

## Bioavailability of iron to *Trichodesmium* colonies in the western subtropical Atlantic Ocean

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### Abstract

*Trichodesmium* provides new nitrogen (N) to marine surface waters via N<sub>2</sub> fixation, a process that requires a substantial amount of iron (Fe). Organic ligands in seawater that bind Fe could either increase or reduce the bioavailability of Fe. Electrochemical techniques indicate that these naturally occurring ligands have Fe-binding constants similar to those of siderophores and porphyrins, suggesting that these chelators play an important role in determining the bioavailability of Fe to cyanobacteria. We conducted Fe uptake experiments using model ligands labeled with <sup>55</sup>Fe to compare the bioavailability of inorganic Fe(III), porphyrin-bound Fe(III), and siderophore-bound Fe(III) to field-collected *Trichodesmium* colonies. Inorganic Fe(III) and siderophore-bound Fe(III) were more bioavailable to *Trichodesmium* colonies than was porphyrin-bound Fe(III). Furthermore, the bioavailability of the siderophore-bound Fe(III) can be characterized by the functional groups of the siderophore. The dihydroxamate siderophore and an uncharacterized ligand from a cultured *Synechococcus* sp. increased the bioavailability of Fe compared to the trihydroxamate siderophores. Except for experiments with desferrioxamine B, dark incubations resulted in lower Fe uptake rates for all treatments, relative to parallel lighted incubations. This suggests that light enhances the photochemical dissociation of most of the ligand complexes or that light energy is required for the active transport of Fe complexed to the model ligands. The Fe uptake rate of *Trichodesmium* colonies also differed slightly on the basis of colony morphology, with higher uptake rates with “puffs” than “tufts.” These experiments show that *Trichodesmium* colonies are capable of discriminating between Fe bound to different organic complexes.

Cyanobacteria are ubiquitous in marine environments and play a significant role in the global carbon (C) and N cycles (Capone et al. 1997). The bioavailability of Fe is an important factor affecting the productivity of cyanobacteria, as Fe is necessary for essential metabolic processes such as photosynthesis and N<sub>2</sub> fixation (Wilhelm 1995). Although cyanobacteria require relatively low amounts of Fe compared to C, N, and phosphorus (P), their biological Fe requirements are not always met because of the low inputs of Fe that are common to the open ocean and the low solubility of Fe in

seawater. Cyanobacteria typically have a higher Fe quota than other phytoplankton (Wilhelm 1995); however, N<sub>2</sub> fixers such as *Trichodesmium* have an even higher Fe quota, with Sanudo-Wilhelmy et al. (2001) reporting N:Fe ratios of *Trichodesmium* in the central Atlantic Ocean near 5,000. Sanudo-Wilhelmy et al. (2001) estimate that N<sub>2</sub> fixation may require 2.5–5.2 times more Fe than organisms relying on NH<sub>4</sub><sup>+</sup> alone. Thus, an oceanic deficiency of bioavailable Fe has been suggested to regulate the primary production, N<sub>2</sub> fixation, and biomass of many species of cyanobacteria (Rueter 1988).

Most of the Fe in seawater exists in the particulate form because of the low solubility of Fe(III) in oxygenated seawater. However, this insoluble portion of the Fe pool is thought to be relatively unavailable for biological uptake, as most organisms can only assimilate dissolved Fe (Bruland et al. 1991). There are very low levels of dissolved Fe in the open ocean, and nearly all of the “soluble” Fe in seawater appears to be bound in organic complexes of unknown origin and chemical composition (Rue and Bruland 1995; Wu and Luther 1995). It is possible that the chemical nature of these organic ligand–Fe complexes either increase or reduce the bioavailability of Fe to cyanobacteria (Hutchins et al. 1999).

