Molecular phylogeny of *Prochlorococcus* ecotypes

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ABSTRACT

Prochlorococcus is the dominant phototroph in the temperate oceans. It is found throughout the euphotic zone, at light intensities spanning over four orders of magnitude. This growth range is larger than observed for any single culture of *Prochlorococcus* and is likely due to the co-existence of multiple ecotypes adapted for optimal growth at different light intensities. Here we report the 16S rDNA sequences of four *Prochlorococcus* isolates from the Pacific Ocean, MIT9201, MIT9202, MIT9211 and MIT9215, which have been physiologically characterized as high or low B/A ecotypes based on their chl b/a_2 ratios and light-dependent growth responses. The three low B/A isolates are closely related and cluster together with other low B/A isolates in a previously identified "high-light adapted" clade. The fourth Pacific isolate, a high B/A ecotype, is not a member of this clade. Sequences specific to each physiological type will be useful in probing field populations to determine the spatial and temporal distribution of the ecotypes of *Prochlorococcus*.

INTRODUCTION

The marine cyanobacterium *Prochlorococcus* (Chisholm *et al.*, 1988; Chisholm *et al.*, 1992) is abundant (often 10^5 cells ml^{-1}) and widespread in the tropical and subtropical regions of the open ocean. *Prochlorococcus* is

found throughout the euphotic zone and often comprises 40-60% of the total chlorophyll in the Pacific (Chavez *et al.*, 1991; Letelier *et al.*, 1993; Campbell *et al.*, 1994; Shimada *et al.*, 1995b), the north Atlantic (Goericke & Welschmeyer, 1993, Veldhuis *et al.*, 1993), and the Red Sea and Somalia Upwelling region (Veldhuis & Kraay 1993, Lindell & Post 1995).

We refer to *Prochlorococcus* as a cyanobacterium, despite its original description as a "prochlorophyte" (Chisholm *et al.*, 1988) – referring to oxygenic photosynthetic prokaryotes that possess chl *b* and lack phycobilins (Lewin, 1981). Molecular phylogenies constructed using both the 16S ribosomal RNA gene (16S rDNA) (Urbach *et al.*, 1992) and the gene for the large subunit of RNA polymerase (*rpoC1*) (Palenik & Haselkorn 1992) demonstrate that *Prochlorococcus* is most closely related to marine *Synechococcus,* and phylogenetically distinct from other prochlorophyte lineages. The close relationship between *Prochlorococcus* and *Synechococcus* is also apparent in phylogenies constructed using the *psbA* and *psbB* genes and portions of the *petB* and *petD* genes (Hess *et al.*, 1995; Urbach *et al.*, 1998), and in the possession of phycoerythrin genes by some strains of *Prochlorococcus* (Hess *et al.*, 1996; Ting *et al.*, 1998). Further, *Prochlorococcus* has a suite of pigments distinctly different from that of two other previously designated prochlorophytes *Prochloron* and *Prochlorothrix* (Chisholm *et al.*, 1992). It is now clear that the chl *b* phenotype has evolved independently at least four times (La Roche *et al.*, 1996) and that these organisms, along with traditional cyanobacteria, should probably be reclassified as Oxyphotobacteria (Pinevich *et al.*, 1997). Although phylogenetic analyses using the gene for the large subunit of Rubisco (*rbcL*) place one isolate of *Prochlorococcus* with the gamma proteobacteria (Shimada *et al.*, 1995a), this likely reflects a horizontal gene transfer event, and not the true organismal phylogeny (Delwiche & Palmer, 1996). Two other *Prochlorococcus* isolates possess *rbcL* sequences which branch within the cyanobacterial clade (Pichard *et al.*, 1997) and one strain of *Synechococcus* has a proteobacterial-like *rbcL* sequence as well (Watson & Tabita, 1996).

