food delicacy and an herbal ingredient by the Chinese for centuries. A number of studies have been performed in China on the ecology, physiology, morphology, and culturing of *N. flagelliforme* (Gao 1998). However, cultivation of the organism has not been successful, and many of its biological characteristics are poorly understood.

Nostoc flagelliforme is often exposed to desiccation, but like other terrestrial *Nostoc* species, it recovers metabolic activity within hours after rehydration (Scherer et al. 1984, Dodds et al. 1995). In nature, after rain or the accumulation of morning dew, the cyanobacterial mats are immersed or masked with water. Such an aqueous film can be alkaline, because the soils in which the alga grows are strongly alkaline (pH 8.0–9.5) (Hu et al. 1987, Diao 1996). The dissolved inorganic carbon (DIC) in the aqueous film that covers the alga is predominantly bicarbonate (>90%). Many aquatic microscopic cyanobacteria can actively take up extracellular bicarbonate by a process that acts to concentrate CO_2 within the cells (Coleman and Colman 1981, Badger et al. 1985, Colman 1989, Kaplan and Reinhold 1999). *Nostoc flagelliforme* is terrestrial and usually exposed to air, and most of the inorganic carbon that it fixes is in the form of CO_2. However, under moist or wet conditions, the major form of inorganic carbon available to *N. flagelliforme* is bicarbonate. Therefore, it is important to evaluate the ability of *N. flagelliforme* to use bicarbonate.

Nostoc flagelliforme mats were collected at Siziwangqi, Inner Mongolia in July 1999 and stored under dry conditions at room temperature (20–25° C) before being used for experiments in October 1999. Samples were immersed in BG-11 medium (Stanier et al. 1971) at 25° C and 45 μmol photons·m^{-2}·s^{-1} for 10 h to allow full photosynthetic recovery (Gao et al. 1998). About 80 mg (fresh weight) of pretreated sample was placed in a chamber with 6 mL BG-11 medium, and photosynthetic oxygen evolution was measured using a Biological Oxygen Monitor (YSI Model 5300) at 625 μmol photons·m^{-2}·s^{-1} (photosynthetically active radiation) and 25° C. The medium was "CO_2"-enriched (DIC 3.3 mM) and buffered at final concentrations of 25 mM MES, pH 6.4, HEPES, pH 7.4, or Tris, pH 8.0 and 9.0 (all buffers from Sigma). The theoretical production rates of CO_2 derived from uncatalyzed dehydration of HCO_3^- for the above pH conditions were estimated according to Briggs (1959) and Miller and Colman (1980). A rate of oxygen evolution greater than the theoretical rate of CO_2 production is considered as evidence for the ability of *N. flagelliforme* to use external HCO_3^- for the fixation of CO_2. To determine the role of external carbonic anhydrase on oxygen evolution, the carbonic anhydrase inhibitor, acetazolamide, which is generally assumed not to penetrate algal cells (Haglund et al. 1992a,b), was added to the pH 8.0 and 9.0 medium to a final concentration of 0.1 mM. To determine the effects of Na^+ on photosynthesis and on the pH-drift patterns, four kinds of media were prepared: control medium (base medium + 0.15 M NaCl), Cl^--free medium (base medium + 0.15 M Na_2SO_4), Na^+-free medium (base medium + 0.15 M LiCl), and $Na^+ + Li^+$ medium (base medium + 0.075 M NaCl + 0.075 M LiCl). Modified BG-11, in which Na^+ salts were substituted by corresponding K^+ salts and DIC was enriched to 3.3 mM, was used as the base medium. In the pH-drift experiment, 0.12 g dry alga was immersed in 15 mL media in sealed glass vials of 25 mL, which were maintained in an incubator at 115 μmol

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photons·m^{-2}·s^{-1} and 20° C. The initial DIC concentration and pH were 3.3 mM and 8.0, respectively. The pH of medium in the vials was monitored at intervals of about 10 h.

Analysis of the relationship between photosynthesis and the external pH showed that *N. flagelliforme* could efficiently photosynthesize in an alkaline medium (Fig. 1). CO_2 amounts were reduced from 40% to about 0.2%, whereas HCO_3^- amounts were raised from 58% to 95% of total DIC when the pH increased from 6.4 to 9.0. The theoretical rates at which CO_2 can be derived from HCO_3^- at pH 7.4, 8.0, and 9.0 were 0.96, 0.29, and 0.07 μmol CO_2·mL^{-1}·min^{-1}, whereas the observed rates of oxygen evolution were about 8.01, 10.76, and 11.07 μmol O_2·mL^{-1}·min^{-1}, respectively. Therefore, the rates of CO_2 generation from the uncatalyzed dehydration of HCO_3^- were not fast enough to account for the observed rates of photosynthetic oxygen evolution, implying that *N. flagelliforme* is capable of using external HCO_3^- to drive photosynthetic CO_2 fixation.

