*VIE MILIEU*, 1997, 47 (4) : 367-374

# RECENT ADVANCES IN THE USE OF MOLECULAR TECHNIQUES TO ASSESS THE GENETIC DIVERSITY OF MARINE PHOTOSYNTHETIC MICROORGANISMS

*F. PARTENSKY, L. GUILLOU, N. SIMON, D. VAULOT*

*Station Biologique, CNRS et Université P.-et-M.-Curie (Paris 6), 29682 Roscoff cedex, France*

18S RRNA
FLOW CYTOMETRY
MOLECULAR PROBES
PHOTOSYNTHETIC PICOPLANKTON
PHYLOGENY
*PROCHLOROCOCCUS*

ABSTRACT. – Despite its important ecological role, especially in oligotrophic parts of the world oceans and seas, the picophytoplankton (i.e. photosynthetic cells smaller than 2-3 µm) remains poorly known at the taxonomic level. In this paper, we have summarized major results of our own and collaborative work concerning the taxonomy of two of the major types of organisms which constitute the picophytoplankton : the eukaryotic picoplankton and the recently discovered, prokaryotic genus *Prochlorococcus*. For picoeukaryotes, we have developed a method using fluorescently labeled class – (or lower taxonomic levels) – specific gene probes, which hybridize with 18S rRNA molecules that possess the target sequence. Labeled cells can then be detected by flow cytometry. Although this method proved useful to characterize new isolates in culture, it still needs to be improved before it can be applied to natural samples. Alternative methods have been developed to study the intra-generic diversity of *Prochlorococcus*, including RFLP (restriction fragment length polymorphism) and sequencing of photosynthetic genes in several strains of *Prochlorococcus*. These experiments demonstrated that there was a very large genetic diversity between the different isolates available in culture. Two genes, the *pcb* gene encoding the major chlorophyll *a/b*-protein complex and the *cpeA* and *cpeB* genes encoding a novel type of phycoerythrin, proved to be useful as genetic markers for future field studies on the diversity of this important micro-organism.

18S RRNA
CYTOMETRIE EN FLUX
SONDES MOLECULAIRES
PICOPLANCTON PHOTOSYNTHETIQUE
PHYLOGENIE
*PROCHLOROCOCCUS*

RÉSUMÉ. – Malgré son rôle écologique important, tout particulièrement dans les zones oligotrophes des océans mondiaux, le picophytoplancton (c'est-à-dire les cellules photosynthétiques de taille inférieure à 2-3 µm) reste mal connu au niveau taxonomique. Dans cet article, nous avons résumé les principaux résultats de travaux réalisés dans notre laboratoire ou en collaboration et concernant la taxonomie des deux types majeurs d'organismes constituant le picophytoplancton : les picoeucaryotes et *Prochlorococcus*, un genre procaryotique récemment découvert. Pour les picoeucaryotes, nous avons développé une méthode utilisant des sondes spécifiques de Classes (ou de niveaux taxonomiques inférieurs), qui sont marquées avec des fluorochromes. Elles s'hybrident à l'intérieur des cellules avec les molécules d'ARNr 18S, si celles-ci possèdent la séquence-cible. Les cellules peuvent ainsi être détectées par cytométrie en flux. Bien que cette méthode se soit avérée utile pour caractériser des souches en culture, elle nécessite quelques améliorations supplémentaires avant de pouvoir être appliquée aux échantillons naturels. Des méthodes différentes ont été développées pour étudier la diversité intra-générique de *Prochlorococcus*. Celles-ci incluent la RFLP (restriction fragment length polymorphism) et le séquençage de gènes photosynthétiques chez plusieurs souches de *Prochlorococcus*. Ces expériences ont démontré qu'il y avait une très large diversité génétique entre les différentes souches disponibles en culture. Deux gènes, le gène *pcb* qui code pour la partie protéique du principal complexe chlorophylle *a/b*-protéine et les gènes *cpeA* and *cpeB* qui codent pour un nouveau type de phycoérythrine, apparaissent être de bons marqueurs génétiques pour de futures études sur la diversité de cet important microorganisme *in situ*.

