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Marine Cyanobacteria

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Contents

Cyanobacterial diversity in marine ecosystems as seen by RNA polymerase (*rpoC1*) gene sequences

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Cyanobacteria comprise one of the major eubacterial lineages. The diversity within this lineage, including both that of morphology (singles cells, branching filaments, akinetes, etc.) and physiology (nitrogen fixation, heterotrophy, motility, etc.) has fascinated microbiologists. Cyanobacteria have also provided model systems for understanding those processes that occur more broadly than the lineage itself such as photosynthesis, nitrogen fixation, pattern development, and circadian rhythms (Bryant, 1994).

Cyanobacteriologists have been greatly interested in the relationships between morphology or unique physiological processes and evolution. What characteristics are related to the evolution of specific groups within the cyanobacterial lineage? For example true branching seems to have evolved once and is characteristic of only one lineage, the Group V cyanobacteria (Rippka *et al.*, 1979; Giovannoni *et al.*, 1988) although this idea has been more recently questioned based on its low bootstrap values in 16S rRNA data (Wilmotte, 1994). In contrast, light harvesting by chlorophyll *b* (in cyanobacteria referred to as prochlorophytes) seems to have arisen multiple times within the cyanobacteria (Urbach *et al.*, 1992; Palenik & Haselkorn, 1992).

These relationships between morphology and physiological characteristics and the evolution of various groups has been investigated using a number of phylogenetic tools, some based on protein sequences, some on 16S rRNA. Reviews of these approaches can be found in Packer & Glazer (1988); Wilmotte (1994). We have been pursuing these questions using as another

tool, DNA sequences of a fragment (*rpoC1*) of the protein DNA-dependent RNA polymerase (Palenik & Haselkorn, 1992; Palenik, 1992; Palenik & Swift, 1996). Cyanobacterial specific primers allow cyanobacterial *rpoC1* sequences to be per amplified from DNA obtained from water samples, cyanobacterial symbiont (tunicate) hosts, and nonaxenic cultures.

Environmental variables such as light, temperature, and nutrients seem to be influencing cyanobacterial genetic diversity in marine ecosystems, but to an extent that is still poorly characterized. This influence is felt at different levels. For example, the presence of nitrogen fixing groups of cyanobacteria is likely to be related to the concentration of dissolved nitrogen sources or N:P nutrient ratios (Karl *et al.*, 1997). More subtle are the changes in the presence of different groups within what we think of as the dominant abundant lineages. We are just beginning to see that there are "ecotypes" or species within the genera *"Synechococcus"* and *"Prochlorococcus"* and these "ecotypes" are associated with particular ecological niches.

In the oligotrophic ocean environment covering much of the globe, large changes in some environmental parameters occur with depth. For example in the oligotrophic regions of the California Current (Eppley, 1986), light flux drops by 3 orders of magnitude from the surface to the bottom of the cyanobacterial growth range (0-150 meters), and large shifts in light quality occur. Nutrient gradients are also large with undetectable nitrate through much of the upper water column up to a few micromolar concentration at depth. Temperature gradients are smaller with the range in the California Current from around 20°C at the surface to about 13°C at the bottom of the euphotic zone. This picture is then complicated by a seasonal mixing cycle in many environments including the California current. During the winter, the water column is mixed through the euphotic zone, while during summer and fall the water column is generally stratified. During stratification, different parts of the cyanobacterial population are presumably under very different selection pressures with high light, low nutrients at the surface and lower, blue light and higher nitrate conditions at depth. We would expect the most genetic differentiation in the cyanobacterial population thus between surface and deep samples at this time. However, since seasonal mixing changes these selective pressures so drastically it is possible that they do not exist long enough for specific "high" or "low" light cyanobacterial species or "ecotypes" to have developed. Sequence data from cyanobacterial DNA-dependent RNA polymerase (*rpoC1*) genes obtained from marine isolates and from bulk seawater DNA samples can be used to describe and understand cyanobacterial diversity as a function of depth and geographical region in order to answer such questions.

Recently, a total of 15 cyanobacterial strains were isolated from the oligotrophic edge of the California Current from two depths (5 and 95 m) (Toledo & Palenik, 1997). RNA polymerase (*rpoC1*) gene sequences of the strains revealed two major genetic lineages, distinct from other marine or freshwater cyanobacterial isolates, or groups seen in shotgun cloned sequences from the oligotrophic Atlantic Ocean. One group, the California Current low phycourobilin group represented by 6 isolates in a single lineage (CC9311) was less diverse than the California Current high phycourobilin group with 9 isolates in three relatively divergent lineages (represented by CC9317, CC9318, and CC9305-3). The former group was found to be the closest known genetic group to *Prochlorococcus*, the chlorophyll *b* containing marine cyanobacterial group. Both groups included strains obtained from surface (5 m) and deep (95 m) samples, thus there was no clear correlation between sampling depth and isolation of genetic groups.