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Electrochemical techniques have provided some limited insight into the possible origin of these organic complexes. The conditional stability constants of these organic ligands are similar to those of porphyrins and siderophores (Rue and Bruland 1995; Witter et al. 2000). Porphyrins are circular tetrapyrroles that can be released into the water column through zooplankton grazing or cell lysis (Hutchins et al. 1999), whereas siderophores are high-affinity Fe(III)-chelating ligands that are released by some prokaryotic marine organisms to scavenge Fe during periods of Fe limitation (Wilhelm and Trick 1994). Some common types of porphyrins found in photosynthetic organisms are cytochromes and chlorophylls. The three main types of functional groups in siderophores that have been identified from cultured marine prokaryotes are hydroxamates, catecholates (Wilhelm 1995; Granger and Price 1999), and  $\beta$ -hydroxy aspartate/catecholates (Reid et al. 1993). Macrellis et al. (2001) provide direct evidence for the presence of siderophores in the ocean with the detection of hydroxamate and catechol Fe-binding functional groups in seawater. The production of siderophores and the release of porphyrins may be important in determining the bioavailability of Fe to all marine phytoplankton.

The productivity of *Trichodesmium*, a nonheterocystous filamentous cyanobacterium found throughout the surface waters of oligotrophic tropical and subtropical oceans, relies heavily on the bioavailability of Fe. Although *Trichodesmium* can occur as free trichomes (or filaments) in the water column, natural populations of trichomes typically aggregate to form macroscopic colonies (Letelier and Karl 1996). Several hundred trichomes aggregate to form either spherical colonies (puffs) or fusiform colonies (tufts) (Capone et al. 1997). When environmental conditions are ideal, these *Trichodesmium* colonies are capable of forming massive surface blooms (Capone et al. 1998). It has been estimated that these *Trichodesmium* blooms are responsible for approximately one fourth of the total  $N_2$  fixation in the ocean (Rueter et al. 1990) and that this organism is the largest source of new N in the surface waters of the north Atlantic Ocean (Lipschultz et al. 2002). Recent evidence, however, indicates that unicellular diazotrophs make a large contribution in some regions as well, especially in the oligotrophic Pacific (Zehr et al. 2001). The regions in the North Atlantic with the greatest seasonal flux of atmospheric Fe dust tend to have an excess of nitrate compared to phosphate, which is presumed to be the result of diazotrophy (Michaels et al. 1996). Berman-Frank et al. (2001) suggest that Fe limitation restricts the  $N_2$  fixation of *Trichodesmium* in much of the subtropical and tropical oceans. Given the significant role of *Trichodesmium* in global C and N cycles, it is important to determine what regulates the bioavailability of Fe to *Trichodesmium*.

In this paper, we report the effects of model ligand complexation on Fe assimilation in *Trichodesmium* colonies. We chose model ligands to represent the possible organic ligands that bind Fe(III) in seawater on the basis of functional groups of siderophores isolated from marine cultures and intracellular porphyrin-containing compounds (Witter et al. 2000). We compared the bioavailability of inorganic Fe(III), porphyrin-bound Fe(III), and siderophore-bound Fe(III) to *Trichodesmium* colonies collected from four sites in the western

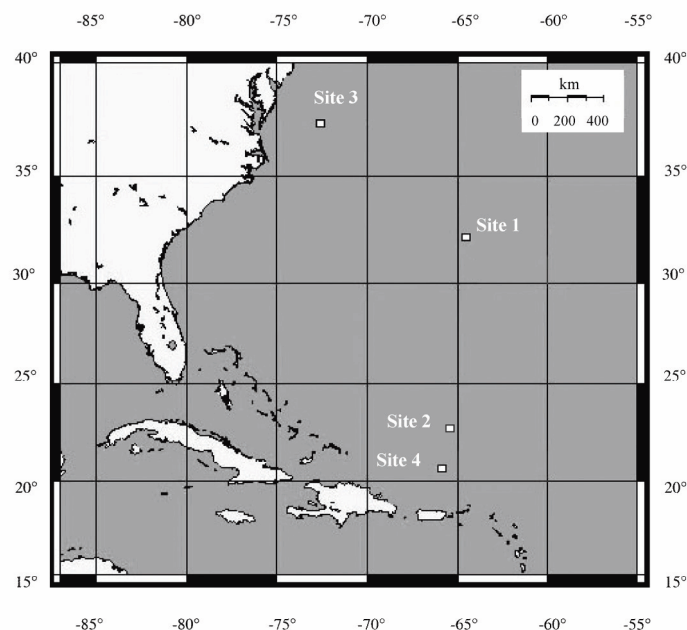


Fig. 1. Collection sites in the western subtropical Atlantic Ocean for *Trichodesmium* colonies used in our Fe uptake experiments.

subtropical Atlantic Ocean, including regions in the Gulf Stream and the northern and southern portions of the central gyre. We also investigated the effects of light on the bioavailability of inorganic Fe(III) and Fe(III) complexed by these model ligands.

