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Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*

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Abstract

Prochlorococcus is the most abundant phytoplankter throughout the photic zone in stratified marine waters and experiences distinct gradients of light and nitrogen nutrition. Physiologically and genetically distinct *Prochlorococcus* ecotypes partition the water column: high-B/A (low-light adapted) ecotypes are generally restricted to the deep euphotic zone near or at the nitracline. Low-B/A (high-light adapted) ecotypes predominate in, but are not limited to, NO_3^--depleted surface waters, where they outnumber coexisting *Synechococcus* populations. The niche partitioning by different *Prochlorococcus* ecotypes begs the question of whether they also differ in their nitrogen (N) utilization physiology, especially with respect to NO_3^- utilization. To explore this possibility, we studied the capabilities of different *Prochlorococcus* and *Synechococcus* strains to grow on a variety of N sources. We found that all the isolates grew well on NH_4^+ and all were capable of urea utilization, occasionally at a lower growth rate. None of the *Prochlorococcus* isolates were able to grow with NO_3^-. Four high-B/A *Prochlorococcus* isolates grew on NO_2^-, but all others did not. Whole genome analysis of the low-B/A *Prochlorococcus* MED4 revealed that the genes required for NO_3^- uptake and reduction were absent. The genome of the high-B/A *Prochlorococcus* MIT 9313 also lacked the NO_3^- utilization genes but has homologs of genes required for NO_2^- utilization consistent with its physiology and ecology. Thus, the utilization of different N sources in the marine environment is partitioned among closely related ecotypes, each with adaptations optimized for the environment where these sources are available.

Since the first description of unicellular cyanobacteria as an abundant component of the marine phytoplankton community (Johnson and Sieburth 1979), the genus *Synechococcus* has been established as a major primary producer in the surface ocean. *Synechococcus* presence has been reported from waters in the (sub)tropics, temperate, and even polar regions and is often the dominant phytoplankter in both nutrient-deplete stratified, and nutrient-rich mixed waters (Partensky et al. 1999; Vincent 2000). The versatility of marine *Synechococcus* spp. has been related to its ability to grow over a wide range of light intensities and spectral quality (Kana and Glibert 1987) and to utilize a wide variety of nitrogen (N) resources. Different isolates of marine *Synechococcus* spp. have been reported to utilize NH_4^+, NO_3^-, NO_2^-, urea, and amino acids as the sole N source while employing systems for active uptake of these compounds (Glibert et al. 1986; Paerl 1991; Lindell et al. 1998; Collier et al. 1999). Under N deprivation, *Synechococcus* will degrade the abundant light-harvesting pigment protein phycoerythrin as an internal N source (Wyman et al. 1985).

Synechococcus in (sub)tropical and temperate stratified waterbodies are accompanied by abundant populations of *Prochlorococcus.* This genus is closely related to *Synechococcus* phylogenetically but is distinguished by a photosynthetic apparatus that employs a chlorophyll *a*/*b* (Chl *a*/*b*) light-harvesting antenna rather than phycobilisomes. Although *Prochlorococcus* is geographically limited to latitude ranges of 40°S–40°N , it occupies the full extent of the photic zone, in contrast to *Synechococcus*, which is most often

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Table 1. Isolation information for *Synechococcus* strains from the MIT Cyanobacterial Culture Collection used in this study. Isolates were obtained from seawater that was enriched with NH_4^+ as the sole N source.

Isolate name	Isolation location	Isolation depth	Isolator	N source utilization
MIT S9220	Equatorial Pacific	Surface	B. Binder	NH_4^+, NO_2^-
MIT S9214	South Pacific	Surface	B. Binder	NH_4^+, NO_3^-, NO_2^-
MIT S9451	Sargasso Sea	65 m	L. Aref	NH_4^+, NO_3^-, NO_2^-
MIT S9452	Sargasso Sea	65 m	L. Aref	NH_4^+, NO_3^-, NO_2^-
MIT S9502	Equatorial Pacific	1 m	E. Mann	NH_4^+, NO_3^-, NO_2^-
MIT S9504	Equatorial Pacific	20 m	E. Mann	NH_4^+, NO_3^-, NO_2^-
MIT S9505	Equatorial Pacific	Surface	E. Mann	NH_4^+, NO_3^-, NO_2^-
MIT S9508	Equatorial Pacific	Surface	E. Mann	NH_4^+, NO_3^-, NO_2^-

