

Prochlorococcus* molecular ecology: the potential to assess genotype, phenotype and productivity *in situ

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ABSTRACT

The unicellular picophytoplankton *Prochlorococcus* is the most abundant photosynthetic organism in oligotrophic oceans between the latitudes 40°N and 40°S. It uniquely possesses divinyl derivatives of chlorophyll a and b but lacks phycobilisomes, though some strains contain trace amounts of phycoerythrin. In order to understand how this organism responds to, and is influenced by, changes in its environment *in situ*, i.e. the role of external gradients of light and nutrients, we have begun to analyse both the genetic diversity and phosphorus (P) status of natural populations. Two physiologically and genetically distinct *Prochlorococcus* ecotypes have already been identified in cultured isolates [1] with the phylogenetic positioning of strains within each ecotype correlating with depth of isolation rather than geographic origin [2, 3]. During recent cruises in the North Atlantic, depth series of DNA and protein samples were collected and the distribution of the major ecotypes determined. Genetic diversity was assessed using the 16S rRNA and the photosynthetic *psbA* genes, whilst antibodies raised against a protein biomarker, PstS [4], allowed determination of P status. Analysis of genotypic differences down the water column was examined both by dot blot hybridisation analysis of PCR products and by restriction fragment length polymorphism (RFLP) analysis. At one location, (37°N 20°W) during summer 1996, an obvious stratification of the major ecotypes was observed. One group dominated at the surface, above 60 m (high-light adapted clade), and the other dominated at depth, below 60 m (low-light adapted clade). Thus, external gradients of light intensity (or quality) and/or nutrients clearly correlate with different genetically defined groups of *Prochlorococcus* *in situ*. Further work is being performed to assess the relative abundance and distribution of individual lineages in this and another water column (31°N 64°W), whilst development of a quantitative assay of PstS expression is being realised in order to determine more accurately the nutrient status of specific *Prochlorococcus* populations.

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3. Scanlan et al., (1996). *Eur. J. Phycol.* 31, 1-9.
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Introduction

Prochlorococcus cyanobacteria, photosynthetic prokaryotes containing divinyl chlorophylls a and b, occur as dominant primary producers throughout oligotrophic oceanic waters spanning roughly the latitudes 40° N and 40° S (for a recent review see [7]). In order to

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measure accurately the total depth integrated productivity of such picophytoplankton in situ over this broad spatial area we need to consider the potential for individual genotypes to contribute differently to this total and how this is affected by external factors e.g. gross water column structure. This underlies a need to accurately enumerate those individual genotypes adapted to specific niches in the water column, and to assess their capacity for CO₂ fixation under a range of light and nutrient regimes. Although much information has been gained from cultured isolates there is still a need to assess genotype, nutrient status and productivity in situ. The use of oligonucleotide probes targeted to ribosomal RNAs (rRNAs) and antibody probes targeted to components of the nutrient acquisition machinery represent potential tools to assess genotype and physiological state respectively, in situ. We present here further evidence for the differential distribution of major *Prochlorococcus* genotypes in situ. We also assess the use of these molecular probes with regard to productivity measurements and suggest ways they might be used in answering questions of how individual genotypes invade and occupy a specific niche in the water column.

