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Detection of micro-organisms in the environment

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Introduction

Microbiologists have long suffered from the common observation that only a fraction of organisms observed under the microscope will cultivate using standard techniques. This limitation, which is both a limitation of present culture techniques and our poor knowledge of microbial physiology, has severely hampered our understanding of the structure and diversity of naturally occurring microbial communities. The problem can be further amplified after successful culture as morphology and physiological traits of isolated species are often ambiguous and provide few clues for identification. Recently, nucleic acid technology such as gene amplification and sequencing methods allied to comparative sequence analysis to determine evolutionary relationships have become new powerful tools in the arsenal of the microbial ecologist. Their use is rapidly giving rise to new sub-disciplines of microbiology such as molecular ecology and molecular phylogenetics. In 1990, two scientific articles showed both the power of these techniques and the limited nature of our understanding of natural microbial diversity [1,2]. Both scientific reports used molecular techniques to show that not only were numerous uncultured bacteria present in a natural community (bacterioplankton of the Sargasso Sea and a cyanobacterial mat from a hot spring, respectively) but also the predominant groups of bacteria in the community were uncultured. Since then, many similar studies have been performed on diverse environments and in every case the presence of unknown micro-organisms has been detected [3]. These analyses support the earlier observation that, as yet, fewer than 20% of extant micro-organisms have been described [4] and asks the question how well do we actually understand or appreciate the diversity of micro-organisms and their natural communities. Also, as micro-organisms are ubiquitous on this planet, we must question our knowledge of the Earth's biota.

Rationale of the molecular approach

The advantage in the use of molecular techniques in microbial ecology is that the need for culture is bypassed by directly isolating phylogenetically informative genes from the environment of choice. The first step is to use cell lysis methods to extract gross DNA present in any sample type. Specific purification steps may be necessary to remove contaminating natural substances such as humic acids which can inhibit gene amplification reactions [5]. The DNA preparation is then used as a target for gene amplification technology such as the PCR [6]. The use of PCR provides both sensitivity and scope in sample design, i.e. small volume samples can be analysed allowing the design of feasible multi-sampling programmes. The small subunit rRNA gene (SSU rDNA) sequence is usually the target of choice for PCR amplification from the gross DNA extractions. SSU rRNA molecules share a highly conserved secondary structure that reflects an evolutionary constraint with respect to their common function in all micro-organisms. However, their primary structure or nucleotide sequence is composed of a patchwork of highly conserved and variable domains [7]. SSU rRNA sequence domains which show universal conservation are the targets of PCR

Abbreviation used: SSU rDNA, small subunit rRNA gene.

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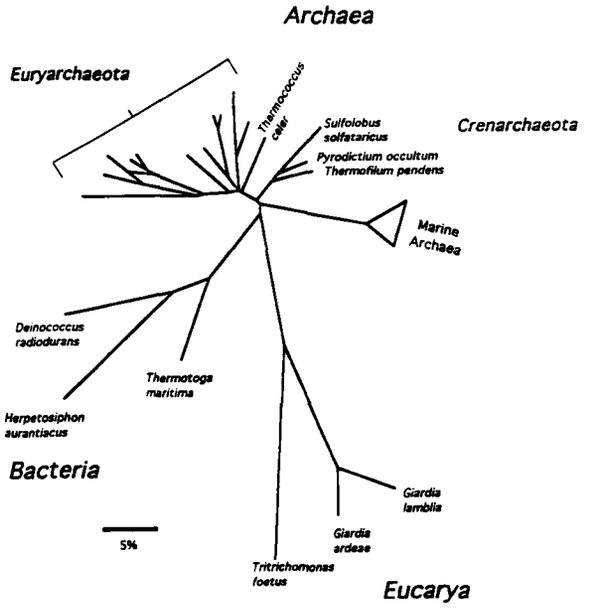
amplification primers which are designed to amplify all SSU rDNA sequences present in a sample. Therefore, one can recover SSU rDNA fragments from species that are unknown or prove uncultivatable. The PCR amplification products which are composed of a heterogenous population of SSU rDNA sequences from different micro-organisms – in theory, and with an efficient cell lysis method, reflecting the microbial population structure – are then segregated into clonal lineages of single SSU rDNA fragments by cloning the PCR amplification products via plasmid vectors into *Escherichia coli.*