## I. INTRODUCTION

Molecular biology techniques have been introduced in oceanography less than a decade ago and have already led to spectacular advances. "Historically", Giovannoni *et al.* (1990) made one of the first significant steps in this new field in the early 90's by isolating total DNA from a natural sample collected in the Sargasso Sea and amplifying the 16S rRNA gene, using the Polymerase Chain Reaction (PCR). After having cloned and sequenced these genes, they were able to build a phylogenetic tree of the bacterioplanktonic community. They showed that (1) a large genetic diversity existed, even if the gene pool analyzed came from a single water sample, and (2) none of the sequenced genes exactly matched those already present in data banks. These authors also designed a radio-labeled oligonucleotide probe complementary to the sequenced genes and showed by specific hybridization that one of the unknown populations (SAR 11), related to the α-proteobacteria, accounted for 12% of the total 16S rRNA pool, as estimated using a universal probe. The outstanding conclusions of this work were (1) that it is not necessary to isolate and culture populations to trace their occurrence and (2) that the gene sequences obtained from the field can be directly compared to that of other natural communities or previously isolated organisms. More recently, similar approaches have allowed to discover that a significant fraction of the bacterial community of coastal temperate areas of Atlantic and Pacific oceans (DeLong 1992), aphotic oceanic zones (Fuhrman *et al.* 1993) and frigid waters of Antarctica (DeLong *et al.* 1994) was constituted of Archaea, prokaryotes which were previously thought to be restricted to environments displaying extreme conditions such as deep sea hydrothermal vents or hyperhaline habitats.

Most papers published to date on the application of molecular methods to marine biology have aimed at studying the genetic diversity and/or phylogenetic relationships within natural communities of micro-organisms (mainly heterotrophic bacteria) using rRNA sequences. The choice of the rRNA (most often 16S for plastids and prokaryotes, 18S for eukaryotes) is justified because it is a universal marker which comprises both variable and conserved regions, which can be selectively targeted depending on the taxonomic resolution needed (e.g. Class, Family, Genus or species). Genes encoding rRNA are those for which the sequence database is the most important, which greatly facilitates gene sequence comparisons and the search for specific genetic markers. In addition, rRNA constitutes more than 95% of the total cellular RNA content and possesses a rather large size (> 1.5 kb). This molecule is also ecologically relevant since the cell content of rRNA, which can be quantified using *in situ* hybridization, provides an index of the metabolic activity and growth rate of a population (DeLong *et al.* 1989; Simon *et al.* 1995). Much fewer studies have investigated either other structural genes such as *rpoC* encoding one of the subunits of the RNA polymerase (Palenik 1994), or ecologically more important, but not as ubiquitous, genes such as the *nifH* gene, which encodes the dinitrogenase reductase and is implicated in the fixation of atmospheric nitrogen ($N_2$; Kirshtein *et al.* 1993).

There are several other important marine planktonic communities, besides heterotrophic bacteria, which diversity remains poorly known at the genetic level. Tremendous advances in the knowledge of those communities may also be expected from the application of molecular techniques. Among them, the picophytoplankton (i.e. photosynthetic cells smaller than 2-3 μm in size) not only plays a very important role in the biogeochemical cycling of carbon, especially in oligotrophic parts of the world oceans and seas, but it also constitutes an essential compartment of the microbial food webs. In the present paper, we briefly summarize the major results of our own or collaborative work concerning two of the major types of organisms which constitute the picophytoplankton : the eukaryotic picoplankton (Simon *et al.* 1994) and the recently discovered, prokaryotic genus *Prochlorococcus* (Chisholm *et al.* 1988, 1992). These two groups account, about equally, for up to 80% of the photosynthetic biomass in vast areas of the intertropical open oceans (Campbell *et al.* 1994, Partensky *et al.* 1996). The approaches and methods we are developing to study the genetic diversity within these two groups are different because we are dealing with distinct taxonomic levels (intra-classes *vs.* intra-generic) and cell sizes. Thus, picoeukaryotes diversity can be studied using fluorescently labeled class– (or lower taxonomic levels)– specific gene probes which hybridize with 18S rRNA molecules possessing the target sequences. Marked cells can then be detected by epifluorescence microscopy or flow cytometry. In contrast, for *Prochlorococcus*, this method is not as adequate due to the smallness of cells. *Prochlorococcus* intra-generic diversity must therefore be studied by alternative methods such as comparing gene sequences from different clones or from amplified DNA fragments from natural samples. The approach we are developing for *Prochlorococcus* is therefore similar to that used previously by others to study heterotrophic bacteria, but with a focus on photosynthetic genes, which have the advantage of being absent from heterotrophs.