## Methods

The ligands used in all the experiments were prepared using trace metal clean methods and were deferrated as needed; details of preparation protocols are presented in Hutchins et al. (1999) and Witter et al. (2000). The model ligands chosen for the Fe uptake experiments were desferrioxamine (a trihydroxamate siderophore), ferrichrome (a trihydroxamate siderophore), rhodotorulic acid (a dihydroxamate siderophore), protoporphyrin IX (a porphyrin compound), and an uncharacterized ligand from the cultured marine cyanobacterium *Synechococcus* sp. PCC 7002. PCC 7002 is a phycocyanin-dominated isolate from the subtropical Atlantic (Puerto Rico) coastal waters and is capable of producing multiple siderophores (Trick and Wilhelm 1995). The same ligand stocks were used for all experiments and were prepared from concentrated stock solutions stored in the freezer. Each ligand was equilibrated with  $^{55}\text{Fe}$  for at least 3 h in chelexed ultraviolet-oxidized Sargasso Sea water, resulting in a final concentration of  $25 \text{ nmol L}^{-1}$  ligand chelated to  $5 \text{ nmol L}^{-1}$   $^{55}\text{Fe}$  in the incubation containers. Each experiment included an inorganic  $^{55}\text{Fe}$  treatment ( $^{55}\text{FeCl}_3$  in 1 N HCl), which had the same concentration of uncomplexed  $^{55}\text{Fe}$  as the chelated treatments.

*Trichodesmium* colonies were collected in a trace metal clean plankton net (Aquatic Research Instruments) at four sites in the Atlantic Ocean (Fig. 1). The sampling occurred at sites in the northern and southern portions of the central

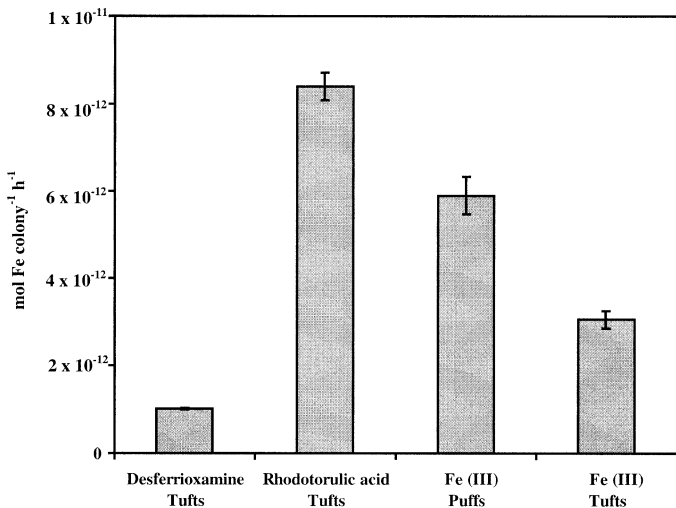


Fig. 2. The bioavailability of inorganic Fe(III) and siderophore-bound Fe(III) to *Trichodesmium* colonies collected at site 2 on 21 October 2001. *Trichodesmium* tufts had a greater uptake rate of Fe from the dihydroxamate siderophore than did the inorganic Fe(III). The inorganic Fe(III) was more available to *Trichodesmium* puffs than to tufts. The Fe bound to the trihydroxamate siderophore was less available than the other treatments. All of the treatments were significantly different (ANOVA,  $P < 0.05$ ). The error bars indicate the standard error of the mean of the three replicates within each treatment.

gyre during cruises in the fall of 2001 (Figs. 2–4) and in the Gulf Stream during June 2000 (Fig. 5). By necessity, experiments were conducted opportunistically at sampling sites where there was a high abundance of *Trichodesmium* colonies. Oceanographic characteristics of each collection site were relatively similar with respect to salinity and surface seawater temperatures (data not shown). Additional parameters were not measured because of sampling restrictions and time constraints. Because of the low abundance of *Trichodesmium* puffs found at each site, most of the experiments were performed on *Trichodesmium* tufts.

