OPINION

组学时代下的可培养技术重要性

The importance of culturing bacterioplankton in the 'omics' age

Stephen Giovannoni and Ulrich Stingl

Abstract | Progress in the culturing of microorganisms that are important to ocean ecology has recently accelerated, and technology has been a factor in these advances. However, rather than a single technological breakthrough, a combination of methods now enable microbiologists to screen large numbers of cultures and manipulate cells that are growing at the low biomass densities that are characteristic of those found in seawater. The value of ribosomal RNA databases has been reaffirmed, as they provide nucleic-acid probes for screening to identify important new species in culture. The new cultivation approaches have focused on specific targets that ecological studies suggest are significant for geochemical transformations, such as SAR11. Here, we review how to cultivate marine oligotrophs and why it is worth the effort.

At a time when metagenomic studies are capturing the limelight, why is culturing important? In short, cultures are important because they provide complete genomes and the means to test the hypotheses that emerge from genomic data. Cultured bacterial and archaeal isolates that have yielded complete genome sequences have also proved to be important for evaluating metagenomic data — a recent paper that presented environmental whole-genome shotgun-sequencing data from Craig Venter's Global Ocean Survey relied heavily on complete genome sequences from bacterial plankton to evaluate metagenomic data, because procedures for reconstructing genomes from whole-genome shotgun sequences are not yet reliable[1,2]. Cultures, supported by predictions from genomes, together with the information about natural variation that is provided by metagenomics are a powerful combination. On the one hand, live cells allow the study of the whole organism, rather than trying to infer physiological properties from an incomplete list of parts. On the other hand, genomic and metagenomic data can reveal hidden metabolic potential, metabolic pathways, regulatory circuits and conservation among cultured and uncultured bacteria (FIG. 1).

The TIMELINE lists selected important events in marine microbial cultivation. Historically, there has been debate about whether heterotrophic microbial cells can grow at the extremely low nutrient concentrations that are found in natural ecosystems[3]. However, now there are many strains that have been cultured which replicate well in

mimic：模仿，似

sterilized seawater, but do not grow in media that contain concentrated organic nutrients (BOX 1). The main challenge of designing artificial media to mimic natural seawater is the accurate reconstruction of the complex composition of dissolved organic carbon (DOC) and trace elements (TABLE 1). To easily obtain dense, turbid bacterial or archaeal cultures, most researchers have relied on media that contain high concentrations of organic carbon. ZoBell's Marine Medium[4], which is still widely used for culturing heterotrophic bacteria, has 170-fold more DOC than natural seawater. To make matters worse, most of the DOC in seawater is derived from recalcitrant matter, so that the difference in useable organic carbon probably exceeds three orders of magnitude (TABLE 1).

The first culture of an obligate oligotroph was obtained from a freshwater sample in 1981 (REF. 5). In the same year, an influential review of oligotrophy was published[6]. Subsequently, several research groups, most notably that of Don Button, developed methods for the successful cultivation of oligotrophs and began working with cultures of marine bacteria that could be grown in autoclaved seawater[7–9]. The laboratory of S.G. modified Button's methods by reducing the size of cultures to microtitre dish volumes, implementing screening methods and adopting clean procedures that had been developed by oceanographers for handling seawater[10]. Additional modifications included segregating containers that were used for culturing cells, avoiding detergents and using plastics such as polycarbonate and Teflon that do not inhibit the growth of bacterial cultures. These methods, which have been referred to as high-throughput extinction-culturing methods, led to the isolation into pure culture of the most important new heterotrophic marine strains that have been cultured so far.

Other approaches to cultivation have used microencapsulation techniques[11], chambers that allow the exchange of small molecules with the environment[12], floating filters[13,14], micromanipulation[15] or, simply, low-nutrient agar plates[16] to cultivate novel microorganisms. Although the details of these approaches differ, they are all based on the same principles of re-creating the low-nutrient conditions of natural environments and overcoming the tendency of rapidly growing cells to overwhelm species that divide less often. These approaches have promise, as do traditional enrichment strategies that target organisms with unusual metabolisms[17]. Although genomic information has not been a major factor in the isolation of new marine organisms, it has been exploited to design isolation strategies for bacteria that are present in different environments[18,19], and it is only a matter of time before genomic or metagenomic information contributes to the isolation of a marine bacterial or archaeal species.