Further work on *rpoC1* gene sequences PCR amplified from DNA samples from the same region clearly show the presence of the "low phycourobilin group", but less clearly the other ("high phycourobilin") group in surface seawater samples (Ferris & Palenik, manuscript in prep.). There is a cluster of DNA library strains that appear related to the high PUB group (clones G 11, 12, 23, 40, 96, 105 in Fig. 1), but we are still unclear on what constitutes a definable clade in these organisms. These results may be because the two approaches of isolation and environmental library preparation are providing different information about these groups, with the later likely to be more related to abundance than the former. *Synechococcus* clades may have some

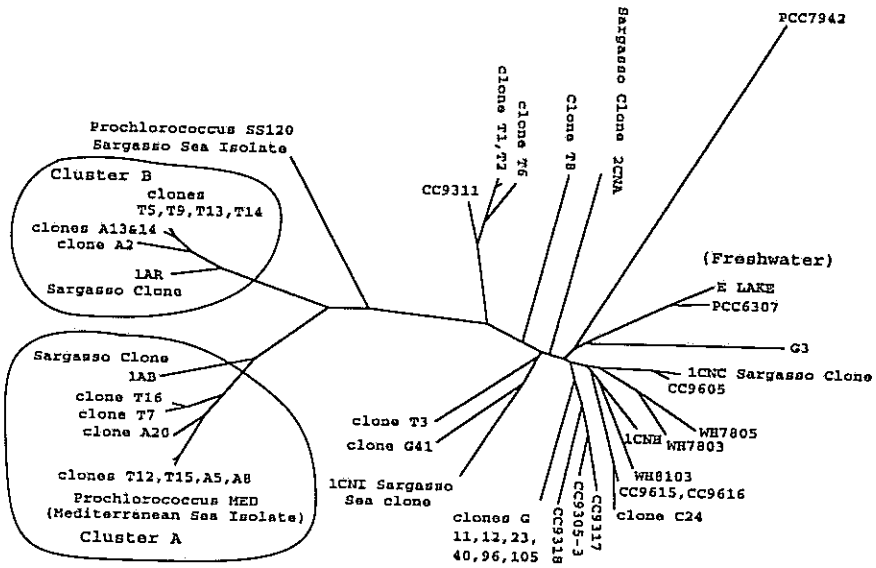

Figure 1. An *rpoC1* DNA fragment library from a sample from the oligotrophic California Current (station 77.100, 1996, 3 meters). Also included are California Current isolates (CC), Woods Hole Culture Collection(WH) and other isolates and Sargasso Sea DNA clones (1AR, 1AB, 1CNC, 2CNA, 1CNH, 1CNI). The sequence data (270 bp) were analyzed using distance matrix (Jukes-Cantor) and the neighbor-joining methods using the program PHYLIP (Felsenstein, U. Washington). The data show at least two *Prochlorococcus* (cluster A and B) and five *Synechococcus* groups. Subsequent libraries have shown that the 1AB cluster is typically surface associated while the 1AR group is more typically found deeper in the water column.

strains that are abundant (clones G 11, 12, 23, 40, 96, 105, for example) while other strains are in low abundance in surface waters (CC9317, CC9318, CC9305-3, for example) but easily amenable to isolation. Alternatively at one site there may be changes in strain abundance within a clade at different times due to viral selection, grazing, and random drift.

New *Synechococcus* clades are also being uncovered in addition to the two described above. These are represented by CC9616 and CC9605 in Fig. 1.

Sequence data from DNA samples also clearly shows the presence of different clusters of *Prochlorococcus* distributed in different parts of the water column. A surface cluster corresponding to the high light *Prochlorococcus* clade was described physiologically by Partensky *et al.* (1993) and Moore *et al.* (1995) and genetically by Scanlan *et al.* (1996) and Urbach *et al.* (1998), and further in this book. In our data it is seen as a large number of clones that cluster with a Mediterranean isolate (MED strain) that was demonstrated to be a member of this cluster. We propose referring to this group as *Prochlorococcus* cluster A, with potential subdivisions of this cluster being referred to as A-1, A-2, etc. An apparent deep *Prochlorococcus* cluster is also present, and we propose to refer to these as *Prochlorococcus* cluster B. This terminology is consistent with our previous work showing several clusters of *Prochlorococcus* in the Sargasso Sea (Palenik, 1994). This Sargasso sample was from 60m and not surprisingly appears to have contained both high and low (or A and B cluster) clones at least based on homologies to clusters seen in the California Current.