RESULTS

Using flow cytometry to study picoplankton in the field, we and others have observed distinct co-occurring populations of *Prochlorococcus*, based on their different mean chlorophyll fluorescence intensities (Campbell & Vaulot, 1993; Veldhuis & Kraay, 1993; McManus & Dawson, 1994; Partensky *et al.*, 1996). Although these populations could have been derived from the mixing together of genetically identical *Prochlorococcus* cells acclimated to different past light and/or nutrient regimes, several lines of evidence suggested they were physiologically and genetically distinct populations. The latter interpretation was supported by physiological experiments defining two different types of *Prochlorococcus* (Partensky *et al.*, 1993; Moore *et al.*, 1995). A comparative study of two isolates, the type strain SS120 (CCMP1375) and MED4 (CCMP1378), demonstrated that saturating growth irradiances are significantly lower for SS120 than for MED4: SS120 is photoinhibited above 140 $\mu E\ m^{-2}\ s^{-1}$, and ratios of chlorophyll *b* to divinyl chlorophyll *a* (chl a_2

(Goericke & Repeta, 1993)) are almost an order of magnitude higher in SS120 over all irradiances measured (Moore *et al.*, 1995). Consistent with these data, biochemical characterization of the two strains indicates that the pigment complexes of SS120 are bigger and more abundant at low light, and bind seven times more chl *b* than those of MED4 (Partensky *et al.*, 1997).

The coexistence of physiologically and genetically distinct types of *Prochlorococcus* was definitively shown recently when we flow-cytometrically sorted and cultured the high and low fluorescence cells from coexisting *Prochlorococcus* populations in two locations of the North Atlantic Ocean (Moore *et al.*, 1998). At low irradiances the high fluorescence isolates, MIT9303 and MIT9313, have relatively high growth rates and a high chl b/a_2 ratio, similar to SS120, whereas the low fluorescence isolates, MIT9302 and MIT9312, have a lower growth rate and a low chl b/a_2 ratio, similar to MED4 (Moore *et al.*, 1998).

The physiology of these six isolates and four isolates from the Pacific Ocean (MIT9201, MIT9202, MIT9211, MIT9215) suggests the genus *Prochlorococcus* can be divided into at least two ecotypes based on pigment content and light-dependent physiological response (Moore & Chisholm, 1999). Pigment content has also been used to distinguish isolates of *Synechococcus* (*e.g.* high and low PUB types; see Waterbury *et al.*, 1986). The distinction between the two groups of *Prochlorococcus* is best defined using their chl b/a_2 ratios (Fig. 1) since at any particular irradiance there is no overlap in this parameter.

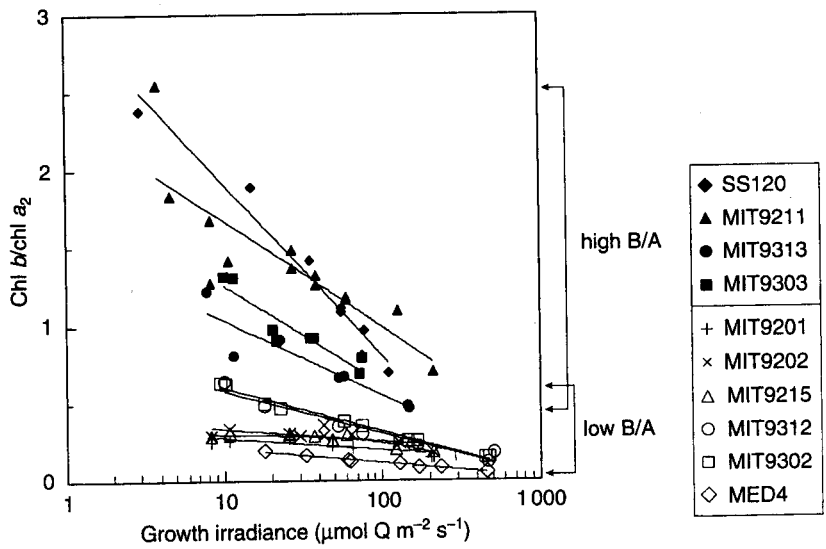

Figure 1. Chlorophyll b/a_2 ratio of different *Prochlorococcus* isolates grown over a range of light intensities (for isolation information see Moore & Chisholm, 1999). The isolates can be grouped loosely into two clusters, those with low chl b/a_2 ratios (low B/A ecotype) and those with high b/a_2 ratios (high B/A ecotype). The high B/A ecotype appears to have two sub-groups within it. Data from Moore & Chisholm, 1999.