restricted to the upper half of the euphotic zone (Partensky et al. 1999). Depth distributions of *Prochlorococcus* show that populations near the bottom of the photic zone coincide with the nitracline (Lindell and Post 1995; DuRand et al. 2001); thus, NO_3^- and NO_2^- may be available N sources for such populations. Isolates of *Prochlorococcus* can be categorized into two groups based on their light-dependent physiology and molecular phylogeny: high light–adapted isolates, which contain relatively low Chl b/a_2 ratios (low-B/A ecotypes), and low light–adapted isolates, with high Chl b/a_2 ratios (high-B/A ecotypes) (Moore and Chisholm 1999; Rocap et al. 1999). The former evolved more recently than the latter (Urbach et al. 1998; Rocap et al. 2001). Furthermore, *Prochlorococcus* high-B/A ecotypes are generally found only in the deep euphotic zone near the nitracline, whereas low-B/A ecotypes extend to the nutrient-depleted surface waters (West et al. 2001). This niche partitioning has led to the hypothesis that *Prochlorococcus* in the deep euphotic zone may be adapted to utilize ambient concentrations of NO_3^-, NO_2^-, or both, whereas those residing in the high-light, low-nutrient surface mixed layers are adapted to use regenerated NH_4^+. Utilization of different sources of N has been studied to some extent in *Prochlorococcus*, and it is known that some strains utilize both NH_4^+ and urea (Moore et al. 1995; Palinska et al. 2000; Rippka et al. 2000). The axenic *Prochlorococcus* strain PCC 9511, which has an identical 16S rDNA sequence to the low-B/A *Prochlorococcus* strain MED4, is incapable of NO_3^- and NO_2^- utilization (Rippka et al. 2000). This begs the question as to whether or not this is a characteristic of other low-B/A *Prochlorococcus* isolates and whether this limitation in N source utilization extends to the high-B/A ecotypes found predominantly in the deep euphotic zone near the nitracline.

Synechococcus and *Prochlorococcus* populations in stratified waters occupy niches in which availability of combined N sources is not obviously compatible with the current understanding of their ability to utilize these sources. *Synechococcus* is capable of efficient NO_3^- utilization, as indicated by yearly blooms, because of nutrient entrainment during deep mixing in the Sargasso Sea (DuRand et al. 2001) and Red Sea (Lindell and Post 1995). However, during stratified water column conditions, *Synechococcus* establishes a population maximum in surface layers from which NO_3^- is absent. *Prochlorococcus*, on the other hand, does not bloom during spring deep mixing, sets up a deep subsurface maximum under stratified conditions, and is virtually the only phytoplankter with significant populations at the nitracline. However, one strain of *Prochlorococcus* at least is incapable of accessing this resource (Rippka et al. 2000). In order to understand these apparent contradictions and to determine whether there are indeed N utilization differences among members of these genera, we set out to study the genetic and physiological potential of NO_3^- utilization in a number of *Synechococcus* and *Prochlorococcus* strains obtained from different geographic regions and different depths within the photic zone. Special emphasis was attributed to the potential capacity of high-B/A isolates of *Prochlorococcus* to utilize NO_3^- and NO_2^- as the sole N source and, thus, take advantage of the availability of these sources in the deep euphotic zone.

Methods

Isolates and culture conditions—Eleven strains of *Prochlorococcus* spp. and 10 strains of *Synechococcus* spp. were examined in this study. Five strains of low-B/A *Prochlorococcus* ecotype (MED4 [axenic], MIT 9401, MIT 9312, MIT 9215, AS9601), six strains of high-B/A *Prochlorococcus* ecotype (SS120, MIT 9303, MIT 9313, NATL1A, NATL2A, MIT 9211), and eight strains of *Synechococcus* (MIT S9220, MIT S9214, MIT S9451, MIT S9452, MIT S9502, MIT S9504, MIT S9505, MIT S9508) were obtained from the Massachusetts Institute of Technology Cyanobacteria Culture Collection (Table 1). *Synechococcus* WH 8103 and WH 8012 were obtained courtesy of J. Waterbury, Woods Hole Oceanographic Institution (WHOI) Cyanobacteria Culture Collection.

Stock cultures of all strains were maintained at 24°C on a 14 : 10 light : dark (LD) cycle at 25 ± 5 μmol Q m^{-2} s^{-1} (measured with Biospherical Instruments QSL-100 light meter) in a Sargasso Sea water-based media enriched with 800 μM NH_4Cl, 50 μM NaH_2PO_4, 1.17 μM ethylenediaminetetraacetic acid (EDTA), and the following trace metals: 8 nM Zn, 5 nM Co, 90 nM Mn, 3 nM Mo, 10 nM Se, 10 nM Ni, and 1.17 μM Fe. All nutrients were sterilized by filtration, to avoid any chemical change from autoclave sterilization, and added aseptically to autoclave-sterilized seawater.

To examine the strains for growth on different N sources, experimental media were made in which 500 μM NH_4^+ was replaced with 50 μM NH_4^+, urea, NO_3^-, or NO_2^- (referred to as NH_4^+, urea, NO_3^-, and NO_2^- media, respectively). In ad-

dition, the PO_4^{3-} was reduced to 10 μM such that the resulting media would have a N : P ratio of 5 : 1, ensuring that when the cultures reached saturated growth phase, N would be growth limiting. This allowed us to do ''rescue experiments,'' in which we added different forms of N to the N-starved cultures and observed which would allow the cells to exit stationary phase. In all experiments, a no-N control (referred to as no-N) served as a reference point for the different experimental cultures. Growth in the no-N cultures reflected the carryover of NH_4^+ from the standard growth condition. All cultures were grown in replicate in 50-ml glass test tubes. Rescue experiments involved aseptic addition of 50 μM of the appropriate N source to cultures that had reached N-limited stationary phase. Growth response was determined by measuring culture densities at the same time of the LD cycle over several generations using a FACScan flow cytometer (Becton-Dickinson) to enumerate cells or a 10AU fluorometer (Turner Designs) to follow bulk fluorescence. When using bulk fluorescence as a measure of growth, cultures were checked periodically on a FACScan flow cytometer to ensure that changes in bulk fluorescence were due to changes in cell number and not to changes in chlorophyll or phycobilisome fluorescence per cell.