Table I. – Characteristics of probes directed against taxa to which belong various picophytoplanktonic and small nanoplanktonic organisms.

| Level | Probe name | Specificity | Target sequence (5' → 3') | Start position | Reference organism | Reference |
|---|---|---|---|---|---|---|
| Domain | Euk309R | Eukaryotes | AGGTCTGTGATGCCC | | | Lim *et al.* (1993) |
| Supra-class | CHLO01 | Chlorophytes | CACCACCAGGCGTGGAGC | 1152 | *Chlamydomonas reinhardtii* | Simon *et al.* (1995) |
| | CHLO02 | Chlorophytes | AAAGTTGGGGGCTCGAAG | 973 | *Chlamydomonas reinhardtii* | Simon *et al.* (submitted) |
| | NCHL01 | Non-Chlorophytes | CACCACCAGGAGTGGAGC | 1152 | *Chlamydomonas reinhardtii* | Simon *et al.* (1995) |
| Class | PRYMN01 | Prymnesiophyceae | AGCATTTGCCAGGGATGT | 946 | *Phaeocystis globosa* | Lange *et al.* (1996) |
| | PRYMN02 | Prymnesiophyceae | GTCAGGGGCACTCGTATTCC | 877 | *Phaeocystis globosa* | Simon *et al.* (submitted) |
| | PELA01 | Pelagophyceae | AGCGTCGAACAAGGACGT | 957 | *Pelagomonas calceolata* | Simon *et al.* (submitted) |
| | PELA02 | Pelagophyceae | GATTGGGATTGATTGTTGC | 1554 | *Pelagomonas calceolata* | Simon *et al.* (submitted) |
| | CHRY01 | Chrysophyceae | GACCTTGGTCTATTTTGT | 809 | *Ochromonas danica* | Simon unpublished |
| Genus | PHAEO01 | *Phaeocystis* | ACGAGTCCACCTCGACCG | 1488 | *Phaeocystis globosa* | Lange *et al.* (1996) |
| | Micro/Manto01 | *Mantoniella squamata*<br>*Micromonas pusilla* | CGACCTCGTTCTGCGGTG | 219 | *Chlamydomonas reinhardtii* | Knauber *et al.* (1996) |
| | Micro/Manto02 | *Mantoniella squamata*<br>*Micromonas pusilla* | TCATACGCTACTCTTAGCGCAGTGACT | 1330 | *Chlamydomonas reinhardtii* | Knauber *et al.* (1996) |
| | Pycno | *Pseudoscourfieldia marina*<br>*Pycnococcus provasolii* | ATCTCGACTTCGGAAGAGACG | 178 | *Chlamydomonas reinhardtii* | Knauber *et al.* (1996) |
| | CCMP1194 | unidentified coccoid prasinophytes (CCMP* 1194, CCMP 1202 and CCMP 1407) | ACCAATCGCTTCGGCGTTTT | 228 | CCMP 1194 | Knauber *et al.* (1996) |

*Center for the Culture of Marine Phytoplankton, Bigelow Laboratory, MA, USA

## II. DIVERSITY OF THE MARINE EUKARYOTIC PICOPHYTOPLANKTON

Tiny micro-algae forming the eukaryotic picophytoplankton are generally coccoid or flagellated cells with very few specific morphological features. They can hardly be discriminated, even at the Class level, by classical methods such as optical microscopy (Andersen *et al.* 1993, Potter *et al.* 1997). The accurate identification of newly isolated strains therefore often requires a combination of sophisticated techniques, such as electron microscopy, HPLC pigment analyses and sometimes 18S rRNA gene sequence analyses, which need much time and expertise. Consequently, the systematics of these micro-organisms is in constant evolution, with the frequent discovery of new species or taxa. For example, several species belonging to the Chrysophyceae have been moved into the newly described class of Pelagophyceae (Andersen *et al.* 1993). Nowadays, the characterization of taxonomic groups in complex natural assemblages remains problematic even using the sophisticated techniques evoked above and alternative methods are needed in order to circumvent these difficulties.

*In situ* hybridization with 18S rRNA oligonucleotide probes is one of the most promising techniques to study the diversity of this eukaryotic picoplankton. Relevant probes are chosen after examination of 18S rRNA gene sequence alignments and the identification of regions that are specific of a pre-determined taxonomic level. A short oligonucleotide (ca. 20 base pairs) which is complementary to this specific sequence is then designed, and coupled with a fluorescent molecule (generally fluorescein or rhodamine). This probe is then incubated with the cells to be identified (culture or natural population) after permeabilizing the cell wall. Cells which possess the target rRNA sequence become fluorescent and can be discriminated from the control either by epifluorescence microscopy or by flow cytometry (Fig. 1). Protocols are detailed elsewhere (Simon *et al.* 1995; Lange *et al.* 1996; Marie *et al.* 1997).

Probes targeted to algal groups ranging from divisions to species have been designed (Table I) and tested against cultures of pico- or nanophytoplankton by whole cell hybridization. Using appropriate hybridization conditions, fluorescent probes label their intended target specifically, i.e. their complementary sequence in rRNA molecules. A single nucleotide difference can be discriminated provided that stringent hybridization conditions are used (e.g. for CHLO and NCHLO probes).

