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Competing interests statement

The authors declare no competing financial interests.

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OPINION

Whither or wither geomicrobiology in the era of 'community metagenomics'

Ronald S. Oremland, Douglas G. Capone, John F. Stolz and Jed Fuhrman

Abstract | Molecular techniques are valuable tools that can improve our understanding of the structure of microbial communities. They provide the ability to probe for life in all niches of the biosphere, perhaps even supplanting the need to cultivate microorganisms or to conduct ecophysiological investigations. However, an overemphasis and strict dependence on such large information-driven endeavours as environmental metagenomics could overwhelm the field, to the detriment of microbial ecology. We now call for more balanced, hypothesis-driven research efforts that couple metagenomics with classic approaches.

Use of modern molecular techniques as applied to investigations in microbial ecology has gained popularity over the last decade. Here, we define molecular techniques as the use of nucleic-acid base sequencing technology to identify the phylogenetic groups present within mixed microbial populations, their community genotypic potentials and the analytical keys for detecting expression of their diverse functional genes.

Molecular approaches can now be considered an essential component of the complete research microbiologist's toolbox, comparable to something as basic as a microscope. A testament to their increased use can be seen by perusing the abstracts from the Tenth International Society for Microbial Ecology meeting, held in Cancun, Mexico (Aug 2004). Of the 1,223 contributed posters, 60% (734) mentioned usage of one or more molecular techniques.

The widespread use of molecular approaches is here to stay, and these technologies are at the cutting edge of science. At least three recent perspectives[1–3] and a scholarly review[4] recount and predict some of the future directions of the rapidly emerging field of environmental genomics as applied to microbial ecology and its subdiscipline of geomicrobiology.

Cultivation of the uncultured

Although estimates of microbial-species diversity vary substantially even in a single sample[5–7], it is generally agreed that only a fraction of the microorganisms that are present have been cultivated[8]. Whereas genomic and 16S rRNA analyses can help to indicate the existence of certain metabolic functions[9,10], a comprehensive understanding of microbial physiologies will undoubtedly require their cultivation. The challenge that environmental microbiologists face today is that conventional enrichment and cultivation techniques cannot be used to cultivate most environmental bacteria. It could be argued that these traditional strategies use conditions that are different from the habitat of many microorganisms and are an important contributing factor to the failure to cultivate most microorganisms in pure culture[11,12]. Therefore, several novel strategies have been developed.

Janssen and co-workers[13] addressed the issue of high concentration of nutrients in media by using a diluted nutrient broth. This approach, together with long incubation times, enabled cultivation of isolates from bacterial groups that are poorly represented in culture collections[14]. A dilution cultivation method for growing bacteria from oligotrophic environments was developed by Button *et al.*[15] Marine microorganisms were diluted to extinction (1 cell per cultivation container) and inoculated into sterilized seawater. After incubation for typically one week, growth of $>10^4$ cells per ml was examined by flow cytometry. This method has been refined[16] with a high-throughput method of Button's protocol now available. Increased sensitivity for the detection of cells based on the use of microtitre dishes and an automated imaging process enabled the isolation of many new planktonic isolates[17,18]. Another approach is based on encapsulation of single cells into microcapsules for parallel cultivation under low organic nutrient flux, followed by flow cytometry to detect and isolate capsules containing microcolonies[19].

So, most of the recently described cultivation methods try to simulate and mimic the natural environment. This has led to the use of extremely diluted culture media, which only support minimal cell growth, resulting in prolonged incubation times. Accordingly, technology has to be developed to work with minute numbers of cells (for example, microcolonies on plates or within microcapsules). Many of these cultures will require the further refinement of molecular and analytical tools to study them. Information gained from environmental sequencing projects might also facilitate cultivation attempts in the future and thereby help to bridge the gap between environmental genomics and classical cultivation studies.

Classical versus molecular approaches

It was recognized prior to the advent of PCR-based technologies that the number of prokaryotes in culture collections represented only a small proportion (often cited as less than 1%) of the total number of species present in nature. Therefore, the hope to isolate and fully describe most microorganisms present in a complex ecosystem, such as the bovine rumen[20], was perhaps an unrealistic, but necessary, first effort. Nonetheless, obtaining 'representative' pure cultures that carry out important processes in geomicrobiology is still considered a crucial step in illustrating the biological component(s) of newly discovered phenomena, akin to fulfilling Koch's postulates to prove that a specific organism is the aetiological agent of a disease.