Only three of the strains (MED4, WH 8103, and WH 8012) used in this study were axenic (free of heterotrophic contaminants). Thus, there is always the concern that bacterial contaminants in the cultures could be regenerating oxidized forms of N to NH_4^+, which then could be used by the *Prochlorococcus* or *Synechococcus,* giving a false positive for growth on NO_3^- or NO_2^-. To test this, bacteria isolated from the nonaxenic MIT 9313 culture were added to cultures of axenic MED4, and the growth responses of the two cultures were compared when NO_2^- was the only available N source. There was no difference in the growth pattern of the MED4 cultures with and without added bacteria when grown on NO_2^- (Fig. 1). This strongly suggests that the presence of bacterial contaminants does not play a significant role in N utilization in our cultures.

Phylogenetic analysis—DNA extraction, 16S–23S rDNA internal transcribed spacer (ITS) amplification, sequencing, and phylogenetic analyses were all performed as described in (Rocap et al. 2002). Briefly, DNA was extracted from *Synechococcus* strain MIT S9220 using a modified hexadecyltrimethylammonium bromide (CTAB)/phenol chloroform protocol (Ausubel et al. 1992). The ITS/23S fragment was amplified in quintuplicate using primers 16S-1247f (CGTACTACAATGCTACGG) and 23S-1608r (CYACCTGTGTCGGTTT), and the ITS was sequenced bidirectionally using 16S-1247f and primers internal to the PCR product.

The MIT S9220 sequence was aligned manually to ITS sequences from other *Synechococcus* and *Prochlorococcus* isolates using inferred secondary structures of the ITS as a guide (Rocap et al. 2002). Phylogenetic analyses used PAUP* v4.0b8 (Swofford 1999) and 233 positions of the ITS, except calculations of fractional sequence identity between strains of *Synechococcus,* which are based on 434 positions. Distance trees were inferred using minimum evolution as the objective criterion and paralinear (logdet) distances. Maximum likelihood analyses used the HKY85 model of nucleotide substitution with rate heterogeneity and empirical nucleotide frequencies. The gamma shape parameter (0.405155) and the transition/transversion ratio (1.182359) were initially estimated from a distance topology and refined by iterative likelihood searches. Bootstrap analyses (1,000 resamplings) were performed with heuristic searches utilizing random addition and tree bisection–reconnection (TBR) branch-swapping methods. The MIT S9220 ITS sequence has been deposited in GenBank (AF397705).

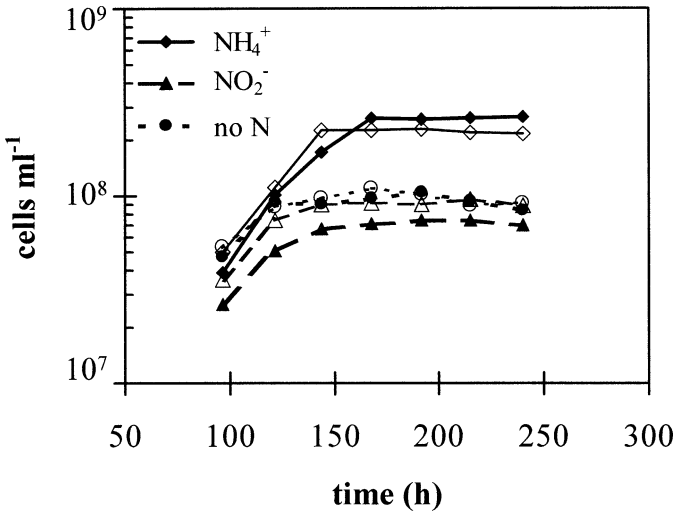

Fig. 1. Growth of an axenic *Prochlorococcus* MED4 culture with (open symbols) and without (closed symbols) added bacteria taken from a nonaxenic *Prochlorococcus* MIT 9313 culture. Solid lines, cultures transferred to NH_4^+ medium; dashed lines, cultures transferred to NO_2^- medium; dotted line, cultures transferred to a medium containing no nitrogen (no-N). Any growth observed in the latter is because of NH_4^+ carryover from the parent culture.