This set of probes still needs to be expanded until there is at least one probe per major class and ideally per major genus or species of the picophytoplankton. As mentioned above, the fina-

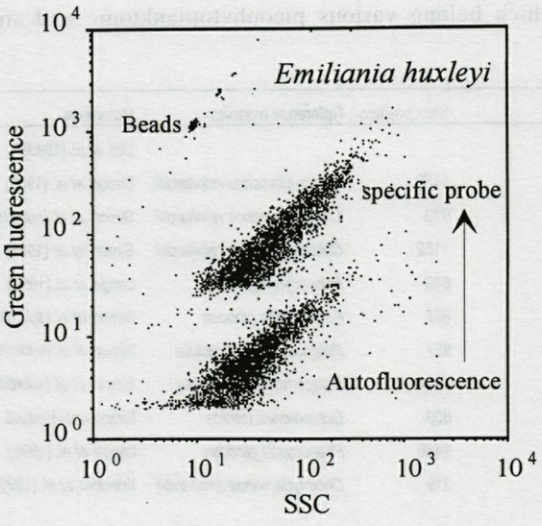


Fig. 1. – Flow cytometric analysis of a nanoplanktonic (*Emiliana huxleyi*) strain before and after hybridization with the CHRO01 probe labelled with fluorescein isothiocyanate (FITC).

lity of this approach is to study the diversity of this complex community in the field. In practice, when applying the *in situ* hybridization methods to natural samples of picoeukaryotes, several drawbacks have to be circumvented, including (1) rRNA content is a function of the status of cells so that cells growing slowly, as it may be the case in oligotrophic waters, have a significantly lower signal than rapidly dividing cells, (2) the loss of red chlorophyll fluorescence, resulting from the permeabilization necessary to allow the probe to enter the cell, makes it difficult to recognize populations in a complex assemblage by flow cytometry and (3) the different centrifugation steps often result in cell loss. Once these problems are solved or minimized by optimizing the different steps of the hybridization protocol (L. Guillou, unpublished), we will be able to tackle intriguing ecological questions. For example, is there a small number of ubiquitous species which dominate the biomass of the photosynthetic picoeukaryotic community in vast areas of the world ocean or numerous species with restricted environmental ranges? Also we will be able to study how given taxa are linked to specific environmental conditions.

## III. DIVERSITY OF *PROCHLOROCOCCUS*

The reasons to study *Prochlorococcus* diversity are slightly different from those justifying the interest for picoeukaryotes. *Prochlorococcus* can easily be identified and counted by flow cytometry without the use of any specific marker, in contrast to eukaryotes (Chisholm *et al.* 1988; Vaulot *et al.* 1990). However, this genus is very ubiquitous since it proliferates in most oceans and seas from 45°N to 45°S. This raises the question of whether widely separated populations are genetically homogeneous. Moreover, in oligotrophic areas, cells of this genus can sustain growth and photosynthesis under a very wide range of irradiances from the surface (100% incident sun light) to depths of ca. 100-200 m receiving less than 0.1% of the incident light irradiance (Olson *et al.* 1990; Campbell and Vaulot 1993). Thus, one can wonder whether this remarkable ability may result from highly efficient adaptation mechanisms of the photosynthetic apparatus and/or of a large genetic diversity. The observation at certain depths of several *Prochlorococcus* populations distinguishable by their mean chlorophyll fluorescence, a parameter linked to the intracellular ratio of divinyl-chlorophyll (DV-Chl) *a* to *b* (Partensky *et al.* 1996), as well as by their different DNA contents (Campbell and Vaulot 1993), strongly suggest that the genus *Prochlorococcus* is in fact constituted by several genetically distinct populations (or "species"?).

With these hypotheses in mind, we initiated an international, collaborative work on the genetic diversity of this micro-organism in culture. The main aims of this work were to study the genetic diversity among isolates, to understand how this diversity may translate at the biochemical and physiological levels and to obtain the molecular tools necessary to study the diversity of natural populations (i.e. *Prochlorococcus*-specific gene sequences).