Strains that represent many of the most abundant marine clades have been isolated into pure culture in recent years, but other important clades still lack any cultured representatives[20,21] (FIG. 2). Some of the uncultured clades originate from phyla that are associated with unusual metabolisms, which suggests that their cultivation could depend on successfully determining their ecological niche. For example, the recent cultivation of *Nitrosopumilus maritimus* SCM-1, which was the first cultured representative of the Group I Marine Crenarchaeota, succeeded because of a specific strategy that excluded bacteria with antibiotics and aimed to isolate archaeal species that can oxidize ammonium to yield energy[22]. The SAR202 clade, which lacks cultured representatives, belongs to the phylum Chloroflexi, which includes photoheterotrophs and anaerobes that have the capacity to oxidize halogenated compounds. The SAR202 clade is one of several that have been relegated to regions that are just below the euphotic zone[23]. Another important group that lacks cultured representatives is the SAR86 group of gammaproteobacteria, which account for approximately 10% of the total prokaryotic microbial community in the ocean surface layer[24]. The discovery of the light-driven, proton pump proteorhodopsin in the fragmentary genome data

subtle不易察觉的，不明显的，微妙的
impediment 阻碍；妨碍；口吃
partition 分割，分区，瓜分

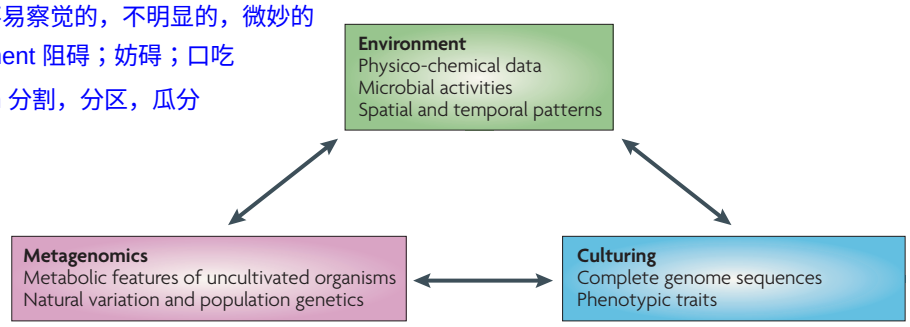


Figure 1 | **Integrated approaches in marine microbiology.** The development of an integrated approach to marine microbiology is emerging that is founded on new model organisms that have a well-established relevance to environmental science.

for SAR86 was the first major success of metagenomics[25–27]. There are now many bacteria in culture that encode proteorhodopsin genes[28–30]. The study of these marine bacterial and archaeal isolates is revealing an important, but sometimes subtle, role for proteorhodopsin that could not have been predicted from metagenomic data or even complete genome sequences.

tool up机床加工

Tooling up for studying oligotrophs

技术已经成熟

Technology that can be used to study oligotrophs at the slow growth rates and low cell densities that are typical of natural seawater is maturing (FIG. 3). However, the main impediment to studying cells that are growing in seawater is low cell densities, which yield little biomass. Methods that use centrifugation are ineffective for concentrating oligotrophic cells from large volumes, so tangential flow filtration is used instead. The cell densities that are typical of oligotrophic cultures are on orders of magnitude that are too low for routine optical-density measurements. Therefore, cell counting is achieved either microscopically, after concentrating cells onto membranes, or by flow cytometry. Perhaps the main technological advances that have benefited the study of oligotrophic microorganisms in culture are more sensitive analytical methods, such as DNA sequencing, microarrays and proteomics, all of which are effective, but barely so; for example, cultures in 20-l carboys often yield only 10^{10}–10^{11} cells. Procedures for genetically manipulating obligate oligotrophic isolates have not yet been developed, largely because plating these cells onto agar-solidified growth media is not feasible, and success will probably require the flow-cytometric detection of cells that express fluorescent protein markers.

The environmental context of these strains raises unique questions that are being answered by scientists who work with cultures. In particular, how do oligotrophs interact with the global ocean DOC pool, and how do the dominant bacterioplankton clades partition nutrient resources? Microbiologists who work with bacteria and archaea that are found in soil, termite guts and extreme environments such as the acidic, metal-laden waters of the Iron Mountain mine (California, United States) face similar questions[19].