The incubations were prepared using trace metal clean techniques in a laminar flow hood. Using acid-washed plastic inoculating loops, the colonies were transferred to an acid-cleaned polypropylene incubation vial containing trace metal clean ambient seawater with either inorganic <sup>55</sup>Fe or a model ligand chelated with <sup>55</sup>Fe. The seawater in the incubation vial was collected from a depth of 20 m with an acid-cleaned Go-Flo bottle attached to a nonmetallic line (in the fall of 2001 cruises), or it was pumped from 10 m using trace metal clean Teflon tubing (June 2000). There were two replicates (with each incubation vial containing 20 colonies) for each treatment for the June 2000 experiment, and the incubation was held under artificial light in the laboratory. All the experiments during the fall of 2001 had three replicates (with each incubation vial containing 50 colonies) per treatment, and the *Trichodesmium* colonies were incubated for 4–6 h in a deckboard incubator during daylight hours with running seawater and blue-tinted Plexiglas panels to simulate in situ light levels (Hutchins et al. 1999). The Fe uptake experiments conducted in the dark incubation vials were run concurrently with the light incubation vials in the

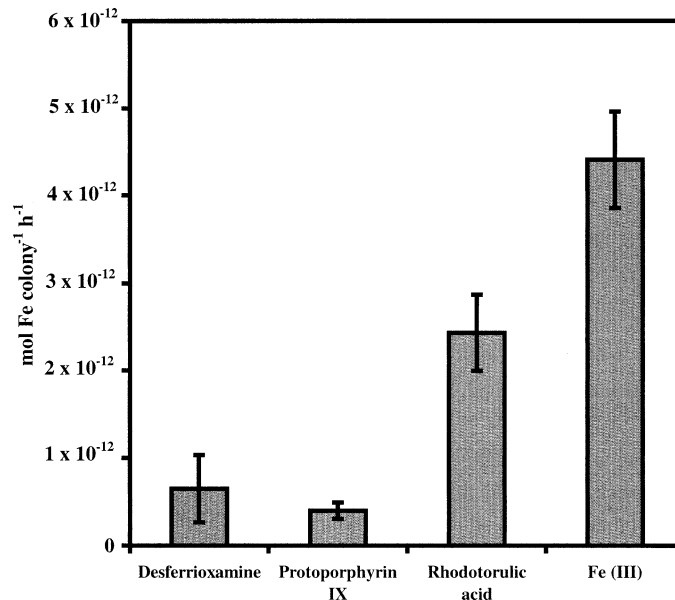


Fig. 3. The bioavailability of inorganic Fe(III), siderophore-bound Fe(III), and porphyrin-bound Fe(III) to *Trichodesmium* colonies collected at site 1 on 27 September 2001. The inorganic Fe(III) was the most available to the *Trichodesmium* colonies. The inorganic Fe(III) and the Fe bound to the dihydroxamate siderophore were more available than the Fe bound to the trihydroxamate siderophore and the porphyrin. The uptake rates of the Fe(III) and the rhodotorulic acid were significantly different from each other (ANOVA,  $P < 0.05$ ), and both had a significantly greater <sup>55</sup>Fe uptake rates than did the desferrioxamine and protoporphyrin IX. The desferrioxamine and protoporphyrin IX were not significantly different from each other. *Trichodesmium* tufts were used for all the treatments. The error bars indicate the standard error of the mean of the three replicates within each treatment.