Genetic and Physiological Heterogeneity in cultured *Prochlorococcus* isolates

Recent studies have demonstrated a relatively high genetic heterogeneity among cultured *Prochlorococcus* strains [12], as has also been found for marine *Synechococcus* isolates [13]. Thus, using a 612-bp *rpoC1* gene fragment (encoding the DNA-dependent RNA polymerase, *rpoC1*) *Prochlorococcus* isolates CCMP1375 and CCMP1378 are between 23-24% dissimilar, whilst *Synechococcus* isolates CC9311 and WH8103 are 19% dissimilar. (At the level of the 16S rRNA gene, though, sequences are only around 3% dissimilar, comparing *Prochlorococcus* isolates NATL2 and MIT9303, and marine *Synechococcus* isolates WH8101 and WH7805 [15], see Fig. 1. Nonetheless, this genetic heterogeneity is now being realised in physiological heterogeneity of *Prochlorococcus* isolates, particularly between strains isolated from surface versus deep waters [6,9,8]. Thus, isolates with high red fluorescence typically contain a high divinyl chlorophyll b to a ratio (chl b₂/a₂) and are adapted to grow and photosynthesise at low light (but higher nutrient?) levels, compared to the dimly fluorescent surface populations. The high chl b₂/a₂ ratio of strains MIT9303 and MIT9313 grown at low irradiance, causes a two-fold increase in chl a₂ - specific photosynthetic efficiency and gives rise to higher maximum quantum yields relative to two surface isolates [5]. Table 1 summarises some of the physiological properties of strains SS120 and MED4 (CCMP1375 and CCMP1378 in Fig. 1), which represent low- and high-light adapted isolates respectively.

***Prochlorococcus* microdiversity in situ**

Prochlorococcus strains adapted to different light environments (and/or nutrient conditions and/or physical properties of the water column) may represent ecotypes of the genus whose distribution in nature mirrors their apparent adaptation to different environments. This is supported by flow cytometric analysis of field samples, where vertical profiles have revealed bimodal red fluorescence distributions suggestive of the presence of multiple *Prochlorococcus* populations [1,5,17]. The study by Moore et al., 1998 [5] is significant since they found that distinct physiological differences were observed between isolates from

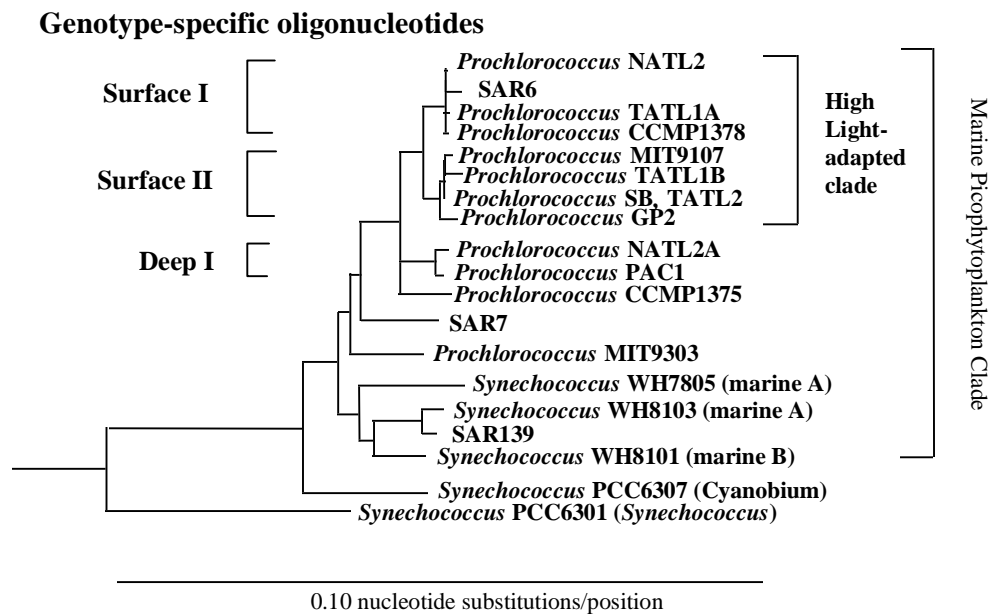
Table 1. Comparison of physiological properties of *Prochlorococcus* strains MED4 and SS120.

	MED4	SS120
chl _b /a ₂ (w/w) ratio	low (0.05 -0.15)	high (0.4 -2.4)
optimal growth irradiance	15-80 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ *	8-30 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ **
major antenna apoproteins	~ 32.5 kDa	34-28 kDa
phycoerythrin	absent	present

* photoinhibited only around 450 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

** photoinhibited at light intensities greater than 37 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

Data in this table derives from [3,6,8].