One group of *Prochlorococcus* isolates, the low B/A ecotype (Moore & Chisholm, 1999), cluster with chl b/a_2 ratios below 0.7 while the other four isolates, the high B/A ecotype, have higher chl b/a_2 ratios, but exhibit more variability between them (Fig. 1). High B/A isolates are also characterized by their higher chlorophyll a_2-specific light harvesting efficiencies and spectrally-weighted average absorption coefficients. High B/A isolates also show decreases in growth rate due to high light exposure at light levels where the low B/A isolates grow maximally. Further, a secondary division can be made within the high B/A isolates: when grown under high light MIT9303 and MIT9313 have higher cell-specific light harvesting efficiencies and maximum photosynthetic rates than SS120 and MIT9211 (Moore & Chisholm, 1999).

Genetic analysis of *Prochlorococcus* isolates indicates that there is a correlation between photophysiology and phylogeny. Characterization of ten isolates by RFLP mapping (using probes to photosynthetic genes) groups them into two clusters, one containing the high B/A isolate SS120 and the other containing the low B/A isolate MED4 (Scanlan *et al.*, 1996). Analysis of 16S rDNA sequences from these isolates (Urbach *et al.*, 1998) reveals a well supported group, deemed the "high-light adapted clade" because it includes the low B/A MED4 and two Pacific isolates, SB and GP2, which have low chl b/a_2 ratios (Shimada *et al.*, 1996). Support for this clade is further strengthened by analyses demonstrating that the low B/A isolates MIT9302 and MIT9312 (each from a coexisting pair of isolates) are also members of this clade (Moore *et al.*, 1998).

The 16S rDNA sequences of four *Prochlorococcus* isolates from the Pacific Ocean further support the correlation of photophysiology with phylogeny. On the basis of their photophysiology three of these isolates (MIT9201, MIT9202 and MIT9215) belong to the low B/A ecotype, while the fourth (MIT9211) is characterized as high B/A (Moore & Chisholm 1999). The three low B/A isolates are more than 99% similar to each other in their 16S rDNA sequence and cluster within the "high-light adapted clade" (Fig. 2). One isolate, MIT9202, has a sequence identical to those of two other isolates, TATL2, from the Tropical Atlantic, and SB from the West Pacific, corroborating previous results demonstrating that *Prochlorococcus* from different oceanic regimes can be genetically similar (Urbach *et al.*, 1998). Although all of the isolates which cluster in the low B/A clade are closely related (at least 98.8% similar to one another), there is a well supported subdivision within this clade. One of these subgroups contains MED4, which has the lowest chl b/a_2 ratio of all the isolates we have characterized (Fig. 1), although no further physiological distinction between the two subgroups is apparent.

Congruent with the larger degree of physiological variability among isolates of the high B/A ecotype, they also exhibit greater genetic variability than the low B/A isolates. The 16S rDNA sequence similarities of MIT9211 with SS120, MIT9313 and MIT9303 are 98.6%, 98.2% and 97.8% respectively. These similarities are lower than those between the low B/A isolates, and are only slightly greater than the similarities between MIT9211 and *Synechococcus* WH8103 (97.4%). The high B/A isolates MIT9303 and MIT9313 share even higher sequence similarities to *Synechococcus* (Moore *et al.*,