Results

Growth of Prochlorococcus *isolates*—Low-B/A *Prochlorococcus* strain MED4 and high-B/A *Prochlorococcus* strain MIT 9313 differed in their N utilization capabilities. MED4 could grow on both NH_4^+ and urea with equal growth rates, whereas MIT 9313 growth rate slowed when provided with urea as the N source (Fig. 2A,C; Table 2). MED4 was unable to use NO_3^- and NO_2^- for growth (Fig. 2A), as reported previously for *Prochlorococcus* strain PCC9511 (Rippka et al. 2000). In fact, NO_2^- appeared to inhibit growth of MED4 (even when carryover NH_4^+ was available), although the inhibition was not always as dramatic as exhibited in Fig. 2A. Like MED4, MIT 9313 could not grow on NO_3^-; however, unlike MED4, it was capable of growth when NO_2^- was supplied as the sole source of N (Fig. 2C). Results from N-starvation rescue experiments were consistent with the findings from the growth experiments. NO_3^- could not rescue MED4 or MIT 9313 from N-starvation, whereas NH_4^+ could (Fig. 2B,D). In addition, MIT 9313 could be rescued from N-starvation with NO_2^-, further supporting the finding that it could grow with NO_2^- as a sole N source (Fig. 2D).

To determine whether the differences in NO_3^- and NO_2^- utilization between MED4 and MIT 9313 extend to other

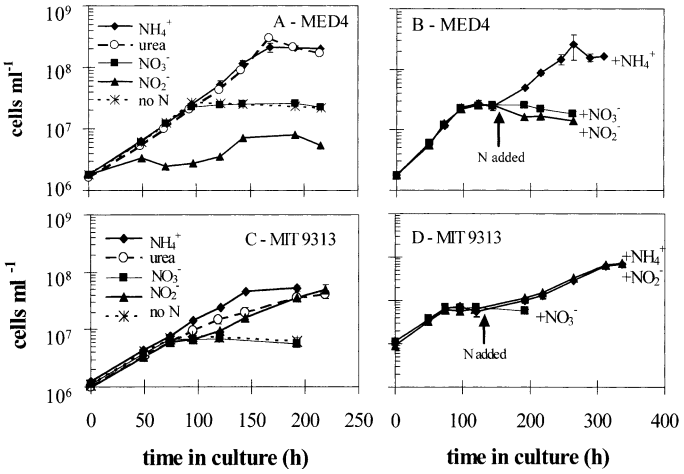

Fig. 2. Growth response of *Prochlorococcus* (A) MED4 and (C) MIT 9313 after transfer from growth on NH_4^+ to a variety of nitrogen sources: NH_4^+, NO_3^-, NO_2^-, urea, and no nitrogen (no-N). Rescue of (B) MED4 and (D) MIT 9313 cultures from nitrogen starvation was attempted by addition of NH_4^+, NO_3^-, and NO_2^- into different cultures. Resumed growth reflects the ability of the strain to utilize the form of N added. Values are mean ± 1 SD of duplicate cultures. When error bars do not show, they are within the size of the symbol.

Prochlorococcus isolates, an additional five high-B/A and four low-B/A *Prochlorococcus* ecotypes were tested for growth on NH_4^+ and NO_3^- as compared to no-N controls. All of these strains could grow on NH_4^+, although with different growth rates, and none could grow on NO_3^- (Fig. 3). Surprisingly, four of the high-B/A strains (MIT 9313, MIT 9303, NATL1A, and NATL2A) were capable of growth when NO_2^- was supplied as the sole source of N, although with a lower growth rate relative to that achieved on NH_4^+ (Fig. 4). The remainder of the strains represented in Fig. 3, including the high-B/A strains SS120 and MIT 9211, are unable to utilize NO_2^- (data not shown).

Growth of Synechococcus *isolates*—In comparison to *Prochlorococcus*, *Synechococcus* WH 8103 was capable of growth on all four N sources tested, although with reduced efficiency on urea, NO_3^-, and NO_2^- relative to growth on NH_4^+ (Fig. 5A; Table 2). Growth rate achieved on urea was only slightly reduced relative to that obtained on NH_4^+ (Fig. 5A; Table 2). This ability to use a wide range of N sources was also observed in experiments with WH 8012 (data not shown) and is consistent with work by other researchers (Glibert et al. 1986; Waterbury et al. 1986; Collier et al. 1999).