So, if a microorganism that carried out a certain function (for example, nitrification, methane oxidation, metal reduction) could be isolated from an environment where these processes were only previously inferred from environmental chemical data, it highlighted the fact that basic biochemistry, not chemistry, was the source. This classical approach then takes the problem out of the realm of geochemistry and proves that a microbiological component is involved. This can be achieved by conducting incubations of natural materials (for example, sediment microcosms) that allow for manipulative additions of substrates, antagonists and/or inhibitors to yield clues that indicate which microorganisms participate in the reaction[21]. This approach can also provide clues as to how to construct stable enrichments and eventually isolate pure microbial cultures from these enrichments. When there are interdependencies, such as interspecies hydrogen transfer, the isolation of the individual components of the defined 'consortia' is more difficult, but is nonetheless a necessary task.

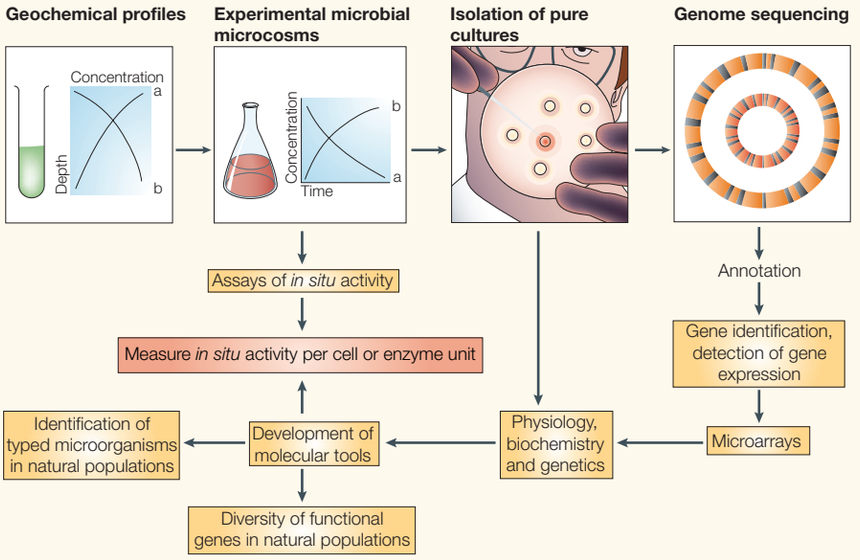

Figure 1 | **A classical approach to geomicrobiological investigations combined with modern methods.** First, geochemical phenomena are identified by geochemists, as exemplified by constituents found in sediment pore-water profiles that indicate involvement of microorganisms. Geomicrobiologists conduct incubation experiments with natural materials, such as sediment slurries, that support a biological basis for the reaction, and eventually isolate pure cultures of microorganisms that can carry out this reaction during their growth. Further research ensues, including the biochemistry and genetics of the process, which can be expanded if the whole genome of this organism (and related species) is sequenced and annotated. Ultimately, the information gained from these approaches can be combined to develop molecular techniques, such as fluorescence *in situ* hybridization–microautoradiography, to probe mixed assemblages of naturally occurring microorganisms. Such techniques can probe fundamental questions, such as which microorganisms in the assemblages carry out the reaction, how many organisms there are, how fast they grow, and what they use as electron donors and acceptors.

The holy grail of these ecophysiological approaches is the isolation of representative pure cultures, which are then available for biochemical and genetic scrutiny, resulting in the synthesis of molecular tools that can detect the presence of these and similar microorganisms in natural ecosystems (FIG. 1).

The advent of molecular techniques has enhanced this classical approach. In the case of methane oxidation, for example, detection and enumeration of type II methanotrophs in acidic peats by use of molecular tags such as fluorescence *in situ* hybridization (FISH) proved that cultured methanotrophs were representative of the overall methanotrophic communities in these systems[22,23]. Although molecular techniques are now commonly used as a stand-alone approach to define complex mixtures or 'guilds' of 'unculturable' or 'too-diverse-to-culture-them-all' microorganisms present in natural assemblages, they can also be readily adapted to improve the isolation of microorganisms previously thought to be unculturable[12,13,16,19,24]. Furthermore, molecular techniques can be applied to study the ecophysiology of natural aggregations of microorganisms by various sophisticated culture-independent methods[25]. Therefore, the knowledge originally gained from classical approaches has 'primed the pump' by coupling subsequent advances in molecular biology and genomics to devise molecular methods (for example, FISH–microautoradiography) to probe natural assemblages (FIG. 1).