SSU rDNA sequence elucidation from the *E. coli* subclones can then be used to prepare a molecular analysis of the original microbial community. Primarily, the information provided by the SSU rDNA sequence is phylogenetic, i.e. comparison of SSU rDNA sequences allows inference of evolutionary relationship. In 1990, Woese et al. proposed a new 'natural' taxonomy for all organisms based on comparative SSU rRNA sequences [8]. The authors proposed the substitution of the five-kingdom classification of life [9] with a new three-domain classification (see Figure 1). The three domains are Bacteria (the eubacteria), Archaea (the archaebacteria) and Eucarya (the eucaryotes). Presently, a rapidly expanding database of over 1000 different SSU rRNA sequences derived from cultured bacteria exists for comparative SSU rRNA sequence analysis [10]. Comparative SSU rRNA sequence analysis can also be used to design DNA hybridization probes to recognize different groups of related micro-organisms. These DNA probes can be used in hybridization experiments to quantify the amount of similar SSU rDNA sequences present among the gross DNA prepared from a particular sample. In this fashion, relative abundances of both cultured and uncultured micro-organisms can be measured in any environment. Activity measurements for different microbial groups can also be assessed by using the DNA probes in hybridization experiments against total rRNA extracted from environmental samples.

Figure 1

Universal phylogenetic tree

This tree is based on small subunit rRNA sequence comparison. The three primary domains, Bacteria, Archaea and Eucarya are noted along with both present subdomains of the Archaea, Euryarchaeota and Crenarchaeota. The placement of the marine Archae on the tree is problematical and they appear equidistant from the Crenarchaeota and the Euryarchaeota. They may represent a third archaeal lineage.



The marine Archaea

One clear example of the surprising and interesting data derived from such molecular approaches to microbial ecology was found in two scientific reports published simultaneously in 1992 [11,12]. These reports revealed the presence of novel Archaea SSU rDNA sequences in the coastal waters of North America. Quantitative estimates indicated that these archaebacteria represented 1–5% of the microbial population and activity in these waters. Originally recognized in 1977 [13], the Archaea have been generally known as extremophiles inhabiting such hostile environments as saturated brines and acidic hot springs. An evolutionary related but phenotypically diverse group of micro-organisms, the Archaea are presently divided into two subdomains, Crenarchaeota (extreme thermophiles) and Euryarchaeota (methanogens, sulphur-reducers and halophiles). The reports of novel archaeal SSU rDNA sequences present in oxygenated oceanic surface waters were striking observations. The evidence suggested that the origins of these sequences were neither anaerobic sediments nor deep-sea hydrothermal vents (known habitats of marine Archaea) but a mesophilic, aerobic environment co-inhabited by bacteria. It is not clear as yet where these archaeal SSU rDNA sequences fit into the phylogenetic tree. The majority of the marine archaeal sequences cluster well together as a related group but the higher-order relationships to the two Archaea subdomains are unclear. They show some relationship to the Crenarchaeota but may yet represent a third archaeal lineage (see Figure 1). A second group of marine archaeal sequences appear to map within the Euryarchaeota

subdomain. To emphasize the significance rather than the novelty of these observations, a recent report has shown that these marine Archaea constitute up to 34% of the prokaryotic biomass in coastal Antarctic surface waters [14]. The previous lack of knowledge of these Archaea in Antarctic waters has been equated with survying 1 km^2 of the African savanna and missing over 300 elephants [15]. Further reports indicating the presence of similar marine archaeal SSU rDNA sequences in the Arctic Ocean, Mediterranean Sea and Atlantic abyssal sediments show a cosmopolitan ecological distribution for these micro-organisms throughout the Earth's largest ecological habitat, the oceans.

Future perspective

The impact of molecular approaches on the study of microbial ecology is correctly being termed a revolution. A new and provocative view of naturally occurring micro-organisms with a diversity far greater than previously thought humbles the discipline of microbiology. With some alarm, we note the evidence that suggests that most cultured micro-organisms represent minute fractions of the natural communities from which they were isolated. In the short term, we can expect a plethora of novel SSU rDNA sequences isolated from different environments and an increasingly more informative and intricate phylogenetic 'Tree of Life'. Also, clearer and more detailed pictures of microbial communities in the environment will be developed. In the longer term, some difficult questions will require explanations. We cannot simply equate close phylogenetic relatedness with shared phenotypic characteristics. Therefore, few physiological assumptions can be made if SSU rDNA sequences similar to those of cultured organisms are isolated from a particular environment. Presently, we can only guess at the physiology of the marine Archaea. However, at least we now know that they exist and we do have 'tools', DNA probes, to track them which should aid their isolation by culture. Perhaps, in the continuing study of the Earth's biodiversity, we will not miss too many more elephants.

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