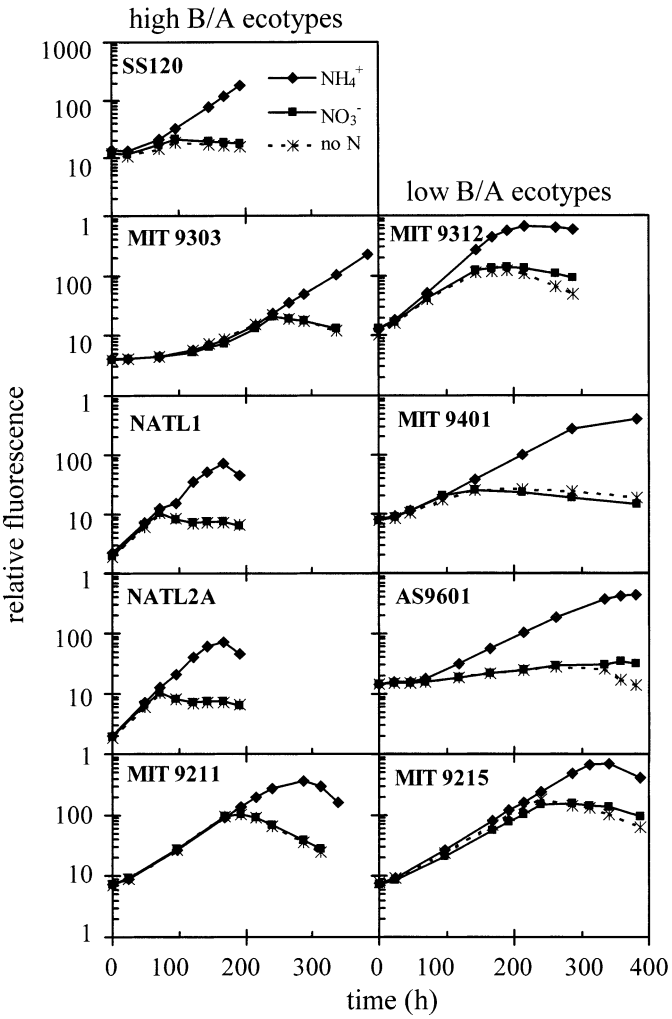

Fig. 3. Representative growth curves for nine different strains of *Prochlorococcus* when transferred into NH_4^+, NO_3^-, or no-N media. None of them could grow on nitrate. Relative bulk fluorescence of the cultures was used to track their growth.

Whenever organisms are isolated into culture, there is always the possibility that particular strains are inadvertently selected for or against through bias in the isolation method. Of the *Prochlorococcus* strains tested in this study, all were isolated and have been maintained without NO_3^- or NO_2^- in the media (Chisholm et al. 1992; Moore and Chisholm 1999), and none are capable of utilizing NO_3^- for growth. On the other hand, *Synechococcus* strains in the WHOI Cyanobacteria Culture Collection were isolated and have been maintained with NO_3^- as the N source, and all cultured

Table 2. Growth rates (d^{-1}) for marine cyanobacteria *Prochlorococcus* MIT 9313 and MED4 and *Synechococcus* WH 8103 and MIT S9220 grown on a variety of nitrogen sources. n.g., no growth.

Cyanobacterial isolate	NH_4^+	Urea	NO_2^-	NO_3^-
Prochlorococcus MED4	0.71±0.01	0.73±0.00	n.g.	n.g.
Prochlorococcus MIT 9313	0.58±0.01	0.25±0.03	0.40±0.04	n.g.
Synechococcus MIT S9220	0.89±0.00	0.23±0.00	0.56±0.04	n.g.
Synechococcus WH 8103	0.85±0.02	0.77±0.01	0.37±0.04	0.55±0.03

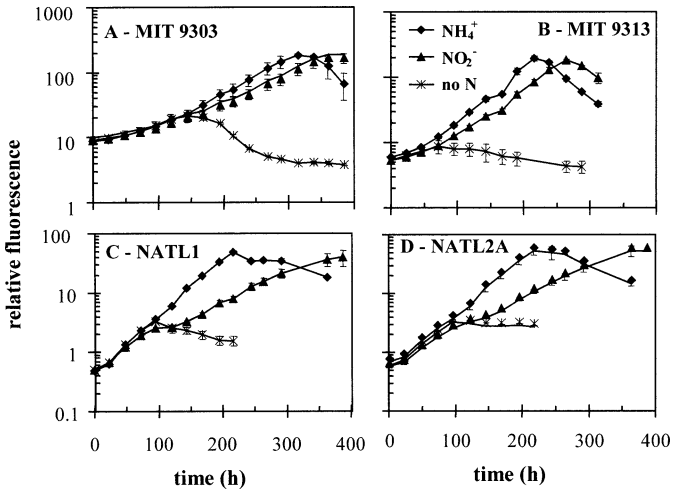

Fig. 4. Growth response of four high-B/A *Prochlorococcus* strains when transferred into media with NH_4^+ or NO_2^- as the source of nitrogen. (A) MIT 9303. (B) MIT 9313. (C) NATL1. (D) NATL2A. Values are mean ± 1 SD of duplicate cultures. When error bars do not show, they are within the size of the symbol.

strains of marine *Synechococcus* are capable of utilizing NO_3^- for growth (Waterbury et al. 1986). Thus, selection during isolation could explain this fundamental difference between these two closely related genera.