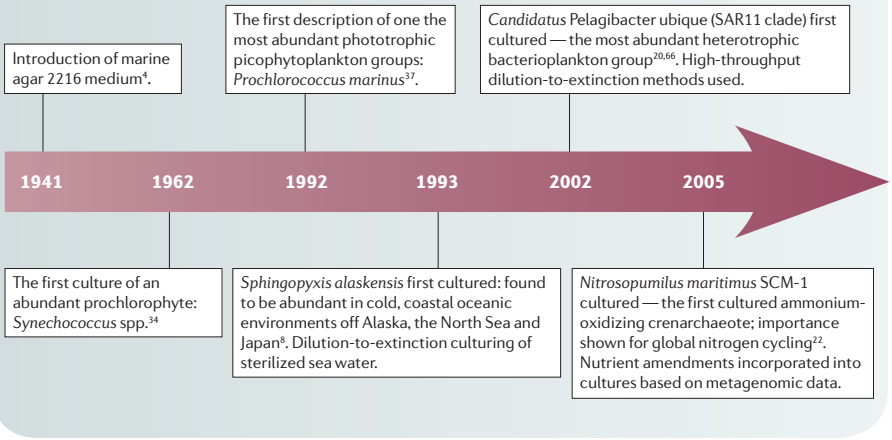
Can we really mimic the ocean environment in physiological experiments conducted in the laboratory or do we need approaches that probe cell functions *in situ*? The answer is that we need to do both. The physical and chemical properties of the ocean environment can be replicated in the laboratory with some accuracy, but the dynamic nature of the oceans — where changing populations interact, water circulates in complex hydrographical patterns and weather is variable — cannot be easily simulated. Chemostats provide a partial solution to the problem, by mimicking steady-state conditions in which growth is substrate limited, which is a better approximation of the ocean environment than the boom-and-bust growth characteristics of batch cultures. Studying the responses of whole plankton communities to short-term confinement and manipulation, which can be thought of as a form of cultivation that is one step removed from nature, can also yield valuable information[10,30]. However, one drawback of using cultivated strains is that it is unknown whether they have evolved adaptations to the culture conditions or whether the initial cultivation has led to the selection of a rare genotype. These issues have not caused major concerns, but might receive more attention as metagenomics studies reveal how isolated strains fit into the landscape of natural variation in the environment.

Cultivation success stories

Most microbiologists would agree that microbial diversity is vast. Below we recount several stories of microbial isolation and research, but the list is anecdotal. Students of the strange and obscure can find numerous accounts of new taxa that have unique properties, an unexplored potential and uncertain significance. Some of these organisms produce novel antibiotics[31], others have a novel metabolism[32] and a few show an evolved capacity for specific biotic interactions[33]. The common theme that underlies the selected organisms that are discussed in this Perspective is their geochemical significance, for which these organisms have received the greatest attention.

The isolation of key oxygenic photoautotrophs from the oceans preceded the recent advances in culturing techniques. The first cultures of cosmopolitan marine *Synechococcus* spp. strains emerged in 1962 (REF. 34), but their importance was overlooked until 1979 (REF. 35). The first observation of the *Prochlorococcus* genus and the description of its first species, *Prochlorococcus marinus* (FIG. 4a), were

Timeline | **Milestones in culturing marine microorganisms**



可以在海洋中生长的微生物却难以在添加有机营养的培养基上生长

Box 1 | **A short history of oligotrophy** 寡营养微生物

The fascination with heterotrophic bacteria that subsist at low nutrient concentrations dates back to work by Martinus Beijerinck at the turn of the century, but it was not until 1979 that the term oligotroph was first used to describe bacteria that are able to grow at 1–15 mg of carbon per litre[63]. Cells that can grow at these low carbon concentrations and in standard and nutrient-rich microbiological media, such as those of the model organism *Sphingopyxis alaskensis*, are facultative oligotrophs. The term obligate oligotroph has been reserved for heterotrophs such as *Candidatus* Pelagibacter ubique, which cannot be easily cultivated at the organic carbon concentrations that are found in most laboratory growth media. The dissolved organic carbon concentration in the ocean rarely exceeds 1 mg per litre, and only a fraction of that is metabolically available[64].

reported in 1988 (REF. 36) and 1992 (REF. 37), respectively. These species were probably no easier to isolate than heterotrophs like SAR11; they also have slow growth rates and require media that is made from natural seawater. However, the fluorescent signatures from their photosynthetic machinery provided a screening tool for their isolation long before ribosomal RNA (rRNA) databases matured as effective tools for the same purpose[36]. Although in this article we do not discuss eukaryotic plankton, they are an important component of the phytoplankton and their cultivation involves similar challenges. The cultivation of unicellular eukaryotic plankton, particularly phytoplankton, has probably enjoyed a more consistent record of success than the cultivation of bacteria and archaea, because many unicellular eukaryotes can be readily identified microscopically. This highlights the importance of assays to the success of cultivation efforts.