**Fig. 1.** Phylogenetic relationships among cultured *Prochlorococcus* and *Synechococcus* isolates. Modified from [15].

sorted high- and low- fluorescence populations, and corresponded with only minor 16S rRNA sequence diversity. Future purification of these isolates would provide valuable material for analysis of nutrient acquisition machinery, uptake kinetics and assimilation properties. This is important since nutrient concentration and possibly composition, together with light intensity and quality, are the major environmental parameters with strong depth related gradients - and hence providing strong selective pressure (see [19]). Although isolation of clonal cultures is paramount for analysis of physiological properties, one criticism of culture related studies is that it may select isolates that are not the dominant clones in situ. In order to directly enumerate and 'characterise' natural *Prochlorococcus* populations we require techniques that are ultimately non-selective or manipulative, whilst at the same time providing information that complements these culture studies. We see as key to this problem the development of techniques that can rapidly and accurately evaluate how an individual *Prochlorococcus* cell, potentially of genotype x, is perceiving its

environment (since even among genetically identical bacterial populations there appear to be different responses to environmental parameters - see [14] and references therein), and how this might be reflected in the capacity of that cell to fix CO₂. Although we give the specific example of carbon fixation here, potentially other biochemical processes can be 'assayed', and given the tremendous biogeochemical role played by the heterotrophic component of the picoplankton, simple radioisotope based uptake coupled with FISH and flow cytometry deserves more attention in this respect.

Molecular tools, biological traffic lights, and *Prochlorococcus* molecular ecology

The approaches we are taking to addressing questions of *Prochlorococcus* community structure and nutrient status in situ, are based largely on molecular techniques (see Fig. 2).

Although these methods may have problems relating to PCR bias or antibody specificity, they have the potential to truly interrogate individual picophytoplankton cells in field samples, and thus they present realistic alternatives to methods which infer the physiological status of cells in nature solely from culture-based studies. In the none too distant future we envisage the potential of flow cytometry coupled with i) fluorescent in situ hybridisation (FISH) using genotype-specific oligonucleotides ii) antibody based indicators of nutrient status, and iii) NaH¹⁴CO₃ incubation of in situ samples to give us a much more complete picture of the molecular ecology of this group of organisms. To some extent several of these tools are already available or have been previously used, though not in tandem. Thus, Li [4] examined primary production of *Prochlorococcus*, *Synechococcus* and picoeukaryotes using ship-board flow cytometric sorting of ¹⁴C-labelled cells, whilst