As a first step, restriction fragment length polymorphism (RFLP) patterns from strains isolated from different locations were compared (Scanlan *et al.* 1996). The technique consisted of extracting chromosomal DNA, digesting it with various restriction enzymes then probing the DNA fragments on a Southern blot with radio-labeled probes for photosynthetic genes. The probes used were heterologous fragments of *rbcL*, *rbcS*, *woxA* and *psbA* genes, encoding the large and the small subunits of the Rubisco, the 33 kDa extrinsic protein of photosystem II (PS II), and one of the reaction center proteins of PS II, respectively. Use of photosynthetic gene probes was necessary to avoid the risk of hybridizing DNA fragments from heterotrophic contaminants from the culture. This study revealed an extensive genetic variation among strains, which were grouped into two main clusters. Unexpectedly, the strains seem to cluster according to isolation depth (i.e. near surface vs. deep isolates) rather than to geographic origin (Scanlan *et al.* 1996). As we pointed out earlier, *in situ* observations suggest that different populations of *Prochlorococcus* characterized by different DV-Chl *a*/*b* ratios may coexist at given sites.

The population having a high DV-Chl *a/b* ratio dominates the upper layer while the one with a low ratio dominates the deeper layer. Thus isolates from different depths can in fact be differentiated by their respective pigment ratios.

The next step was therefore to characterize *Prochlorococcus* strains representative of two extreme pigment types. *P. marinus* SS120, isolated from 120 m in the Sargasso Sea has a DV-Chl *a/b* ratio lower than 1, while *Prochlorococcus* sp. MED4, isolated from surface waters on the Mediterranean Sea, has a ratio about ten times higher (Moore *et al.* 1995). Preliminary physiological studies showed that these strains also exhibit shifted irradiance optima for growth and photosynthesis (Partensky *et al.* 1993 ; Moore *et al.* 1995), which is consistent with the different depths from which they were isolated. Such a large difference in Chl *a/b* ratios implies differences in the content or structure of the light-harvesting complexes (or antennae), since most Chl *b* is concentrated in these complexes. A comparative biochemical study of antenna complexes confirmed this hypothesis (Partensky *et al.* 1997). The major Chl *a/b* complexes of SS120 are made of several, abundant apoproteins in the range 34-38 kDa while for MED4, there is only one major and less abundant antenna apoprotein of 32.5 kDa. Moreover, the relative concentration of these complexes varies more with growth irradiance in SS120 than MED4, suggesting that both the structure and the regulation of antenna complexes differ between these two strains (Partensky *et al.* 1997).

To go further in the comparison of these two *Prochlorococcus* types, a characterization of the antenna genes was initiated. The N-terminal sequence of the major antenna apoproteins from both strains were obtained. They were identical between the two *Prochlorococcus* strains and interestingly showed strong homology with a protein, CP 43' (Laudenbach and Straus 1988), found in abundance in iron-depleted cyanobacteria (Partensky and LaRoche 1994). Using these informations, primers were designed and the corresponding antenna gene (so-called *pcb*, for prochlorophyte chlorophyll *a/b*-protein) was amplified and sequenced in both strains (LaRoche *et al.* 1996). The percentage of identity between the antenna proteins of the two strains, as derived from gene sequences, is only 76 %, which indicates a very large genetic distance. This molecular study also confirmed the homology of *Prochlorococcus* antenna with the cyanobacterial gene *isiA*, encoding CP 43', and provided new insights about the phylogeny of this micro-organism. Importantly, it confirmed its close relationship with Cyanobacteria, as previously suggested by sequence data obtained for the genes encoding the 16S rRNA gene (Urbach *et al.* 1992), a subunit of the RNA polymerase gene (*rpoC*; Palenik and Haselkorn, 1992) and the D1 protein of the reaction center of photosystem II (*psbA*; Hess *et al.* 1995).

The search for genes which could allow to discriminate the different pigment types of *Prochlorococcus* led to another important discovery. *P. marinus* SS120 was found to possess functional *cpeA* and *cpeB*-like genes, coding for the $\alpha$ and $\beta$ subunits of a novel phycoerythrin (Hess *et al.* 1996). This phycobilin-binding protein is part of the phycobilisomes, supra-molecular complexes which play the role of antennae in cyanobacteria. The occurrence of low amounts of phycoerythrin in SS120 cells was shown by immuno-blotting with an heterologous antibody. This protein was also found to bind two types of chromophores, phycourobilin and phycoerythrobilin, similar to the ones found in marine *Synechococcus* cyanobacteria. Unexpectedly, no traces of phycoerythrin at the protein and gene levels were found in *Prochlorococcus* MED4 nor in several other strains isolated from near surface. These data obtained from cultures seem to be corroborated by flow cytometric data from the field, which show that deep *Prochlorococcus* have a significant orange fluorescence attributable to phycoerythrin, while near surface populations have none (Hess *et al.* 1996). The phycoerythrin genes could thus well constitute genetic markers specific of *Prochlorococcus marinus*, a species representative of deep populations, but not of other species.