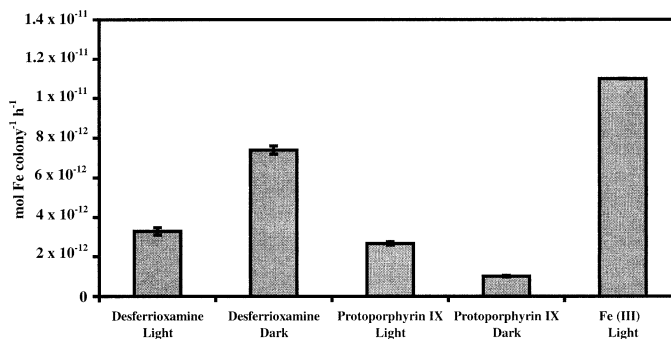


Fig. 4. The bioavailability of inorganic Fe(III), siderophore-bound Fe(III), and porphyrin-bound Fe(III) to *Trichodesmium* colonies collected at site 4 on 27 October 2001. The inorganic Fe(III) was the most available to the *Trichodesmium* colony. The Fe bound to the trihydroxamate siderophore was more available than the Fe bound to the porphyrin (ANOVA,  $P < 0.05$ ). The uptake rate of Fe was lower in the dark porphyrin treatment than in the light incubation, whereas the uptake from the desferrioxamine treatment in the dark was higher than in the light incubation. *Trichodesmium* tufts were used in all the treatments. The error bars indicate the standard error of the mean of the three replicates within each treatment.

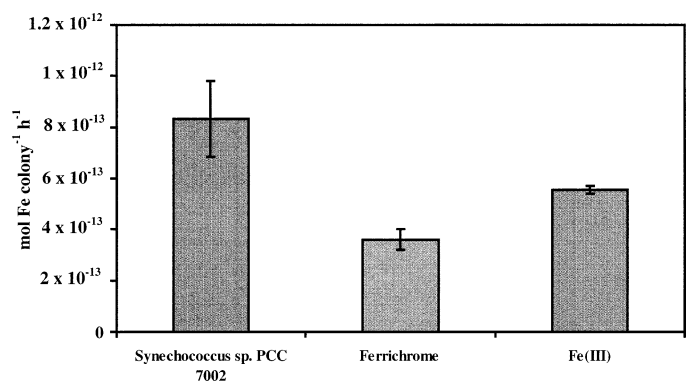


Fig. 5. The bioavailability of inorganic Fe(III) and siderophore-bound Fe(III) to *Trichodesmium* colonies collected at site 3 on 13 June 2000. The Fe bound to the trihydroxamate siderophore was the least available to the *Trichodesmium* colony. The ligand from *Synechococcus* sp. PCC 7002 followed trends similar to the dihydroxamate siderophore because the bound Fe was more available than the inorganic Fe(III) and the Fe bound to the trihydroxamate siderophore. The inorganic Fe(III) was significantly more available to *Trichodesmium* colonies than was the Fe bound to ferrichrome (ANOVA,  $P < 0.05$ ). *Trichodesmium* tufts were used in all the treatments. The error bars indicate the range of the two replicates within each treatment.

deckboard incubator. Because of time restraints, replicate experiments using dark incubation vials were not performed.

The colonies were filtered onto a polycarbonate filter (0.2–0.8  $\mu\text{m}$ ) after the incubation, rinsed with a titanium–ethylene-diamine–tetraacetic acid–citrate reagent to remove any extracellular  $^{55}\text{Fe}$  (Hudson and Morel 1989), and rinsed with seawater. The Fe uptake rate was determined for each incubation vial by measuring the  $^{55}\text{Fe}$  incorporated into the cells by liquid scintillation counting. The Fe uptake rate of each vial was based on a single measurement at the end of the incubation period. For each treatment, the Fe uptake rate per colony was calculated as mol Fe colony<sup>-1</sup> h<sup>-1</sup> (Schmidt and Hutchins 1999). Each incubation vial contained colonies of similar sizes to help alleviate any discrepancy based on colony size. Multiple replicates within each treatment, along with the high number of colonies in each incubation vial, also helped to account for any variations in colony size. Results were analyzed using an analysis of variance ( $\alpha = 0.05$ ) and a Tukey's pairwise comparison.