Interestingly, there is a hint in our sequence data and that of others cited above that the low light adapted strains of *Prochlorococcus* branch more deeply within the *Prochlorococcus* cluster while surface strains appear to have diverged more recently. Is it possible that *Prochlorococcus* first evolved to take advantage of the low light region of the euphotic zone, because of its superior ability to harvest blue light, and subsequently diverged to compete with *Synechococcus* for the high light regimes, because of its ability to make a light harvesting apparatus using less nitrogen? Such a speculation is still only weakly supported, and is one of the many questions remaining to be answered in the study of cyanobacterial ecology and evolution.

REFERENCES

BRYANT D.A., 1994. — *The molecular biology of cyanobacteria.* — Kluwer Academic Publishers, Dordrecht.

EPPLEY R.W., 1986. — *Plankton Dynamics of the Southern California Bight.* — Springer, New York.

GIOVANNONI S.J., TURNER S., OLSEN G.J., BARNS S., LANE D.J., PACE N.R., 1988. — Evolutionary relationships among cyanobacteria and green chloroplasts. — *J. Bacteriol.*, **170**, 3584-3592.

KARL D., LETELIER R., TUPAS L., DORE J., CHRISTIAN J., HEBEL D., 1997. — The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. — *Nature*, **388**, 533-538.

MOORE L.R., GOERICKE R., CHISHOLM S.W., 1995. — Comparative physiology of *Synechococcus* and *Prochlorococcus* -influence of light and temperature on growth, pigments, fluorescence and absorptive properties. — *Mar. Ecol. Prog. Ser.*, **116**, 259-275.

PACKER L., GLAZER A.N., 1988. — *Cyanobacteria.* — Academic Press, San Diego.

PALENIK B., HASELKORN R., 1992. — Multiple evolutionary origins of prochlorophytes, the chlorophyll b-containing prokaryotes. — *Nature*, **355**, 265-267.

PALENIK B., 1992. — Polymerase evolution and organism evolution. — *Current Opinion in Genetics and Development*, **2**, 931-936.

PALENIK B., 1994. — Cyanobacterial community structure as seen from RNA polymerase gene sequence analysis. — *Appl. Environ. Microbiol.*, **60**, 3212-3219.

PALENIK B., SWIFT H., 1996. — Cyanobacterial evolution and prochlorophyte diversity as seen in DNA-dependent RNA polymerase gene sequences. — *J. Phycol.*, **32**, 638-646.

PARTENSKY F., HOEPFFNER N., LI W.K.W., ULLOA O., VAULOT D., 1993. — Photoacclimation of *Prochlorococcus* sp (Prochlorophyta) strains isolated from the North Atlantic and the Mediterranean Sea. — *Plant Physiology*, **101**, 285-296.

RIPPKA R., DERUELLES J., WATERBURY J.B., HERDMAN M., STANIER R.Y., 1979. — Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. — *J. Gen. Microbiol.*, **111**, 1-61.

SCANLAN D.J., HESS W.R., PARTENSKY F.P., NEWMAN J., VAULOT D., 1996. — High degree of genetic variation in *Prochlorococcus* (Prochlorophyta) revealed by RFLP analysis. — *Eur. J. Phycol.*, **31**, 1-9.

TOLEDO G., PALENIK B., 1997. — *Synechococcus* diversity in the California Current as seen by RNA polymerase (rpoC1) gene sequences of isolated strains. — *Appl. Environ. Microbiol.*, **63**, 4298-4303.

URBACH E., ROBERTSON D.L., CHISHOLM S.W., 1992. — Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. — *Nature*, **355**, 267-270.

URBACH E., SCANLAN D.J., DISTEL D.L., WATERBURY J.B., CHISHOLM S.W., 1998. — Rapid diversification of marine picophytoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria). — *J. Mol. Evol.*, **46**, 188-201.

WILMOTTE A., 1994. — Molecular Evolution and Taxonomy of the Cyanobacteria. — *In:* Bryant D.A. (ed.), *The molecular biology of the cyanobacteria.* Kluwer Academic, Dordrecht, 1-25.