Several unexpected and exciting discoveries have been made by applying some basic molecular approaches, such as community 16S-rDNA-gene amplification and sequencing, to well studied ecosystems like the open ocean. For example, Fuhrman *et al.*[26] noticed that abundant archaeal 16S-rDNA signatures constituted most of the clones amplified by PCR with 'universal' primers in deep oceanic waters. DeLong *et al.*[27] quantified the significance of archaeal RNA by bulk hybridization, and Fuhrman and Ouverney[28] used FISH to show that, in some oceanic regions, the free-living archaea are even more abundant than bacteria. Karner *et al.*[29] used monthly FISH measurements over >1 year at a tropical open-ocean station to show that these archaea typically comprise 30–40% of the total bacteria over a huge depth range (300–4,000 m), making them perhaps the

most common organisms in the ocean. These results were surprising because all cultured members of the Archaea were thought to be either fastidious anaerobes (methanogens) or extremophiles, such as thermoacidophiles or halophiles. So what were archaeal sequences doing in the aerobic, non-extreme environment of the open ocean? The biogeochemical function(s) of these marine archaeal organisms were unknown, although it has been speculated that their presence implies that they must be doing something fundamental, like oxidizing hydrogen[30].

It took a combination of classical and molecular approaches to provide a more direct answer. Autoradiography combined with FISH showed that marine planktonic archea import dissolved, free amino acids from the trace levels available, implying that these bacteria have heterotrophic capability[31]. However, stable isotope probing (SIP) analysis of archaeal-specific lipids indicated that they use an inorganic C source[32–34], and experiments revealed archaeal uptake of added ^{13}C-bicarbonate[35]. So, either the group consists of mixotrophs (with a partly autotrophic and partly heterotrophic metabolism) or there is a broad metabolic diversity within the group, with some members primarily heterotrophs and others autotrophs. An intriguing hint about a possible marine archaeal autotrophic pathway, ammonium oxidation, came from the metagenomic shotgun cloning study of Sargasso Sea prokaryotes, which reported an ammonium monooxygenase gene on an archaeal scaffold[36]. It is now clear that these planktonic archaea must be cultured to understand their metabolic function(s). Some data from archaeal species isolated from salt marshes might indicate that they are mundane heterotrophs, albeit heterotrophs which have adapted to tolerate broad ranges of salinity[37]. Clearly, more work needs to be done to determine if these isolates are in any sense 'representative' of the archaeal molecular signals that were originally detected in seawater. Nonetheless, this research serves as a good example of how exciting discoveries that were initially made through the use of molecular approaches can eventually result in a more complete understanding of processes by pairing with classical approaches (FIG. 2).

Molecular techniques are also applied to microbial biochemistry. Again using methanotrophs to illustrate this point, novel forms of particulate methane monooxygenase have been discovered using functional gene probes in cultured methane- and ammonium-oxidizing bacteria[38,39]. Similar advances on the biochemistry of methyl-halide-oxidizing methylotrophs have been made using molecular approaches[40]. Whole genome sequences of geochemically important bacteria, like *Geobacter sulfurreducens*, have yielded surprising new insights into their physiology, revealing unexpected metabolic functions and vastly expanding our appreciation of the complexity of their basic biochemical functionality[41]. For example, motility in *Geobacter* strains was never observed until genomic annotations revealed the presence of genes encoding for both flagella and pili. Subsequent microbiological experiments with *Geobacter metallireducens* showed that this species is chemotactic for Fe(III) or Mn(IV), but that this behaviour was only expressed when concentration gradients of Fe(II) or Mn(II) were present[42]. Similarly, *G. sulfurreducens* was thought to be a strict anaerobe until genomic studies revealed a capacity for the use of oxygen as a terminal electron acceptor[43]. The genome of *G. sulfurreducens* also revealed a large amount of redundancy in c-type cytochromes, which are presumably required to reduce Fe(III) and other metal(loid)s under a wide range of environmental conditions. The benefit of whole-genome annotation to geomicrobiology is now abundantly clear.