The alphaproteobacterium *Candidatus* Pelagibacter ubique (FIG. 4b,c), belonging to the SAR11 clade, is the most noteworthy new isolate of a heterotrophic marine bacterium. The SAR11 clade has a cosmopolitan distribution, occurring throughout the water column at all depths. It accounts for approximately 25% of all microbial plankton cells in the ocean surface layer, and might exceed 50% of the cells found in temperate ocean gyres during the summer months[38]. The 'tricks' to isolating SAR11 were relatively simple: first, we removed competitors using dilution-to-extinction methods; second, we mimicked natural growth conditions by using media that were based on natural seawater; and finally, we used high-throughput screening methods with specific fluorescent probes[20]. P. ubique isolates are obligate oligotrophs that, so far, can only be grown on media that are made with natural seawater, where they can reach densities of approximately 10^6 cells per ml, which is similar to the densities that are attained by native populations. The P. ubique genome is composed of only 1,308,506 base pairs (bp), but this is sufficient to encode all of the functions that are needed for this free-living cell to have a dominant role in the oxidation of the oceanic DOC pool[39]. In contrast to the parasitic bacteria that have small genomes, such as *Chlamydia trachomatis*[40] or *Wolbachia pipientis*[41], P. ubique has complete pathways for most fundamental metabolic pathways, including biosynthetic pathways for all 20 amino acids. P. ubique cells express proteorhodopsins in culture and *in situ,* and are probably a major source of this molecule in ocean systems[25,29].

The Roseobacter clade was the first culturable group of heterotrophic marine bacteria to appear frequently in environmental clone libraries (metagenomes), and has emerged as an important model organism. A comprehensive analysis of three complete and nine draft genomes of diverse *Roseobacter* spp. strains[42] showed that they range in size from between 3.3 to 4.3 million base pairs (Mbp) and contain one or two plasmids (with 86–823 kilobase pairs (kbp)). Although the Roseobacter lineage is a coherent group, based on 16S rRNA analysis, their genomic and physiological diversity is considerable. Only approximately 50% of the coding sequences in each of the three complete genomes were shared with the other two strains. Among the most common *Roseobacter* spp. phenotypes are motility, the ability to degrade aromatic compounds (such as gentisate, homoprotocatechuate, homogentisate, benzoate and phenylacetate) and the ability to demethylate dimethylsulphoniopropionate. More striking than the core genes of the Roseobacter clade are the strain-specific genes. Genes for aerobic anoxygenic phototrophy, lithotrophy by the oxidation of carbon monoxide or reduced sulphur compounds, phosphonate degradation and nitrite reduction are found in some, but not all, members of the Roseobacter clade.

Sphingopyxis alaskenis, which was formerly classified as *Sphingomonas alaskensis*[43], is the best-studied oligotrophic bacterium. *S. alaskensis* strains were isolated by dilution-to-extinction methods using samples from the coastal waters off Alaska, the North Sea and Japan[9,44,45]. The ability to grow under low nutrient conditions, the low maximum-growth rate (0.13–0.16 generations per hour at 23°C) and the ultramicrobial size of this bacterium (less than 0.1 μm^3) led to the early use of *S. alaskensis* as a model for studying oligotrophy (reviewed in REF. 46).

Table 1 | **Elemental composition of sea water and marine broth 2216**

Element	Seawater (grams per litre)	Marine broth 2216 (grams per litre)
Chlorine	19	19.8
Sodium	10.5	8.8
Magnesium	1.35	2.2
Sulphur	0.885	0.73
Calcium	0.4	0.65
Potassium	0.38	0.32
Bromine	0.065	0.054
Carbon	0.028*	4.78‡
Boron	0.0046	0.0047
Silicon	0.003	0.00085
Fluorine	0.0013	0.00109
Nitrogen	0.0005*	0.72‡
Phosphorus	0.0007*	0.045‡
Iron	0.00001	0.0226

*Contains particulate and dissolved components. Note that biologically available organic carbon in seawater is usually less than 12 µg per litre[6]. ‡Assuming a Redfield ratio of 116:16:1 (carbon:nitrogen:phosphorous) for peptone and yeast extract. Data from REF. 4.