When the dry alga was rehydrated in Na^+-replete and Na^+-depleted media in the light, the pH increased with time in the control (NaCl) and Cl^--free (Na_2SO_4) media (i.e. media with Na^+) to reach a pH of about 10.8. In contrast, the pH decreased in Na^+-free (LiCl) medium (Fig. 2). The pH rise in Na^+-replete medium indicated active photosynthetic carbon fixation, whereas the pH decrease in the Na^+-free medium indicated little or no photosynthetic carbon utilization and active respiration. We noted that during the first 10 h the pH of the Na^+-replete medium declined. These results suggest that it takes about 10 h for *N. flagelliforme* to develop the ability to use HCO_3^- after rehydration (Gao et al. 1998). The ability of algae to raise the pH of the external medium to above 9.0 had been considered as an indicator of HCO_3^- use in some macroalgae (Maberly and Spence 1983, Maberly 1990, Haglund et al. 1992a,b). The pH of the medium in which *N. flagelliforme* was growing reached values of over 10.0, which is strong evidence that this cyanobacterium can use HCO_3^- for photosynthesis.

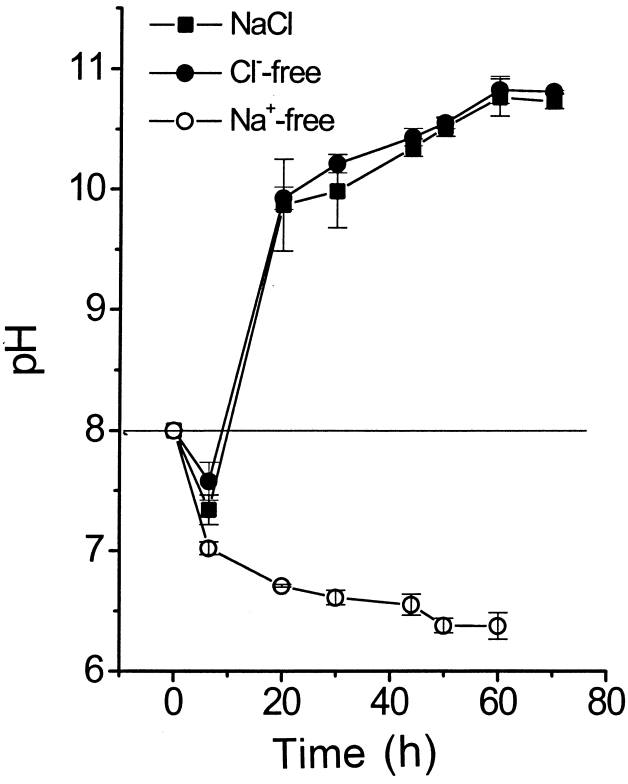

FIG. 2. The pH-drift curves for *Nostoc flagelliforme* at 20° C and 115 μmol photons·m^{-2}·s^{-1} in (●) Cl^--free, (○) Na^+-free, and (■) NaCl-containing media. Vertical bars represent the ± SE of the mean of four replicates.

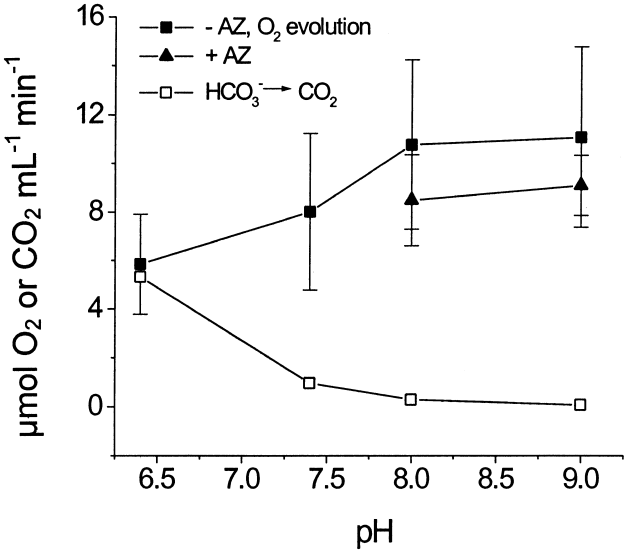

FIG. 1. Observed rates of photosynthetic oxygen evolution by *Nostoc flagelliforme* (▲) with or (■) without acetazolamide (AZ) and (□) the theoretical rates of CO_2 supply derived from uncatalyzed dehydration of HCO_3^- as a function of pH, at 25° C and 625 μmol photons·m^{-2}·s^{-1}. Vertical bars represent the ± SE of the mean of six replicates.