Ecosystem genomic inventories

When genomics is coupled with microarray technology, it provides powerful tools to advance our understanding of physiology, gene expression and regulation in ways unimaginable only a few years ago. Although genome-sequencing projects first focused on pathogens, dozens of microorganisms of primary interest to geomicrobiologists have already been sequenced (see the Genomes Online Database). Sequencing technology is now routine and rapid, although the manual annotation and experimental verification are by far the most difficult steps.

We have now arrived at the post-genomic era, sometimes also referred to as the era of metagenomics. The techniques for amplification, shotgun sequencing, computational assembly and annotation of large genomic databases have advanced so far that they are being applied to investigate natural assemblages of microorganisms. Therefore, it is in principle possible to amplify, sequence and annotate all the genomes of all the prokaryotes present in such assemblages. The two examples that highlight both the promise and the current limitations of this approach are the investigation of the Sargasso Sea[36], and a study of the microbial-mat community found in the highly acidic drainages from the Iron Mountain mine, California (USA)[44]. Whereas both studies provided a glimpse into the genetic diversity of these systems, the Sargasso Sea study[36] was unable to complete a single genome using the obtained data by itself, and relied on existing genomic data from cultured organisms on which to 'hang the contigs'. The identification of 1.2 million genes in the Sargasso Sea was an impressive feat in itself, although this enormous amount of partial sequence data had the unintended consequence of overwhelming the GenBank database.

A scrutiny of the dataset provided by these two studies raises several questions. For example, elevated concentrations of arsenic are found in the waters of Iron Mountain, which would indicate the presence of bacteria that use arsenate as a terminal electron acceptor. However, a BLAST search using the protein sequence of ArrA, the catalytic subunit of the respiratory arsenate reductase, did not reveal any putative *arrA* homologues. In the Sargasso Sea, where there are only traces of arsenic present, the results were just the opposite. Surprisingly, four *arrA* sequences, including that of *Shewanella* sp. SAR-1, were identified. Why should the genes for arsenic respiration occur in the Sargasso Sea (where there are no important amounts of arsenic present) but be absent from Iron Mountain (where there is abundant arsenic)? Certainly, such ambiguities could be related to something as simple as protocol artefacts. However, a technical criticism of these investigations is well beyond the scope of this article, and we have faith that this approach will prove its validity over time.

This paradox also prompts a question: do these large-scale sequencing endeavours truly enhance our understanding of the biogeochemical functioning of complex microbial ecosystems? Or are these investigations merely an extravagant means of answering the question of the famous 'knock-knock' joke, namely 'who's there?' Not that there is anything wrong with this question, as it is vital to expand our understanding of the biota of these realms by use of methodology far broader than either classic or narrowly focused molecular techniques can allow. But at some point, more detail concerning the functions and activities of the organisms that harbour these partially annotated metagenomes is required to further our understanding of these ecosystems.

Clearly, the effort that has been put into compiling environmental metagenomic databases for geomicrobiological purposes

can ultimately only prove useful when this information is paired with hypothesis-driven research. Therefore, a metagenomic database can only reveal the potential for the expression of an identified enzyme. Whether the sequences analysed encode functional enzymes of significance to biogeochemical cycling can only be determined using biochemical approaches.

Therefore, metagenomic analyses are just one aspect of any ecological investigation. Detailed follow-up studies must evaluate if the genes identified are expressed, if the encoded products are functional *in situ*, and if the process has any quantitative significance to the particular ecosystem studied or the cycling of elements, nutrients or energy flux within such ecosystems (FIG. 2). A good example of this was the discovery of genes that encode proteorhodopsin in uncultivated marine bacteria, which indicated that a non-photosynthetic light-driven pathway to generate ATP might be important for bacteria in oceanic surface waters[9,10,45]. A broad diversity of proteorhodopsin genes was also found in the Sargasso Sea study[36]. Subsequent spectroscopic studies of membrane preparations extracted from bacterioplankton proved that proteorhodopsin was present and functional in natural populations, and explored the diversity of cloned proteorhodopsin genes present in bacterial populations from different marine systems[46].