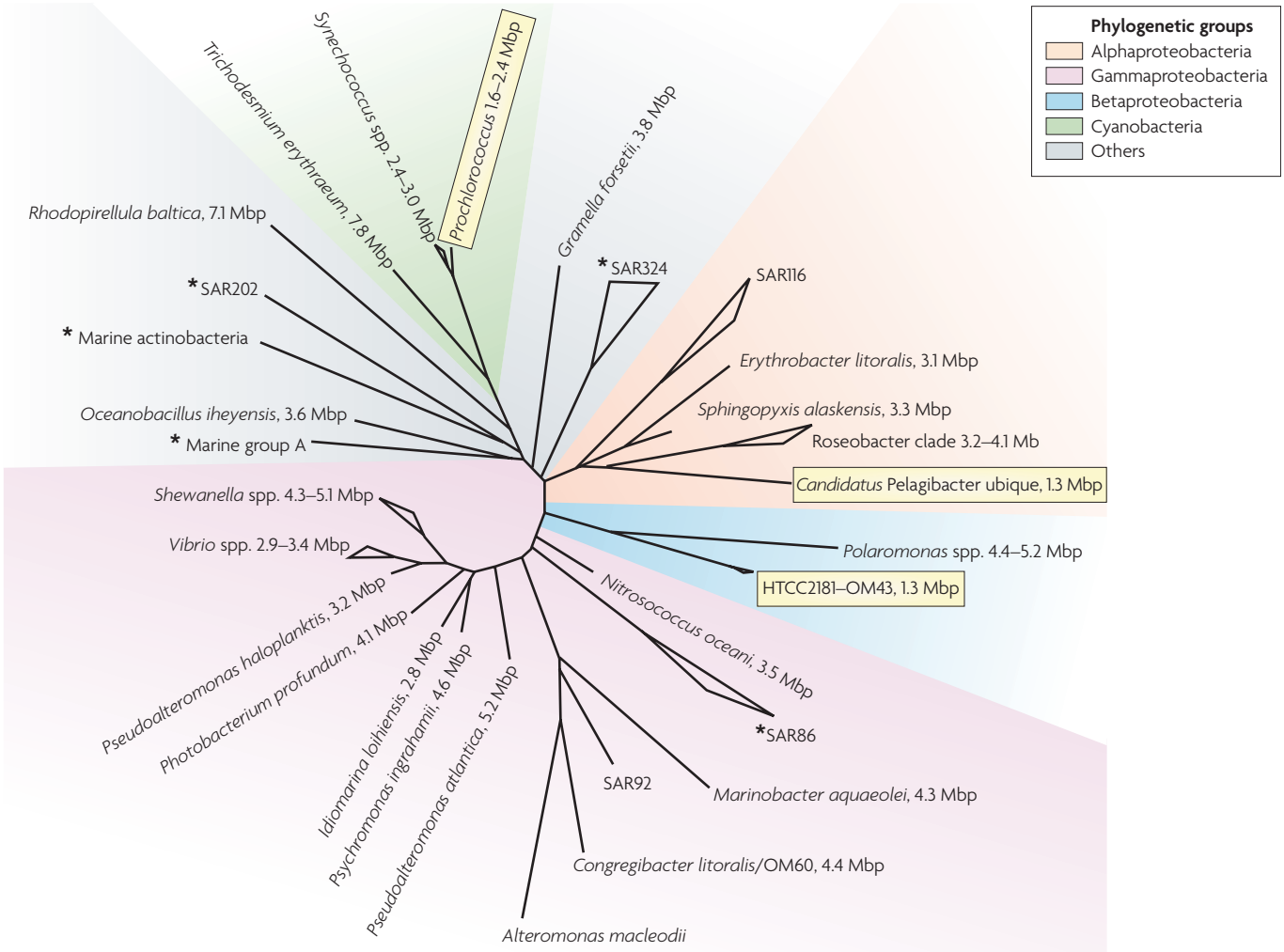


Figure 2 | **Phylogeny of selected marine bacteria.** A phylogenetic tree showing the major bacterioplankton groups and the sizes of their completed genomes. Abundant uncultured groups are marked by an asterisk; taxa with a genome size of 2 million base pairs (Mbp) or less are boxed.

Comprehensive studies on the physiology of *S. alaskensis* have revealed interesting features that were unexpected in oligotrophic bacteria. The genome size is large, 3.1–3.2 Mbp as measured by pulse-field gel electrophoresis[45]. *S. alaskensis* is well adapted to growth in the presence of high nutrient concentrations (up to 800 mg of carbon per litre) without a change in growth rate and is, therefore, a facultative oligotroph. *S. alaskensis* strains have a single rRNA gene and contain approximately 2,000 ribosomes per cell in the early exponential phase; a number that decreases to 200 in the late-log phase[47]. Studies on gene expression in batch cultures, using two-dimensional gel electrophoresis, showed that a large fraction of the proteome undergoes changes when the cells enter the stationary phase[48], whereas few changes were detected in strains growing at different growth rates in glucose-limited chemostats[49].

A loosely connected collection of clades, collectively known as the oligotrophic marine gammaproteobacteria group, contain some interesting isolates that seem to be ubiquitous in seawater, although found in low abundance[16,29,50]. Two isolates from the OM60 clade are particularly interesting because they correspond with metagenomic evidence for anoxygenic bacteriochlorophyll -containing gammaproteobacteria in the oceans. The genomes for these isolates are of average size — 3.58 megabases (Mb) for a draft genome from the HTCC2080 strain[51] and 4.36 Mb for strain KT71 (*Congregibacter litoralis*)[52]. Interestingly, within the OM60 clade, *puf* operons, which encode genes for light-driven cyclic electron transport, are polyphyletic, suggesting that there are multiple, independent acquisitions of these genes[53]. Another unusual feature of these genomes is the fact that there are multiple TonB receptor paralogues. TonB receptors typically function in the transport of large substrate molecules, such as siderophores and vitamins, across the periplasmic membrane, using the electrochemical potential across the cell membrane as the energy source for active transport. Why some marine bacteria seem to rely so heavily on the TonB transport system is one of many questions that have arisen from genome sequencing to which physiological studies that use cultures might provide an answer.