## Results and discussion

The bioavailability of Fe to *Trichodesmium* colonies was altered by the complexation of Fe with various organic ligands. Despite the different sampling locations in the northern and southern portions of the central gyre as well as in the Gulf Stream (Fig. 1), there were similar trends in the bioavailability of Fe to *Trichodesmium* colonies. Variations in the dust deposition and dissolved Fe concentration to these regions may influence the bioavailability of Fe. Larger *Trichodesmium* blooms typically correspond to areas containing higher atmospheric Fe(II) concentrations as well as to regions associated with larger fluxes of atmospheric dust (Falkowski 1997). The high abundance of *Trichodesmium*

colonies at all the sampling sites implies that there were adequate levels of bioavailable Fe in all the regions during the time of sampling.

The *Trichodesmium* colonies were capable of discriminating between inorganic Fe and Fe bound to the several different organic complexes. The most biologically available forms of Fe to *Trichodesmium* colonies were inorganic Fe and Fe bound to the dihydroxamate siderophore “rhodotorulic acid” (Fig. 2). Although the rate of Fe uptake from the dihydroxamate siderophore relative to the inorganic Fe varied between experiments (Figs. 2, 3), the dihydroxamate siderophore always significantly increased the bioavailability of Fe compared to the trihydroxamate siderophores (ferrichrome and desferrioxamine) and the porphyrin (Fig. 3) (ANOVA,  $P < 0.05$ ). Porphyrin-bound Fe was equally or less available for *Trichodesmium* colonies when compared to trihydroxamate-bound Fe (Figs. 3, 4). These experiments suggest that siderophore-bound Fe, particularly from dihydroxamate siderophores, is more accessible to *Trichodesmium* colonies than is porphyrin-bound Fe. These results support the overall conclusion reported by Hutchins et al. (1999), who found that Fe bound to siderophores is more available to cyanobacteria, while Fe bound to porphyrins is more accessible to eukaryotes. Hutchins et al. (1999) performed Fe uptake experiments with laboratory cultures of *Synechococcus* and field incubations with *Synechococcus*-dominated oligotrophic Atlantic assemblages that showed that Fe uptake rates were higher with siderophores than with porphyrins; however, no dihydroxamate siderophores were used in these experiments.

Several cyanobacteria are capable of producing siderophores when exposed to low levels of Fe (Wilhelm and Trick 1994); however, there have been no reports of siderophore production by *Trichodesmium* (Webb et al. 2001). There are many organisms associated with natural colonies of *Trichodesmium*, especially bacteria (Capone et al. 1997). These heterotrophic bacteria associated with the colonies are capable of siderophore production (K. Barbeau et al. and E. Mann et al. unpubl.). These colony-associated bacteria may play an essential role in the acquisition of Fe if *Trichodesmium* is capable of using the Fe bound to these siderophores. The bacterial composition associated with the colonies may differ on the basis of the species of *Trichodesmium* or the colony morphology, which may explain why *Trichodesmium* puffs had a slightly higher uptake rate of inorganic Fe than tufts (Fig. 2). A single experiment, also in the southern portion of the central gyre, comparing the Fe uptake rate of tufts versus puffs for Fe bound to rhodotorulic acid displayed a similar trend (data not shown). Differences in uptake rates between the two colony types could also be related to differences in exposed cell surface area:cell volume ratios; however, because of the complex and highly variable forms of *Trichodesmium* colonies, these are difficult to estimate quantitatively. These observations are based on a limited number of samples; therefore, further experiments are needed to confirm that these differences are not simply due to physiological differences (i.e., growth phase) or variations in prior history (i.e., exposure to Fe sources).