The external carbonic anhydrase inhibitor, acetazolamide, had little effect ($P > 0.1$, *t*-test) on photosynthetic oxygen evolution when the pH of the medium was alkaline (Fig. 1). In addition, the observed rates of photosynthetic oxygen evolution in the presence of acetazolamide were about 29- and 131-fold higher than the rates at which CO_2 could be generated from the uncatalyzed dehydration of HCO_3^- at pH 8.0 and 9.0, respectively. These results suggest the direct transport of HCO_3^- into *N. flagelliforme* cells. The transport of HCO_3^- across the plasma membrane may be either associated with Na^+/HCO_3^- symport (Kaplan et al. 1984), Na^+/H^+ antiport (Katz et al. 1986), or HCO_3^-/Cl^- (Olsnes et al. 1986) or OH^-/HCO_3^- exchange (Lucas 1983). The net photosynthesis of *N. flagelliforme* was dependent on Na^+ rather than Cl^- ions and was inhibited significantly by the presence of Li^+ ions (Fig. 3). These results indicate that HCO_3^- utilization by *N.*

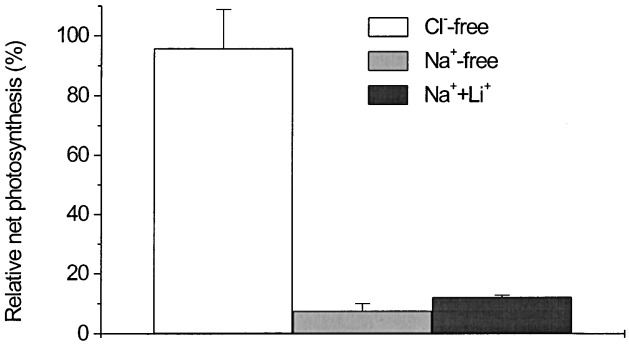

FIG. 3. The effects of Cl^--free, Na^+-free, and $Na^+ + Li^+$ media on rates of photosynthetic oxygen evolution of *Nostoc flagelliforme* at 25° C and 625 μmol photons·m^{-2}·s^{-1}. The medium used for the control was modified BG-11 + 0.15 M NaCl, and the rate was set at 100%. The medium was buffered to pH 8.0 by 25 mM Tris. The rate of photosynthetic oxygen evolution in the control was 10.28 ± 2.35 μmol·O_2· g^{-1}FW·h^{-1} (mean ± SE). Vertical bars represent the ± SE of the mean of four replicates.

flagelliforme may involve a Na^+/HCO_3^- symport system rather than a Cl^-/HCO_3^- exchange system. Stimulation of HCO_3^- transport by Na^+ has also been demonstrated in the aquatic cyanobacteria *Anabaenae variabilis* (Reinhold et al. 1984) and *Synechococcus* sp. (Miller et al. 1984). However, treatment with Na^+-free medium did not affect the photosynthesis of rewetted *N. flagelliforme* in air (Qiu and Gao 1999). In air, CO_2 was determined to be the only carbon source for photosynthesis and Na^+ appeared not to be required for the CO_2 uptake. The dependence of photosynthetic bicarbonate utilization on Na^+ explains the pH-drift patterns in Figure 2. The presence of an Na^+-activated HCO_3^- utilization system apparently caused the rise in pH. Thus, a Na^+/HCO_3^- symport, driven by an Na^+/H^+ antiport, is hypothesized to be critical for photosynthesis in *N. flagelliforme* when it is immersed in water. The energy for HCO_3^- uptake would be derived from the transmembrane Na^+ gradient (Bhaya et al. 2000).

Annual precipitation is less than 300 mm in areas inhabited by *N. flagelliforme*. Therefore, *N. flagelliforme* usually exists in a desiccated state. However, the data presented here indicate that it is still capable of HCO_3^- utilization—a feature common to many aquatic cyanobacteria (Kaplan and Reinhold 1999). Maintenance of such a system would enable *N. flagelliforme* to take the advantage of abundant HCO_3^- when immersed or rewetted in its alkaline soil environment. It has been reported that *N. flagelliforme* grows mainly during the period from June to September (Qian et al. 1989, Wang and Liang 1989), a period when rainfall is responsible for about 60%–70% of the yearly precipitation. Upon hydration, *N. flagelliforme* appears to become efficient in the uptake and utilization of HCO_3^- ions, possibly because of its large surface to mass ratio. It will be interesting to more directly measure the ability of *N. flagelliforme* to take up HCO_3^- under different environmental conditions.

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