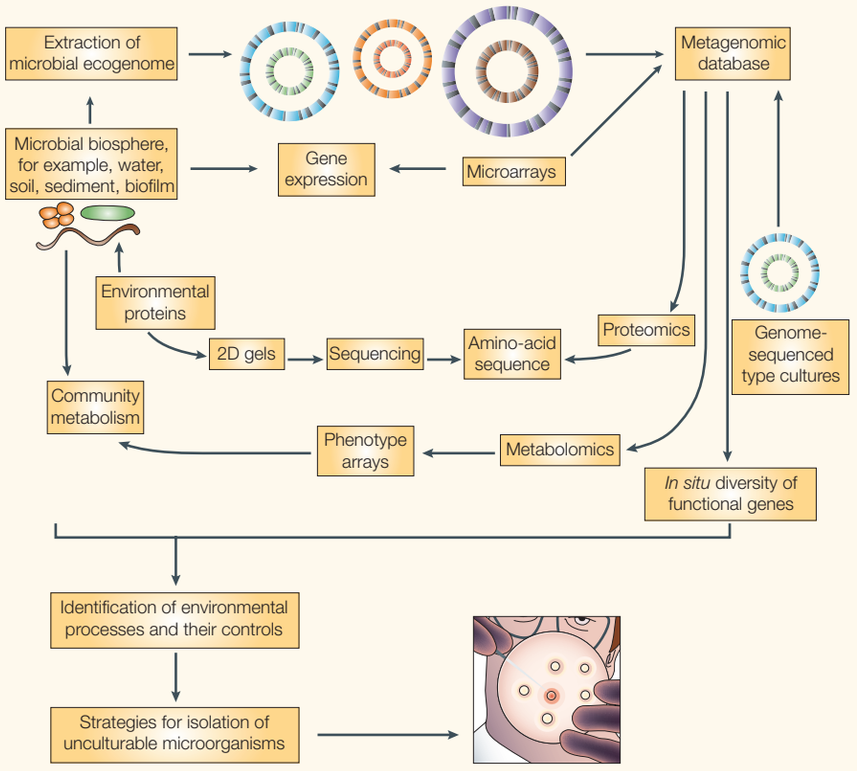

Figure 2 | **A metagenomic approach to geomicrobiological investigations.** Instead of focusing on a single geochemical phenomenon, the entire genome of all the microorganisms present in a natural assemblage is sequenced and annotated. This information should yield the potential of the population to carry out diverse biogeochemical reactions through detailed gene annotations. Specific biogeochemical reactions carried out by the natural assemblage can be detected using various molecular tools, such as microarrays for detection of gene expression or protein gels, amino acid sequencing for detection of synthesized proteins and microarrays for detection of community metabolism. Ultimately, the synthesis of these data could lead to novel techniques and strategies for the isolation of environmentally important microorganisms that have previously been 'unculturable'.

Geomicrobiology plus genomics

Two case studies described below illustrate how combining traditional and environmental genomic approaches can advance our understanding of biogeochemical processes.

Case study 1: anaerobic oxidation of methane. One of the best examples of the successful application of environmental genomics is that of the anaerobic oxidation of methane (AOM)[47–51]. AOM was first documented almost 40 years ago by geochemists[52–54] who hypothesized that sulphate was its oxidant and that AOM had a microbiological basis. This piqued the interest of geomicrobiologists, who began a frustrating 30-year-long effort to unravel this process. Although experimental work using $^{14}CH_4$ radiotracers showed that $^{14}CO_2$ was produced in experiments with incubated anoxic sediments, and that a peak of activity occurred at the base of the sulphate-reduction profile[55], obtaining pure or even mixed cultures proved intractable and considerably impaired characterization of the basic mechanism(s) of AOM. Reverse methanogenesis was first hypothesized by Zehnder and Brock[56], but they did not observe a net consumption of methane. It was not until later, when Hoehler *et al.*[57] carried out prolonged laboratory incubations of marine sediments, that a net consumption of methane was first shown, and reverse methanogenesis was indicated as the initiating mechanism.