Learning from genomes

Cultivated strains can provide unbiased complete genome sequences that offer unique insights into their metabolism and are pivotal for interpreting metagenomic datasets. Most genomes from pelagic microorganisms are average in size (FIG. 2), but a few are exceptionally small, such as the most abundant marine heterotroph P. ubique[39] and the most abundant marine

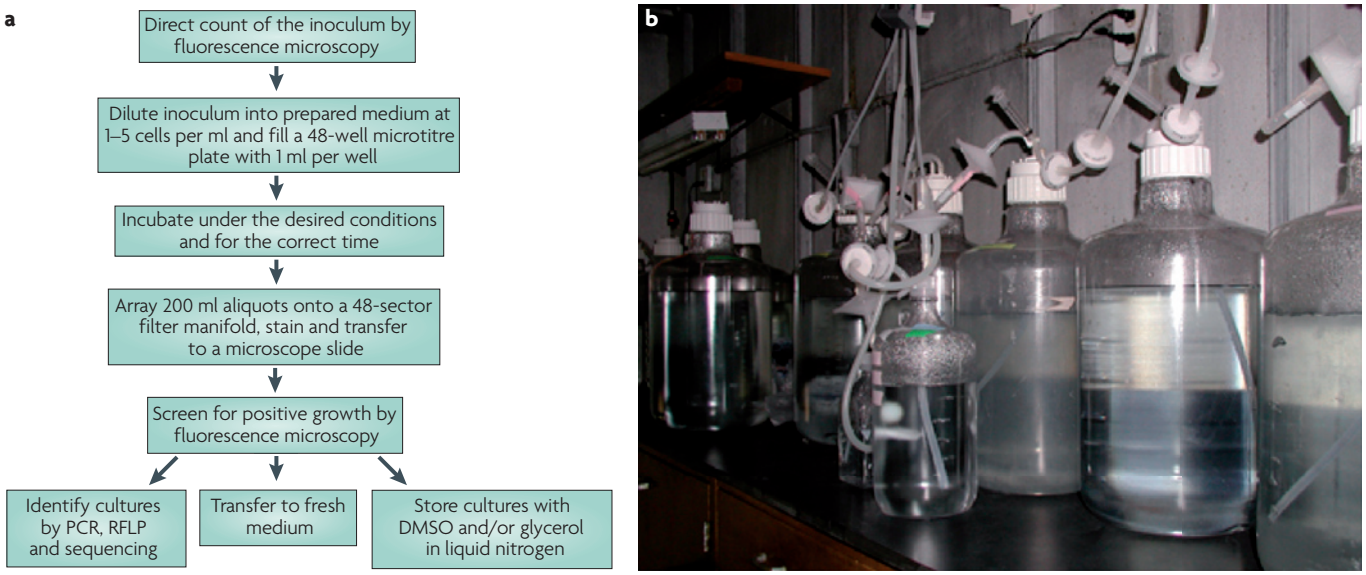


Figure 3 | **Cultivation of oligotrophs. a** | A schematic outline of the high-throughput cultivation procedures that are used in the laboratory of S.G. and U.S. to isolate oligotrophic marine bacteria by dilution-to-extinction methods. **b** | Cultures of oligotrophic marine bacteria growing in 5- and 20-l carboys of autoclaved seawater. With generation times typically one division per day, cultures of oligotrophic bacteria often take 3–4 weeks to reach stationary phase and produce less than 1 mg (wet weight) of biomass from 20 l of seawater. DMSO, dimethyl sulphoxide; RFLP, restriction fragment length polymorphism.

autotroph *Prochlorococcus*[36,54]. The smallest bacterioplankton genomes seem to be extreme examples of streamlining selection. The genome of P. ubique is 1.31 Mbp, which is the smallest reported genome for a free-living heterotrophic cell. *Prochlorococcus* genomes, which range in size from 1.66 to 2.41 Mbp, have the smallest cyanobacterial genomes that have been reported[55]. The essence of the genome streamlining theory is that selection is most efficient in microbial populations that have large effective population sizes, and therefore, the elimination of unnecessary DNA from genomes will be most pronounced in organisms such as bacterioplankton that meet this criterion. Bacterioplankton also inhabit a niche that is frequently limited for the macronutrients nitrogen and phosphorous, which are stochiometrically high in nucleic acids. Therefore, although it can be argued that all bacteria have streamlined genomes to some degree, basic theoretical considerations predict that marine bacterioplankton might exhibit streamlining to a greater extent than other bacterial populations. Alternatively, it could be argued that the marine habitat is simply more uniform than other habitats, with less versatility required, allowing organisms to dispense with much of their genomes. This concept is contradicted by the observation that many marine bacterioplankton, albeit those with smaller population sizes, have average genome sizes.