Further sequencing work is still needed on additional *Prochlorococcus* isolates as well as on related organisms, such as marine *Synechococcus*, to know if *Prochlorococcus*-specific *pcb* primers and *P. marinus*-specific *cpeA/B* primers may be designed in order to amplify DNA from natural *Prochlorococcus* populations and to study their genetic diversity. Elucidation of the extent and role of genetic variability within this genus is essential to understand how environmental stress influences community structure and function. A preliminary study of the variability of natural "cyanobacterial" populations (i.e. including *Prochlorococcus*) from one station and one depth of the Sargasso Sea was recently achieved by amplifying, cloning and sequencing a RNA polymerase gene (Palenik 1994). However, the true extent of the genetic diversity within the genus *Prochlorococcus* in the field and the role of abiotic factors in maintaining this diversity remain poorly assessed.

## IV. CONCLUSION

Although the applications of molecular biology to oceanography are still in their infancy, Falkowski and LaRoche (1991) predicted that they could

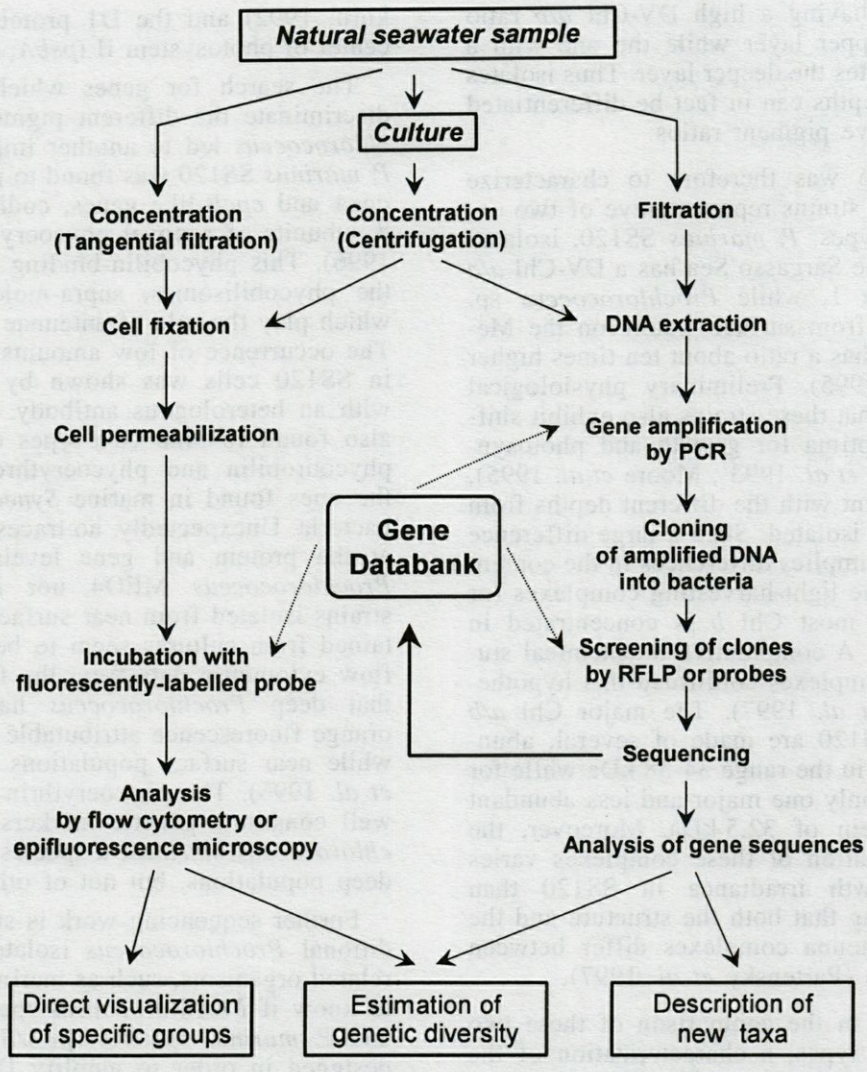


Fig. 2. – Flow chart of the main methods available to characterize natural samples or cultures of marine picophytoplankton. Dotted arrows indicate that new gene sequences may be used to further improve molecular probes or PCR amplification primers.

resolve a number of oceanographic questions, including microbial metabolism, photosynthesis regulation and limitation of phytoplankton growth by nutrients. To date however, most studies have focused on the genetic diversity and/or phylogenetic relationships within natural communities of micro-organisms. This is also the case with our study of picoeukaryotes, but our interest in *Prochlorococcus* largely exceeds the simple knowledge of the extent of its genetic diversity.