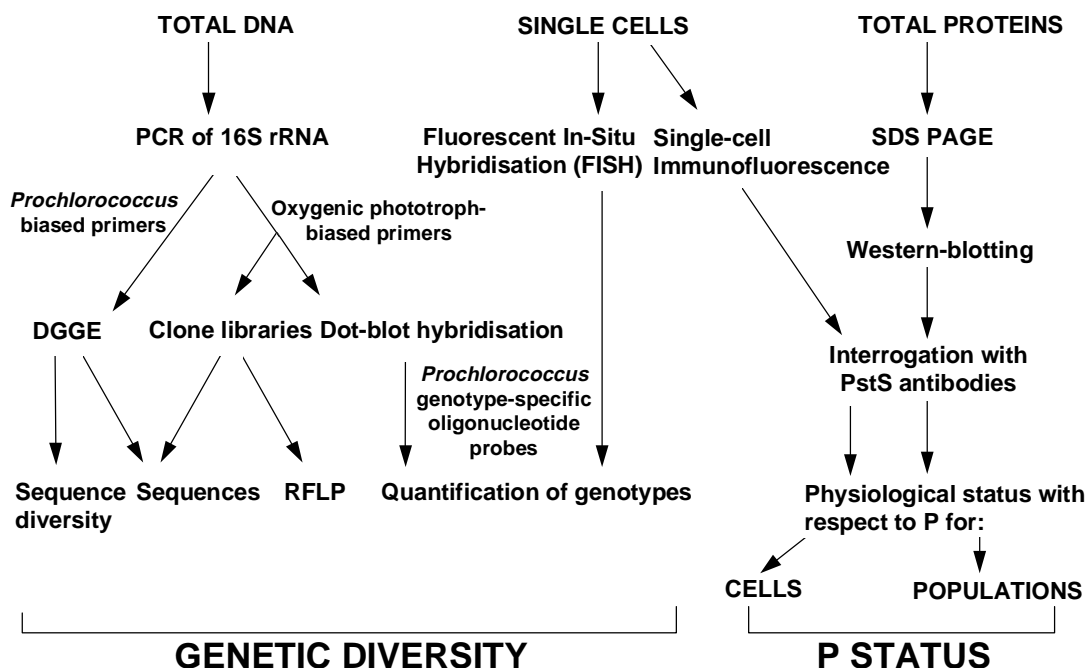


Fig. 2. Molecular ecological approaches to assessing the genetic diversity and nutrient status, with specific respect to P, in natural picophytoplankton populations.

Worden et al., this symposium [20], have begun to use peptide nucleic acid (PNA) probes to target individual *Prochlorococcus* genotypes. We ourselves have developed an immunological assay based on the expression of PstS, encoding a component of the high affinity phosphate acquisition machinery, to assess the P status of individual *Prochlorococcus* and *Synechococcus* cells [11]. Future development of this technology for use with flow cytometry will potentially allow us to have a kind of 'biological traffic lights' for the nutrient status of individual cells, where we might envisage 'red' to indicate the nutrient-starved state, 'amber' - cells which are nutrient stressed, or 'green' those which are nutrient replete. That this can be coupled to primary production measurements of individual genotypes will allow us to directly integrate aspects of C and P or N metabolism.

***Prochlorococcus* community structure and P status in the eastern North Atlantic**

We have recently begun to use the approaches outlined in Fig. 2 to characterise the community structure of a natural picophytoplankton community in situ [18]. DNA samples were extracted from a depth profile at 37°N 20°W during a NERC PRIME cruise in July 1996. At this station *Prochlorococcus* represented between 93-97% of the total picoplankton (see Fig. 3). 16S rRNA gene sequences were retrieved by amplification of total DNA with the PCR, using primers biased for oxygenic phototrophs [16]. Oligonucleotide probes targeting surface or deep populations (presumably high- or low-light adapted [HL or LL] respectively, see Fig. 1) were then used in dot blot hybridisation experiments to determine the relative contribution of specific genotypes at each depth (Fig. 4). The two probes, surface I, which specifically recognises sequences from the HL-adapted *Prochlorococcus* cluster comprising isolates NATL2, SAR6, TATL1A, and CCMP1378, and Deep I, recognising the LL-adapted *Prochlorococcus* strains NATL2A and ENATL1, showed an obvious differential hybridization down the water column. Thus, there was a distinct population shift between Surface I genotypes predominating in the upper 50 m and Deep I genotypes predominating below 60 m.