By this time, molecular techniques were sufficiently advanced to provide help in solving the AOM problem. Microbial communities in which AOM occurred were investigated using molecular approaches in conjunction with new secondary ion mass spectrometry measurements of the $\delta^{13}C$ of cell lipids. The $\delta^{13}C$ lipid data indicated individual microorganisms that were involved in reverse methanogenesis, because their cell carbon was highly ^{12}C-enriched compared to the $\delta^{13}C$-methane that the organisms were presumably feeding upon. Amplified 16S-rRNA-gene sequences from these communities revealed the involvement of previously unrecognized subgroups of methanogen-like archaea, designated ANMEs[58] (anaerobic methane oxidation), which were identified in their natural niche using FISH-imaging microscopic techniques. This allowed the remaining skeptics to see images of the cells that seemed to carry out AOM[59–61] which, when combined with additional sophisticated $\delta^{13}C$ measures of their ^{13}C-depleted cell material[60], provided further convincing proof. Nonetheless, the ANMEs responsible for AOM still cannot be grown in pure culture, owing to the poor energy yield of the process and their lengthy (~6 months) doubling times.

Environmental genomics has provided an important boost to our understanding of how AOM works by linking this process to sulphate reduction[62]. Krüger *et al.*[63] reported that ANME assemblages on the bottom of the Black Sea contained a previously unidentified subunit of the methyl-coenzyme-M reductase, namely a nickel-containing cofactor (F_{430}) with

a molecular mass of 951 kDa, instead of the 905-kDa subunit found in cultivated methanogens. As methyl-coenzyme-M reductase is the final enzyme in forward-running methanogenesis, this novel cofactor might initiate reverse methanogenesis by removing one of the hydrogen atoms from the methane molecule. Hallam *et al.*[64], working with samples from the Eel River Basin, might have found an apparent lesion in the ANME-1 population at the level of methylene-methanopterin reductase that, if verified, might indicate that these AOM microorganisms are unable to carry out forward methanogenesis.

Despite the advances enabled by environmental genomics, the need for isolation of a pure ANME culture to further understand AOM remains. We still do not understand the initial activating mechanism for methane or the molecular currency by which electron equivalents are transferred from reverse methanogenesis to those linked to sulphate reduction. From the perspective of this article, it is vital to note that recent progress on AOM using molecular approaches came only after over 40 years of intensive research on methanogens, methanotrophs and the biogeochemistry of methane in marine ecosystems. If the process of AOM had not been first discovered by the geochemists, then the various ANME communities found at the bottom of the Black Sea and the Eel River Basin would, if examined solely by the community genomics approach, just seem to be large aggregations of otherwise normally functioning methanogens. Their AOM reverse functionality would not be appreciated, nor would any annotation of such a property in the database provide clues that they oxidized, not produced, methane. This point clearly illustrates the need for multidisciplinary approaches, in addition to molecular ones, to tackle difficult problems in biogeochemistry.

Case study 2: anaerobic ammonium oxidation. Similar to AOM[51], the bacterial pathway of anaerobic ammonium oxidation (ANAMMOX) was initially a theoretical process based on evolutionary and thermodynamics considerations by Broda[65]. Interestingly, around the same time, Bender *et al.*[66] hypothesized the stoichiometry of ANAMMOX as a possible bacterial reaction to account for the consumption of nitrate in deep-sea sediments. Experimental evidence for ANAMMOX from denitrifying sewage treatment reactors was first provided by Mulder *et al.*[67], and these reactions were shown to be biological by van de Graaf *et al.*[68] Since then, ANAMMOX activity has been observed in marine sediment[69], in the anoxic water columns of the Black Sea[70] and in the Golfo Dulce in Costa Rica[71], where it can account for a substantial fraction of nitrogen flux.

ANAMMOX bacteria have been difficult to purify and cultivate, owing in part to their slow growth rates — doubling times of approximately 2 weeks. However, molecular techniques were invaluable in this research, as culture-independent analysis of rDNA in samples purified from bioreactors revealed that ANAMMOX organisms were bacteria from a deep branch of the Planctomycetes[72]. They have various unique characteristics. For instance, the ANAMMOX reaction seems to take place in an internal, membrane-bound organelle, named the anammoxosome, which confines the reactive hydrazine (N_2H_4) intermediate. The anammoxosome is surrounded by a bilayer of ladderane molecules, which are composed of concatenated cyclobutane rings[73]. The ladderanes are excellent biomarkers for anammox bacteria[70].