Figure 2 summarizes the two main methods that are available for characterizing marine picophytoplankton. As concerned the picoeukaryotes, the *in situ* hybridization method constitutes a very good tool for rapidly identifying newly isolated strains in culture. However, since current protocols are not optimized for all taxonomic groups, this application often requires a slight optimization of the method for new groups. Moreover, this and other drawbacks still prevent the straightforward use of this method for characterizing individual species or groups within complex natural assemblages of picoeukaryotes. Moreover, the majority of picoplanktonic cells are naked and possess a thin plasmic membrane. Because of their subsequent fragility, it is possible that many groups may escape detection. Therefore, beside improving current *in situ* hybridization methods to make them applicable to natural samples, it is clear that a good assessment of picoeukaryote diversity in the field will also require complementary analyses, such as the sequencing of clones obtained after amplification of 18S RNA genes from natural picoeukaryote populations (Fig. 2).

The study of the diversity within the *Prochlorococcus* genus is in a more prospective stage.

We are still trying to find photosynthetic genes which may constitute good markers to identify the different clades (or species) of *Prochlorococcus* with the aim of studying its genetic diversity in the field.

ACKNOWLEDGMENTS – This work was presented at the "Symposium on the Biodiversity in Dispersive Environment" held in Paris on 18-19 November 1996. It was supported by an 'Action Spécifique' funded the EC Program MAST III, the CNRS-Sciences de l'Univers, the French National Programme on Biodiversity and the ACC-SV7. Laure Guillou's PhD grant was funded by the Région Bretagne. We thank D. Marie for help with flow cytometry measurements.

## REFERENCES

ANDERSEN R.A., SAUNDERS G.W., PASKIND M.P., J. SEXTON 1993. Ultrastructure and 18S rRNA gene sequence for *Pelagomonas calceolata* gen. and sp. nov. and the description of a new algal class, the Pelagophyceae *classis* nov. *J. Phycol.* **29** : 701-715.

CAMPBELL L., NOLLA H.A., D. VAULOT 1994. The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnol. Oceanogr.* **39** : 954-961.

CAMPBELL L. & D. VAULOT 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). *Deep-Sea Res.* **40** : 2043-2060.

CHISHOLM S.W., OLSON R.J., ZETTLER E.R., WATERBURY J., GOERICKE R., N. WELSCHMEYER 1988. A novel free-living prochlorophyte occurs at high cell concentrations in the oceanic euphotic zone. *Nature* **334** : 340-343.

CHISHOLM S.W., FRANKEL S.L., GOERICKE R., OLSON R.J., PALENIK B., WATERBURY J.B., WEST-JOHNSRUD L., ZETTLER E.R. 1992. *Prochlorococcus marinus* nov. gen. nov. sp. : an oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b*. *Arch. Microbiol.* **157** : 297-300.

DELONG E.F. 1992. Archaea in coastal marine environments. *Proc. Natl. Acad. Sci. U.S.A.* **89** : 5685-5689

DELONG E.F., WICKHAN G.S., PACE N.R. 1989. Phylogenetic stains : ribosomal RNA-based probes for the identification of single cells. *Science* **243** : 1360-1363.

DELONG E.F., WU K.Y., PRÉZELIN B.B., JOVINE R.V.M. 1994. High abundance of Archaea in antarctic marine picoplankton. *Nature* **371** : 695-697.

FALKOWSKI P.G., LAROCHE J. 1991. Molecular biology in studies of ocean processes. *Int. Rev. Cytol.* **128** : 261-303.

FUHRMAN J.A., McCALLUM K., DAVIS A.A. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific oceans. *Appl. Environ. Microbiol.* **59** : 1294-1302.

GIOVANNONI S.J., BRITSCHGI T.B., MOYER C.L., FIELD K.G. 1990. Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345** : 60-63.

HESS W.H., PARTENSKY F., VAN DER STAAY G.W.M., GARCIA FERNANDEZ J.M., BÖRNER T., VAULOT D. 1996. Coexistence of phycoerythrin and a chlorophyll *a/b* antenna in a marine prokaryote. *Proc. Nat. Acad. Sci. U.S.A.* **93** : 11126-11130.

HESS W.R., WEIHE A., LOISEAUX-DE GOER S., PARTENSKY F., VAULOT D. 1995. Characterization of the single *psbA* gene of *Prochlorococcus marinus* CCMP 1375 (Prochlorophyta) *Plant Mol. Biol.* **27** : 1189-1196.

KIRSHTEIN J.D., ZEHR J.P., PAERL H.W. 1993. Determination of $N_2$ fixation potential in the marine environment : application of the polymerase chain reaction. *Mar. Ecol. Prog. Ser.* **95** : 305-309.