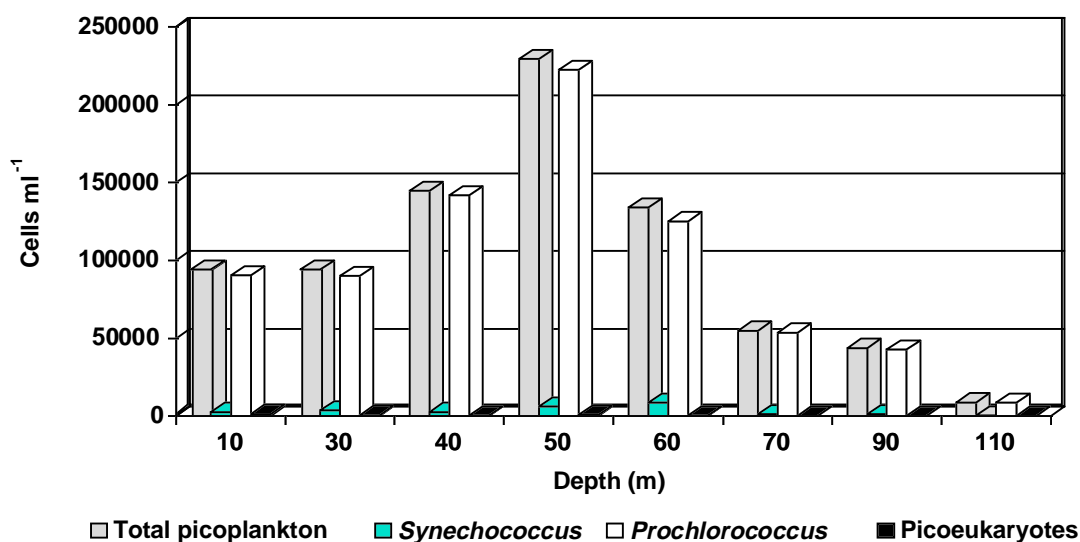


Fig. 3. Flow cytometric analysis of phytoplankton in a water column depth profile in the eastern North Atlantic at 37°N 20°W.

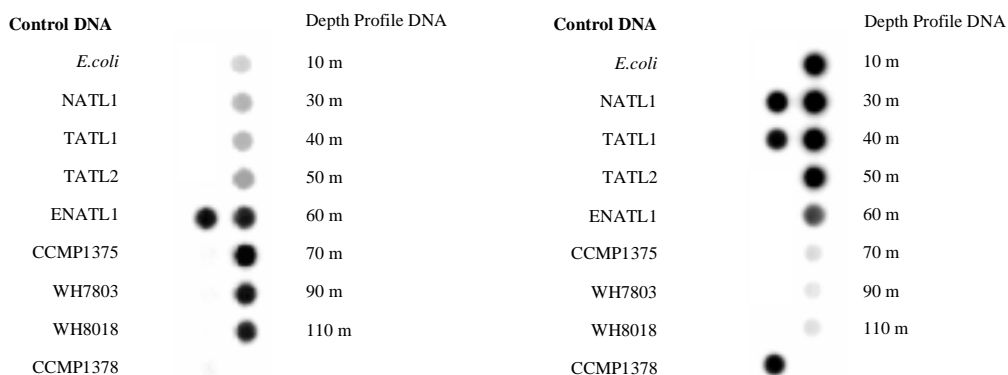
Deep I *Prochlorococcus*-specificSurface I *Prochlorococcus*-specific

Fig. 4. Vertical distribution of *Prochlorococcus* genotypes down a water column as revealed by dot blot hybridisation. The Deep I specific oligonucleotide as well as specifically recognising NATL2 and PAC1 (see Fig.1), also recognises genotype ENATL1 (retrieved from 110 m in this water column) and was used as the LL-genotype control.

Around 50-60 m in this water column seems to correlate with the transition depth at which both populations co-exist. PstS expression was apparent only in the upper 50 m but not at 80 m (West and Scanlan unpublished), a noteworthy observation which correlates well with nutrient profiles in the water column and known concentrations of P which induce PstS expression. Thus we can now begin to address the dynamics of *Prochlorococcus* community structure and nutrient status not only on a spatial scale but also temporally in situ (both in annual and diel cycles). This will allow a much more thorough examination of the environmental parameters in situ which control the relative distribution of specific genotypes and ultimately productivity. Moreover, by studying water columns of varying hydrography there is the potential to determine how such genotypes invade and ultimately successfully dominate a particular niche as the water column changes or stratifies. This observation of a transition in genotype as the gradients of light intensity and nutrient concentration change inversely down a water column is paralleled in observations of the vertical distribution of thermophilic *Synechococcus* populations in a hot spring microbial mat [10]. A further example of niche adaptation, this time in a non-photosynthetic bacterium, the gamma Proteobacterium *Achromatium* sp. [2], suggests that microdiversity among heterotrophic bacterial populations might also equate with significant differences in physiological properties.

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