The diversity question

There is no doubt that the sampling of marine microbial genomes remains skewed towards facultative oligotrophs because obligate oligotrophs are more difficult to grow in the laboratory. Thus, although P. ubique and *Prochlorococcus* seem to have anomalously small genomes, they might be part of a larger class of organisms that are resistant to cultivation. Raes and colleagues[56], working from metagenomic data, estimated the average size of marine microbial genomes to be 1.6 Mbp, but this value was an average for all cells in samples that were dominated by SAR11 and does not provide information about the range of genome sizes. Similarly, genome-reconstruction methods, such as the extreme-assembly technique of Rusch and colleagues[1], could not provide that information.

Recently, large datasets that total more than 6,000 16S rRNA sequences from more than 40 different marine locations have been released[1,57]. Although, in general, phylogenetic analyses of these new data corroborated earlier studies on microbial plankton diversity, a vastly more detailed, but controversial, picture of the biogeography and microdiversity of marine microorganisms emerged.

Pommier and colleagues[57] reported on 16S rDNA clone libraries from nine, mainly coastal, oceanic locations around the world. Nearly 70% of all observed operational taxonomic units (OTUs; based on a 97% cut-off) were endemic to one of the locations, which led the authors to propose that most marine habitats contain functionally different microorganisms that are adapted to their specific environment. Most of the observed endemic OTUs were rare (1–2 clones per location). The recent report by Rusch and colleagues[1] was based on whole-genome shotgun sequencing and is, therefore, not affected by PCR bias, which can result in overestimates of microdiversity[53]. Rusch and colleagues also used a 97% cut-off-value threshold for the delineation of taxa, which split the SAR11 clade into six OTUs and five more 'remotely SAR11-like' OTUs. Similarly, the same group delineated nine OTUs within the gammaproteobacterial SAR86 clade, which is the second most abundant bacterioplankton clade.

It is clear that as yet there is no agreement about the complexity of marine microbial diversity. If the assessments of Pommier and colleagues[57], and Rusch and colleagues[1] are correct, then it will be necessary to study a large number of isolates in culture to understand marine ecosystems. However, we suspect that defining taxa using a fixed threshold of 97%, instead of relying on clade structure, and disregarding seasonal variation in the ecotype composition of communities, might have led both groups to overestimate the number of endemic strains. Many of the abundant marine microbial clades are

diverse collections of evolutionary lineages that differ by as much as 10% in their 16S rDNA sequence but have evolved from common ancestral strains[58]. How much of the observed variation is neutral, and how much is related to speciation, remains a crucial, but unresolved, question.

Until the relationships between speciation and sequence diversity are resolved it will be unknown how many cultured strains and genomes are needed to provide a thorough picture of microbial diversity in the ocean water column. However, most workers in the field accept that at least the major clades are divided into multiple variants, known as ecotypes. Ecotypes are predicted to occupy similar ecological niches, but owing to minor differences in phenotypic properties, such as optimal growth temperature, they have different distributions in time and space. For example, there is evidence for at least three ecotypes of SAR11 in the Sargasso Sea[58,59] — one ecotype that dominates the upper 300 m in the spring and two different ecotypes that replace the spring ecotype in the summer and which specialize in the upper and lower regions of the water column when it becomes stratified. The best-studied ecotypes are those of *Prochlorococcus*, which are fundamentally similar but differ significantly in genome size and physiological properties, which thus impacts their success in the water column[55,60].

Glossary

Bacterioplankton
The bacteria that inhabit the water column of lakes and oceans, either freely suspended or attached to particles.

Euphotic zone
A transitional region of the water column where light levels are low and labile organic matter is in even shorter supply than at the surface, but where macronutrients, such as iron, phosphorous and reduced forms of nitrogen, are readily available.

Heterotrophic
The acquisition of metabolic energy by the consumption of living or dead organic matter.

Oligotrophic
An aquatic environment that has low levels of nutrients and algal photosynthetic production (for example, high mountain lakes or the open ocean).

Pelagic
Relating to or occurring in the water column.