KNAUBER D.C., BERRY E.S., FAWLEY M.W. 1996. Ribosomal RNA-based oligonucleotide probes to identify marine green ultraphytoplankton. *J. Euk. Microbiol.* **43** : 89-94.

LANGE M., GUILLOU L., VAULOT D., SIMON N., AMANN R.I., LUDWIG W., MEDLIN L.K. 1996. Identification of the class Prymnesiophyceae and the genus *Phaeocystis* with ribosomal RNA-targeted nucleic acid probes detected by flow cytometry. *J. Phycol.* **32** : 858-868.

LAUDENBACH D.E., STRAUS N.A. 1988. Characterization of a cyanobacterial iron stress-induced gene similar to *psbC*. *J. Bacteriol.* **170** : 5018-5026.

LAROCHE J., VAN DER STAAY G.W.M., PARTENSKY F., DUCRET A., AEBERSOLD R., LI R., GOLDEN S.S., HILLER R.G., WRENCH P.M., LARKUM A.D., GREEN B. 1996. Independent evolution of the prochlorophyte and green plant chlorophyll *a/b* light-harvesting proteins *Proc. Nat. Acad. Sci. U.S.A.* **93** : 15444-15248.

LIM E.L., AMARAL L.A., CARON D.A., DELONG E.F. 1993. Application of rRNA-based probes for observing nanoplanktonic protists. *Appl. Environ. Microbiol.* **59** : 1647-1655.

MARIE D., SIMON N., GUILLOU L., PARTENSKY F., VAULOT D. 1997. Flow cytometry analysis of marine picoplankton. *In* Current Protocols in Cytometry. Edited by S. Demaggio : (in press).

MOORE L.R., GOERICKE R., CHISHOLM S.W. 1995. The comparative physiology of *Synechococcus* and *Prochlorococcus* isolated from the subtropical open ocean : Growth regulation by light and temperature. *Mar. Ecol. Prog. Ser.* **116** : 259-275.

OLSON R.J., ZETTLER E.R., ALTABET M.A., DUSENBERRY J.A., CHISHOLM S.W. 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep-Sea Res.* **37** : 1033-1051.

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

PALENIK B., HASELKORN R. 1992. Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* **355** : 267-269.

PARTENSKY F., BLANCHOT J., LANTOINE F., NEVEUX J., MARIE D. 1996. Vertical structure of

picophytoplankton at different trophic sites of the subtropical northeastern Atlantic Ocean. *Deep Sea Res.* I **43** : 1191-1213.

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 Physiol.* **101** : 295-296

PARTENSKY F., LAROCHE J. 1994. N-terminal sequence of the light-harvesting antenna of the marine prochlorophyte *Prochlococcus marinus* is similar to that of CP43', a pigment-protein complex induced during iron-depletion in *Synechococcus* cyanobacteria. Proceed. VIII Intern. Symp. on Phototrophic prokaryotes, Urbino, 10-15 Sept. 1995 : 39.

PARTENSKY F., LAROCHE J., WYMAN K., FALKOWSKI P.G. 1997. The divinyl-chlorophyll *a/b* complexes of two strains of the oxyphototrophic marine prokaryote *Prochlorococcus* – characterization and response to changes in growth irradiance. *Photosynth. Res.* **51** : 209-222.

POTTER D., LAJEUNESSE T.C., SAUNDERS D.W., ANDERSEN R.A. 1997. Convergent evolution masks extensive biodiversity among coccoid picoplankton. *Biodiv. Conserv.* **6** : 99-107.

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

SIMON N., BARLOW R.G., MARIE D., PARTENSKY F., VAULOT D. 1994. Flow cytometric analysis of oceanic photosynthetic picoeucaryotes. *J. Phycol.* **30** : 922-935.

SIMON N., CAMPBELL L., NOLLA H., VAULOT D., MEDLIN L. Oligonucleotide probes for the identification of three algal groups by fluorescent whole-cell hybridization. *Aqu. Microb. Ecol.* (submitted).

SIMON N., LEBOT N., MARIE D., PARTENSKY F., VAULOT D. 1995. Fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes for identifying small sized phytoplankton by flow cytometry. *Appl. Env. Microbiol.*, **61** : 2506-2513.

URBACH E., ROBERTSON D.L., CHISHOLM S.W. 1992. Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* **355** : 267-269.

VAULOT D., PARTENSKY F., NEVEUX J., MANTOURA R.F.C., LLEWELLYN C. 1990. Winter presence of prochlorophytes in surface waters of the northwestern Mediterranean Sea. *Limnol. Oceanogr.* **35** : 1156-1164.

*Reçu le 7 mars 1997; received March 7, 1997*
*Accepté le 8 avril 1997; accepted April 8, 1997*