Fe uptake experiments conducted in dark incubation vials resulted in significantly lower (ANOVA,  $P < 0.05$ ) uptake

rates of Fe from protoporphyrin IX than those in incubation vials exposed to light (Fig. 4). Similar trends were observed with rhodotorulic acid and inorganic Fe(III) at other locations in the southern portion of the central gyre (data not shown). This indicates that either light induces active uptake or that light enhances the photochemical dissociation of most ligand complexes. Barbeau et al. (2001) conducted  $^{59}\text{Fe}$  uptake experiments on natural communities of plankton in the north Atlantic Ocean and concluded that the photolysis of Fe(III)–siderophore complexes increased the bioavailability of Fe compared to inorganic  $^{59}\text{Fe(III)}$ . Exposure to light caused the reduction of Fe(III) to Fe(II) and the photooxidation of the ligand, which ultimately enhances the amount of the siderophore-bound Fe that is available for biological uptake because of the creation of a lower-affinity Fe(III) ligand (Barbeau et al. 2001). Variability in solar and artificial radiation during the incubations may be one factor contributing to the differences we sometimes observed in uptake rates using the same ligands in separate experiments.

In contrast to the dark incubation treatments mentioned above, we found that the availability of Fe from the same desferrioxamine stock in the dark incubation vials was significantly higher than the availability of Fe from the desferrioxamine in the light incubation vials (Fig. 4). Although our results are based on a single experiment, the preliminary results of Trick et al. (pers. comm.) also suggested an increase in the uptake rate within the 0.2- to 1.0- $\mu\text{m}$  class fraction in their dark incubations of subtropical Pacific Ocean samples to which desferrioxamine B had been added. Barbeau et al. (2001) suggest that different ligand structures have different photochemistry, which affects chemical and biological reactivity; however, the reason for this apparent enhanced uptake from desferrioxamine in the dark is not known. The light may destroy the complex, causing the Fe to oxidize to a hydroxide precipitate form, making the Fe less available. Alternatively, the organism assimilating the compound (e.g., a colony-associated heterotrophic bacterium) may be very light sensitive and thus take up more desferrioxamine-bound Fe in the dark.

The ligand from *Synechococcus* sp. PCC 7002 followed the trend of the dihydroxamate siderophore, with both displaying comparable or higher Fe uptake rates in *Trichodesmium* colonies than the inorganic Fe(III) (Fig. 5). *Synechococcus* PCC 7002 produces at least four different siderophores during Fe limitation, including two catechol-type ligands, one hydroxamate ligand, and one unidentified type of ligand (Trick and Wilhelm 1995). Although the detailed structures of the ligands from *Synechococcus* sp. are unknown, this experiment suggests that the hydroxamate ligand is a dihydroxamate, because it followed trends similar to those of rhodotorulic acid.

The chemical form of chelated Fe appears to play an important role in regulating the bioavailability of Fe to *Trichodesmium* colonies in the north Atlantic Ocean. The Fe uptake experiments were performed for 2 yr in several regions of the Atlantic Ocean, including the Gulf Stream and the northern and southern portions of the central gyre. Regardless of the station where they were collected, the bioavailability of Fe to *Trichodesmium* colonies followed similar trends. Determining the bioavailability of Fe from these model ligands

to *Trichodesmium* cultures in the laboratory is also important, as cultures lack the bacterial diversity associated with colonies at sea. This would allow us to compare differences in the trends of Fe acquisition between laboratory cultures and *Trichodesmium* colonies in the field and thus differentiate between the Fe uptake capabilities of the cyanobacterium in isolation and the uptake mediated or modified by associated bacteria. This comparison is essential to unequivocally demonstrate that *Trichodesmium* is capable of distinguishing between inorganic Fe and organically bound Fe, as it is plausible that the selectivity for the different Fe chelators by *Trichodesmium* colonies is solely due to the heterotrophic bacteria associated with the colony and that *Trichodesmium* alone is only capable of Fe(III) uptake.

In addition, Fe uptake experiments that use siderophores isolated from colony-associated bacteria of *Trichodesmium* would be valuable. Because of the possible symbiotic relationship between the colony-associated bacteria and *Trichodesmium*, the siderophores produced from these associated bacteria could potentially increase the bioavailability of Fe to *Trichodesmium* (K. Barbeau et al. and E. Mann et al.). Such a mutualistic association would be advantageous to both organisms, since Fe acquired through the activities of symbiotic bacteria would assist the host colony in providing C and N supplies to the symbiont. Given the significant role of *Trichodesmium* in global C and N cycles, it is important to determine what controls the bioavailability of Fe to *Trichodesmium* in the ocean.

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