In the process of isolating our *Prochlorococcus* strains using NH_4^+ media, *Synechococcus* cells were often isolated and maintained in parallel. This offered us the opportunity to examine whether or not these *Synechococcus* isolates had the capability of using NO_3^-, like the strains in the WHOI Cyanobacteria Culture Collection, and to this end we examined eight of our *Synechococcus* isolates (Table 1) to see if they could grow on NO_3^- and NO_2^-. Seven of the eight isolates could grow on NH_4^+, NO_3^-, and NO_2^- as sole N sources (data not shown). However, one isolate (*Synechococcus* MIT S9220 from the Equatorial Pacific) could not use NO_3^- for growth (Fig. 5B), nor could it be rescued from N limitation with NO_3^- (data not shown). This strain could also use urea as an organic N source, although with significantly reduced growth efficiency (Fig. 5B; Table 2). Fluorescence excitation spectra indicate that this strain contains a low ratio of phycourobilin to phycoerythrobilin (L. R. Moore, unpubl. data). In phylogenetic analyses of this strain, based on the 16S–23S rDNA ITS, MIT S9220 branches with strain WH 7805 (Fig. 6), although this branching pattern does not have high bootstrap support and the two strains are only 84% identical in their ITS sequences. Thus, we conclude that MIT S9220 represents a seventh clade of marine cluster A *Synechococcus.*

Discussion

All strains of the low-B/A (high light–adapted) *Prochlorococcus* ecotype (MED4, MIT 9215, MIT 9312, MIT 9401, and AS9601) tested so far are incapable of using NO_2^- and NO_3^- for growth. None of the six high-B/A *Prochlorococcus* strains could utilize NO_3^- for growth, although four strains

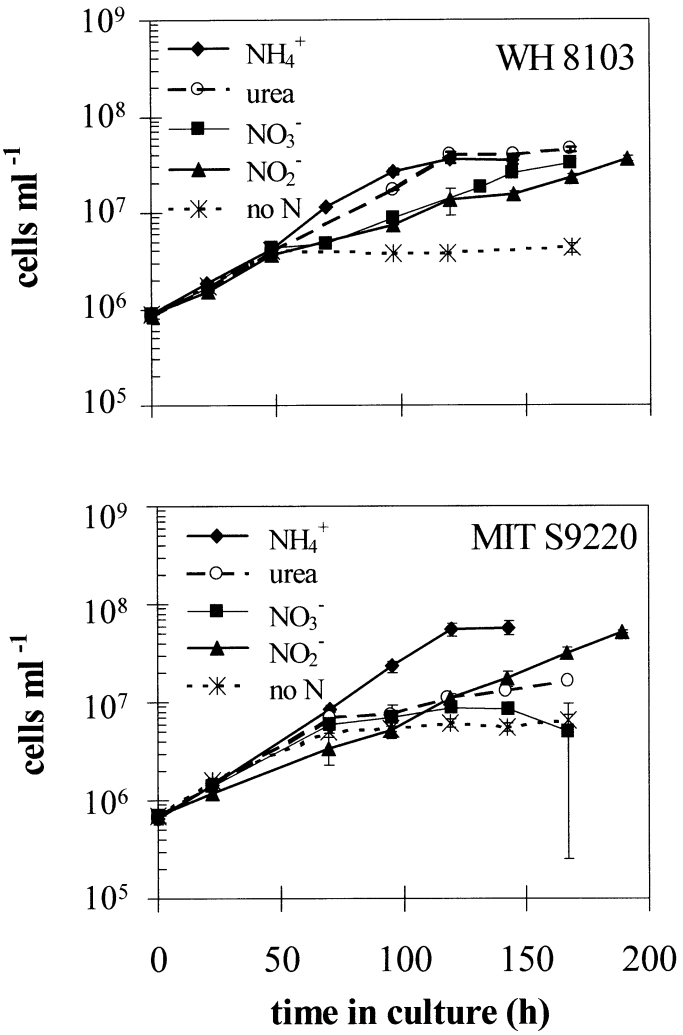

Fig. 5. Growth response of *Synechococcus* strains (A) WH 8103 and (B) MIT S9220 when NH_4^+, NO_3^-, NO_2^-, urea, or no nitrogen (no-N) was provided as the source of nitrogen. Values are mean ± 1 SD of duplicate cultures. When error bars do not show, they are within the size of the symbol.

(MIT 9303, MIT 9313, NATL1A, and NATL2A) could grow using NO_2^-. All *Synechococcus* strains examined, which include strains containing both high and medium levels of phycourobilin, can grow on both NO_3^- and NO_2^-, except for MIT S9220, one of the strains isolated on NH_4^+ media. To our knowledge, this is the first report of a marine *Synechococcus* that cannot utilize NO_3^- for growth. Several freshwater thermophilic cyanobacteria have been reported that are deficient in NO_3^- assimilation (Miller and Castenholz 2001). There is only one other report of a marine phytoplankter that cannot utilize NO_3^- for growth, the Texas brown tide alga *Aureococcus anophagefferens* strain TBA-2 (DeYoe and Suttle 1994). Thus, inability to utilize NO_3^- is not a common feature among phytoplankton.