Further rDNA sequencing identified two distinct genera of candidate planctomycetes, Candidatus *Brocadia anammoxidans* and Candidatus *Kuenenia stuttgartiensis*, from the denitrifying reactors. These are provisional designations, as neither bacterium has been isolated in pure culture. However, *K. stuttgartiensis* is currently being sequenced from mixed cultures in which this organism is dominant. The studies in the Black Sea revealed another distantly related planctomycete, named Candidatus *Scalindua sorokinii*[70]. Efforts to concentrate and purify these organisms have progressed through the development of unique culturing approaches, although it still remains a challenge to bring them into pure culture (G. Kuenen, personal communication).

The ANAMMOX example shows the important synergisms among experimental approaches of geochemistry, biogeochemistry, microbial and molecular ecology and even industrial microbiology — ANAMMOX pilot plants for nitrogen removal have been built — in identifying a novel pathway of metabolism, finding its occurrence in diverse environments, quantifying its importance and identifying the organisms responsible through genomics, even when these organisms are presently unobtainable in pure cultures. However, it is improbable that, in the absence of the original observations of anaerobic NH_4^+ disappearance in reactors and the physiological investigations that followed, genomics alone would have identified this novel process.

Linking genes to function

The links between gene sequences and function require well-characterized proteins from cultured microorganisms, because genomic information cannot be used unless it is already known which sequences relate to which proteins and functions. For example, the discoveries of such geomicrobial processes as the respiratory reduction of oxyanions of selenium and arsenic were made using classic approaches[74,75], as there was no annotated genomic database to identify functional genes. At present, after decades of investigation, we have accumulated a wealth of known, identified proteins and their sequences and functions. However, despite this progress, we still do not know the function of large portions (typically a third or more) of whole genomes from well-studied organisms. In some cases, the function is clearly present but the corresponding proteins and genes have not yet been found, such as the protein and genes for carbonic anhydrase in a *Synechococcus* sp. and *Prochlorococcus* spp.[76–78]. Within that same cyanobacterial clade, as a further example, the gliding motility mechanism found in some *Synechococcus* spp. has not been identified, despite the availability of whole genome sequences and multiple motile and non-motile strains for comparison. So, although the genomic work might move in leaps and bounds owing to newly available high-throughput gene sequencing, interpretation of the genomic data will be sharply limited by slower progress in the characterization of genes after organisms are brought into culture.

A call for future balanced approaches

In this article, we have attempted to stress the excitement and sense of invigoration that molecular approaches, including the emerging field of metagenomics, have brought to current research in microbial ecology and geomicrobiology. We can now view how the microbial world functions and by which organisms it is populated in ways that were unimaginable a mere 10 years ago. As this field grows, there is an increasingly recognized need to improve and develop techniques, expand communication between researchers and facilitate the training of students and postdoctoral researchers. These needs are currently being met by formal workshops sponsored by various scientific committees, such as SCOPE, and by societies, such as the American Academy of Microbiology[79]. But community metagenomics is primarily a computer-based informational system that is constrained in part by the validity of the database entries.

Therefore, community metagenomics, along with other related fields of computational biological databases (for example, proteomics and metabolomics), is constrained by the need to identify and annotate genes and proteins of unknown functions, as well as to test the validity of those that have already been annotated in the database. Such work cannot be successfully accomplished without continued efforts made with classical approaches, which include ecophysiology, isolation of new microbial species and biochemistry.

Nonetheless, metagenomics and its related fields are at the forefront of research and therefore tend to be strong magnets for rising young scientists and graduate students. They are also an important draw for funding from various agencies and private research foundations. But in doing so, there is a danger that metagenomics will eclipse the classic approaches to geomicrobiology and might give rise to the perception that such techniques are passé and unnecessary.

We advocate that it is essential to continue research, training and funding in the classic approaches to microbiology, as well as in the new molecular techniques. When such combined approaches are taken, the future for the field of geomicrobiology looks extremely bright. Our views, therefore, are best summarized by the following sentiment recently expressed by Professor Hans Trüper[80]: "Microbiology students today are under the spell of molecular biology or genomics and too often disregard the enormous width of microbiology and its applications. A good microbiologist should try to look at all aspects of his [her] science and use the chance to work in different fields of it during his [her] career."

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