Tangential flow filtration
A technique that re-circulates the retentate (the part of a solution that does not cross the membrane) along a membrane surface that is only permeable to water and low-molecular-weight compounds.

Lessons from oligotrophs

What new insights of general significance are we likely to get from culturing marine microorganisms? It is simply too soon to answer this question. The availability of methods to work with these organisms, and the will to explore them in a research framework that maintains close links with their ocean ecology, might indicate that a renaissance is occurring in this field, but data have only just begun to appear. One important question is: do oligotrophs share common features of metabolism that set them apart from other organisms? Button's metabolic models[61], which are based on cell architecture and kinetics, suggest that they do, but these are largely untested. Have many marine oligotrophs evolved small genomes or are *Procholorococcus* and P. ubique (FIG. 4) anomalies? The descriptions of more complete genomes will be informative, but cultures are also required for those difficult-to-cultivate organisms. Breakthroughs in assembling genomes from single cells or metagenomic data might also help to resolve this question. How much of the observed natural variation among marine organisms is neutral, and how much of this natural variation simply supports the concept of functional diversification into niche space? The case of *Prochlorococcus* has provided insight into this issue, but a general ecological theory that explains genetic variation and satisfactorily relates it to taxonomy and systematics has yet to emerge.

Finally, why are these organisms so difficult to cultivate? So far, no single theme has emerged that explains why some organisms are more difficult to cultivate than others. Much of the recent success has come from new methods that work for organisms that grow slowly or, at best, achieve low cell densities in culture. It seems likely that using media that is derived from natural seawater can circumvent the need to define specific growth factors. As many of the current approaches still rely on autoclaving, they might not be successful with strains that require unstable growth factors. In addition, some organisms require interactions with other organisms and grow in consortia. There are several clear examples of this in the microbiological literature, although no consortia have been detected in the oxic water column as of yet. Most microbial plankton in the marine water column are found as suspended, free cells that do not appear to physically interact with other organisms. However, aggregates known as marine snow are densely colonized by microbial cells, and may provide a habitat where co-evolved microbial interactions are a factor that affects the success of culturing[62].

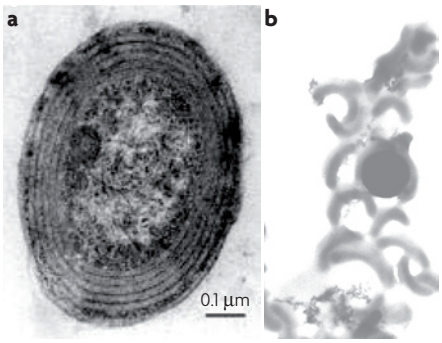


Figure 4 | **Micrographs of important oligotrophic marine bacteria. a** | A transmission electron micrograph of *Prochlorococcus marinus*. The image is courtesy of F. Partensky, National Centre for Scientific Research, France. **b** | A transmission electron micrograph of negatively stained *Candidatus* Pelagibacter ubique (SAR11) cells in culture. The spherical object is a latex bead that has a diameter of 0.514 microns. Image **b** reproduced, with permission, from *Nature* REF. 20 © (2002) Macmillan Publishers Ltd.

It is inevitable that continuing efforts will result in the successful cultivation of representatives from the major microbial plankton clades that have so far been elusive. Cultivation does not seem to be a matter of chance, as the application of the same methods routinely results in the isolation of the same species from seawater. Rather, it seems more likely that new strains will require imaginative media designs that are based on the interpretations of metagenomic and ecological data.

In this context, it will be important to know if the main properties of a clade can be elucidated from the analysis of a single strain or whether the clades comprise such diverse organisms that several model organisms are needed. This question is unsolved and will be one of the more exciting tasks for microbial ecologists. What seems certain is that the quest to cultivate additional strains will continue as marine microbiology moves into the age of functional genomics. Today, 'systems biology' refers to approaches that consider the cell as an integrated sum of its parts. The phrase could assume a new meaning in oceanography, where cells are viewed as components of larger ecosystems and the ultimate goal is to understand biological processes on vast scales.

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doi:10.1038/nrmicro1752

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Acknowledgements
This paper was supported by National Science Foundation grant DEB-0207085 and by a grant from the Gordon and Betty Moore Foundation.

Competing interests statement
The authors declare competing financial interests: see web version for details

DATABASES
Entrez Genome: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome
Prochlorococcus marinus
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Candidatus Pelagibacter ubique | *Chlamydia trachomatis* | HTCC2080 | KT71 | *Nitrosopumilus maritimus* SCM-1 | *Sphingopyxis alaskensis* | *Wolbachia pipientis*

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Stephen Giovannoni's homepage: www.mcb.oregonstate.edu/giovannoni/

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