After these experiments were conducted, the complete genomes of two *Prochlorococcus* strains and one *Synechococcus* strain were sequenced (http://www.jgi.doe.gov/JGI_microbial/html/index.html), allowing us the opportunity

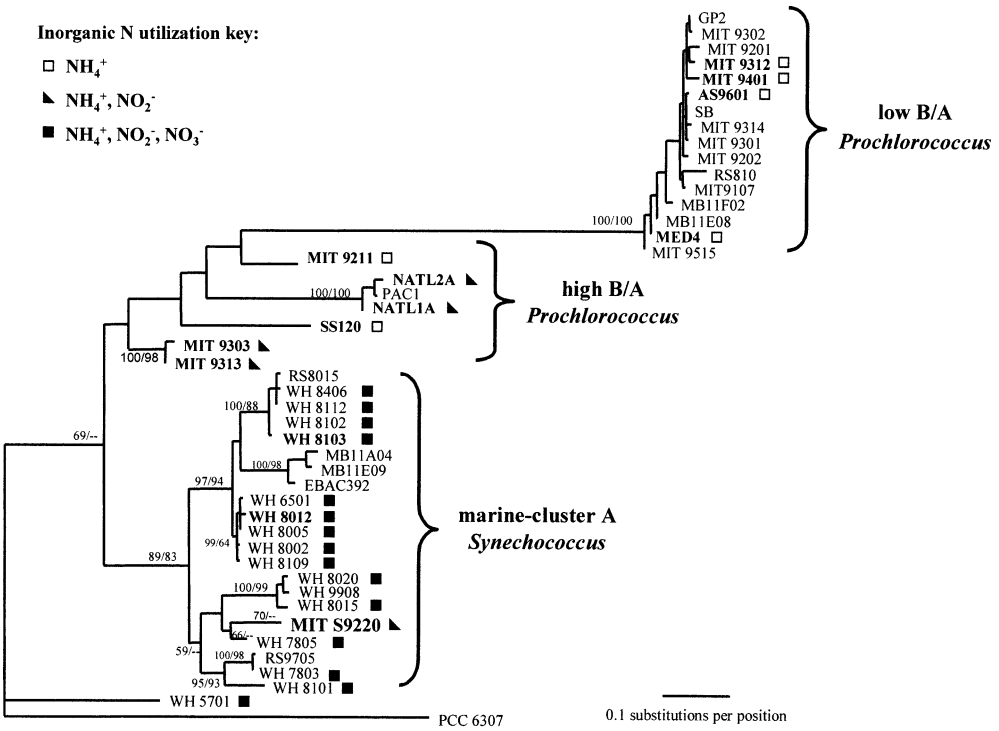

Fig. 6. Evolutionary relationships of *Synechococcus* strain MIT S9220 to other *Synechococcus* and *Prochlorococcus* isolates and environmental sequences from Monterey Bay. Names in bold font indicate strains that were examined in this study for their N utilization capabilities. Symbols to the right of each strain indicate its ability to grow on NH_4^+; on NH_4^+ and NO_2^-; or on NH_4^+, NO_2^-, and NO_3^-. The tree shown was inferred by maximum likelihood using 233 positions of the 16S–23S rDNA internal transcribed spacer. Bootstrap values (from distance/parsimony) are listed next to each node with values <50 not shown. The tree is rooted with Cyanobium cluster *Synechococcus* PCC 6307.

to explore the genetic capabilities of these strains. The complements of N source utilization genes differ considerably between these strains and are consistent with their respective N utilization physiology. Both *Prochlorococcus* MED4 and MIT 9313 lacked the genes required for nitrate utilization in marine cyanobacteria—NO_3^- permease (*napA*), NO_3^- reductase (*narB*)—and the genes for synthesis of the NO_3^- reductase cofactor molybdopterin (Post et al. in prep.). However, the genome of MIT 9313, a high-B/A isolate capable of growth on NO_2^-, was found to carry open reading frames with >80% identity to the putative nitrite reductase gene, *nirA,* of *Synechococcus* WH 8103 (GenBank AF065403) and *Synechococcus* WH 8102, along with the WH 8102 *cysG* gene required for the synthesis of the nitrite reductase cofactor sirohaeme. The WH 8102 genome contained the full gene complement for NO_3^-/NO_2^- utilization consistent with the growth potential of the closely related *Synechococcus* WH 8103.

The N physiology of marine cyanobacteria in this study is generally consistent with their phylogenetic relationship (Fig. 6). The low-B/A *Prochlorococcus* ecotypes, which represent the most recently evolved lineage (Urbach et al. 1998; Rocap et al. 2002), have lost the ability to utilize NO_3^- and NO_2^-. The four high-B/A *Prochlorococcus* strains that can utilize NO_2^- represent two of the four high-B/A phylogenetic clusters (I and IV) (Rocap et al. 2002). Thus, the loss of NO_2^- utilization ability (and genes) may have occurred independently at least three times during the evolution of the *Prochlorococcus* lineage. Alternatively, the loss of NO_2^- utilization capability may have occurred once during the evolution of the low-B/A *Prochlorococcus* lineage, and the inability of SS120 and MIT 9211 could be the result of frameshift or point mutations in *nirA* that have occurred since they have been in culture. No *Prochlorococcus* isolates in this study could grow on NO_3^-, and neither MIT 9313 nor MED4 contain the genes to utilize NO_3^-, implying that the ability to use NO_3^- was lost early in the evolution of *Prochlorococcus* from *Synechococcus.* One strain of *Prochlorococcus,* PAC1, appears to have the capacity for NO_3^- uptake and utilization, although NO_2^- utilization has not been tested (L. Campbell, pers. comm.). This strain falls into the high-B/A cluster I with NATL1A and NATL2A, two strains that were capable of growth on NO_2^- but not NO_3^-. It is possible that PAC1 could have reacquired the ability to utilize NO_3^- through horizontal gene transfer.