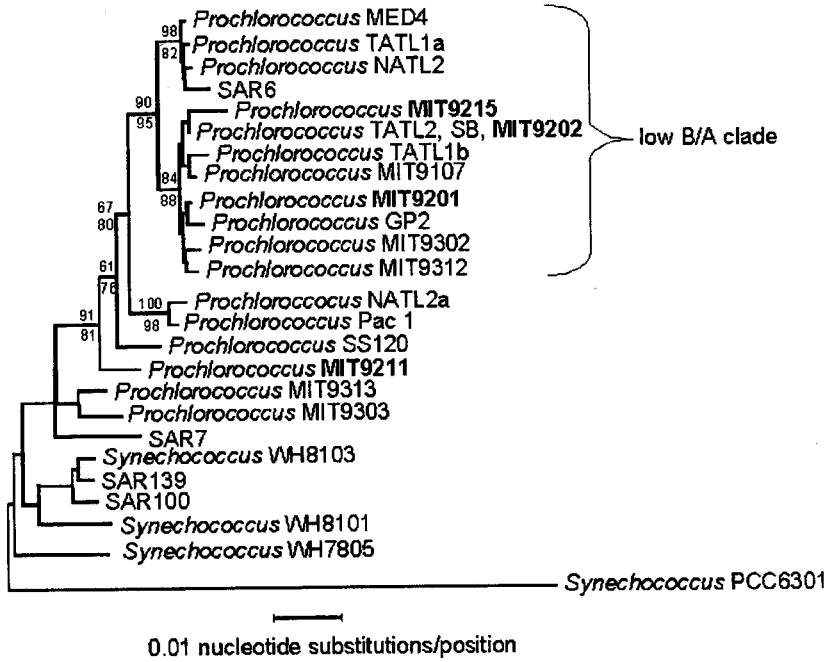

Figure 2. Phylogenetic relationships of *Prochlorococcus* and *Synechococcus* isolates and environmental sequences (SAR) from the Sargasso Sea (Giovannoni *et al.*, 1990) inferred from 16S rRNA gene sequences. Sequences of Pacific isolates MIT9201, MIT9202, MIT9211 and MIT9215 (in bold) were determined using previously described methods (Moore *et al.*, 1998). All analyses (tree construction and similarity values cited in text) employed 1070 unambiguously determined and aligned bases of the 16S rDNA. The phylogenetic framework was inferred by a distance analysis, using minimum evolution as the objective function. Bootstrap values from distance and parsimony analyses (above and below the branches, respectively) are presented out of 100 resampled datasets; values below 50 are not shown. The freshwater cyanobacterium *Synechococcus* PCC6301 was used to root the tree.

1998). The high B/A isolates do not cluster together in a single clade but are on separate, basal branches of the tree (Fig. 2), consistent with the possible subdivisions within this ecotype suggested by their photophysiology.

CONCLUSION

Future directions include further consideration of the taxonomic status of the ecotypes we have described. It is possible that the high and low B/A ecotypes represent different species of *Prochlorococcus*. However, a definitive determination will require either sequence data from a more variable locus to provide more resolution between the high B/A isolates and marine *Synecho-*

coccus, or axenic cultures of each ecotype in order to perform other taxonomic analyses such as DNA-DNA hybridizations and determinations of % GC content and fatty acid composition (Wayne *et al.*, 1987; Priest & Austin, 1993; Stackebrandt & Goebel, 1994; Tamaoka, 1994). At present we adopt the convention used for marine *Synechococcus* (Waterbury *et al.*, 1986) and designate our isolates simply as strains of the genus *Prochlorococcus*. Although we have definitively described two ecotypes, we consider this a minimum number. As described above, there are undoubtedly subdivisions within both the high and low B/A ecotypes. Future investigations will likely reveal additional ecotypes not currently represented in culture and/or further ecotypic differentiation based on susceptibility to cyanophages, nutrient utilization efficiencies, or trace metal toxicity (Mann *et al.*, 1997).

A second direction we are actively pursuing is determining the distributions of the two ecotypes in the field. It has been suggested that low-light adapted, high chl b/a_2 *Prochlorococcus* predominate in the deeper portion of the euphotic zone and that high-light adapted, low chl b/a_2 *Prochlorococcus* predominate in surface waters (Campbell & Vaulot, 1993; Goericke & Repeta, 1993; Moore *et al.*, 1995). Analyses of natural populations, by examining sequences from environmental clone libraries (Ferris & Palenik 1998) and using molecular probes designed to take advantage of the correlation of physiology with phylogeny (N. West & D. Scanlan, pers. comm.), are consistent with this hypothesized distribution. Repeated observations of multiple phylotypes in single water samples (Palenik, 1994; Ferris & Palenik, 1998; Urbach & Chisholm, 1998) indicate that further work is needed to determine how the ecotypes are distributed under different environmental conditions. It is clear that the distribution of multiple *Prochlorococcus* ecotypes in the same water column would result in greater integrated production than could be achieved by a single ecotype (Moore *et al.*, 1998). The existence of multiple ecotypes allows for survival over a broader range of conditions than could be achieved by a physiologically and genetically homogenous population and undoubtedly contributes to the dominance of *Prochlorococcus* in the world's oceans.

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