Recently, we attempted to isolate *Prochlorococcus* from the Red Sea using NH_4^+, NO_3^-, and NO_2^- as sole sources of N in the isolation media. No *Prochlorococcus* isolates were obtained from seawater enriched with NO_3^- media. *Prochlorococcus* cells from a single tube containing NO_2^- as the sole N source have been maintained on NO_3^- media for over 6 months. However, these are consortium cultures, and

growth of the *Prochlorococcus* cells may be due to cycling of NO_3^- by other cells in the cultures. Efforts to get these *Prochlorococcus* cells into a pure culture are underway in order to verify their apparent NO_3^- utilization and to determine phylogenetic relationship to other *Prochlorococcus* and *Synechococcus.* Examination of NO_3^- utilization genes in PAC1 and determination of the phylogenetic position of the potential NO_3^--utilizing *Prochlorococcus* strain from the Red Sea may resolve the evolutionary history of NO_3^- and NO_2^- utilization in the *Prochlorococcus* genus.

This study of *Prochlorococcus* and *Synechococcus* N utilization can be related to the current understanding of horizontal and vertical distributions of natural *Prochlorococcus* and *Synechococcus* populations. It has been observed that *Synechococcus,* but not *Prochlorococcus,* growth rates correlate with NO_3^- concentration (Partensky et al. 1999). Low-B/A *Prochlorococcus* ecotypes, which predominate in nutrient-depleted surface-mixed layers of stratified open ocean waters (West et al. 2001), depend on the input of recycled N sources, such as NH_4^+ and urea. Cell division measurements of natural populations of *Prochlorococcus* in NO_3^--depleted surface waters indicate that they grow at or near their maximal growth rates (Furnas and Crosbie 1999; Partensky et al. 1999), implying the rate of input of NH_4^+ and urea is not limiting growth. High-B/A *Prochlorococcus* ecotypes are generally restricted to the deep euphotic zone of stratified water columns (West et al. 2001) and, thus, can take advantage of the higher concentrations of NO_2^- often present at these depths (Olson 1981). Thus, not only do high-B/A and low-B/A *Prochlorococcus* partition the water column with respect to depth because of differences in their light utilization capabilities, but also because of differences in their N utilization capabilities. *Synechococcus,* which can utilize NO_3^- and NO_2^-, do not extend as deeply in the water column as *Prochlorococcus* (Partensky et al. 1999). This, too, has been explained in terms of their differences in light absorption and utilization (Morel et al. 1993; Moore et al. 1995; Moore and Chisholm 1999). However, it is further possible that *Synechococcus* are limited to surface waters because they cannot balance the high cellular N required for their N-rich phycobilisomes, since the low light levels in the deep euphotic zone may not provide enough energy to reduce NO_2^- to NH_4^+ (Carr and Mann 1994).

The ubiquity of *Prochlorococcus* in open ocean waters and the general inability of *Prochlorococcus* to use NO_3^- means that some *Prochlorococcus* populations would not contribute to ''new production,'' which is traditionally defined as primary production dependent on new N sources such as NO_3^-, NO_2^-, and N_2 (Dugdale and Goering 1967). Evidence in a variety of oceanic regimes indicates that natural populations of *Prochlorococcus* are not abundant in nitrate-rich, winter-mixed waters (Lindell and Post 1995; Liu et al. 1998; DuRand et al. 2001). Moreover, *Prochlorococcus* populations do not appear to bloom in response to seasonal or periodic NO_3^- inputs, whereas *Synechococcus* populations do (Glover et al. 1988; DuRand et al. 2001). Instead, *Prochlorococcus* populations bloom after thermal stratification has been established and NO_3^- input has been depleted (Olson et al. 1990; Lindell and Post 1995; DuRand et al. 2001). However, there is evidence that a measurable percentage of exported carbon can be attributed to *Prochlorococcus* (Goericke et al. 2000). Thus, *Prochlorococcus* is not only a part of the microbial loop in surface waters, but it also contributes to the export of carbon into deeper waters via the biological CO_2 pump driven by NH_4^+, urea, and NO_2^- as opposed to NO_3^-.

The observation that different ecotypes of *Prochlorococcus* and *Synechococcus* have different repertoires of N acquisition, which have clear genomic and ecological underpinnings, adds a new dimension to how we think about picophytoplankton population dynamics. N, in addition to light, plays a critical role in determining the dynamics between closely related ecotypes of these two genera, and contributes to the collective stability of these populations in the world's oceans.

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