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Towards uncultured-microbe genomics

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Abstract

DNA libraries from uncultured soil-microbes have revealed insights into the functional and genomic diversity of soil bacteria.

Significance and context

Over the past decade, molecular techniques have revealed a diversity of microorganisms not amenable to study by conventional culture-based techniques. At the same time, molecular biology has revealed more and more about already well-known organisms, culminating in the current public availability of the complete nucleotide sequences for around 30 prokaryotes. Since the existence of many uncultured (and perhaps unculturable) bacteria in diverse habitats was revealed by ribosomal RNA sequences, however, relatively little has been learned about these mysterious organisms - our knowledge of their ecology and metabolism remains extremely patchy. At least one previous report has suggested using libraries of environmental DNA clones - DNA isolated directly from soil - to investigate the biology of these organisms outside culture, but Rondon *et al*. demonstrate significant expression of genes from environmental DNA cloned into bacterial artificial chromosome (BAC) libraries. This is the first technique that promises easy access to at least some of the functional diversity of environmental microbes by direct screening for particular metabolic activities, and allows microbiologists access to the full arsenal of molecular biological techniques for investigating this diversity.

Key results

The authors constructed two BAC libraries from environmental DNA samples, termed metagenomic libraries. The first library contained a total of approximately 100 Mb of environmental DNA with a mean fragment size of 27 kb, while the second, constructed using a refined technique, contained 24,576 cloned fragments of mean size 44.5 kb - making a total of around 1000 Mb of environmental DNA. Antibacterial, lipase, amylase, nuclease, and hemolytic activity could all be detected from the smaller of these libraries, and DNase and antibacterial genes from two clones were successfully sequenced. Seven of the clones from the smaller library had detectable 16S rRNA genes, which could be used to place the clones in a wide diversity of bacterial phyla - low G+C Gram-positives, cytophagales, proteobacteria and acidobacteria.

Methodological innovations

Environmental DNA was extracted using fairly standard methods, although pulsed-field gel electrophoresis was used to isolate only high-molecular weight DNA for use in the second library. The major advance in this paper is the use of BAC vectors, which allows significant expression of heterologous environmental DNA in *Escherichia coli*, making it possible to screen the library for novel functional genes using standard, quick biochemical assays. The authors also use an interesting PCR protocol involving competitive oligonucleotides to amplify 16S rRNA genes in the presence of *E. coli* DNA, which they intend to describe in detail elsewhere.

Conclusions

A strategy of BAC cloning of large environmental DNA fragments allows both traditional and functional genomic studies of uncultured microorganisms to be carried out.

Reporter's comments

As with any new technique, there are many unanswered questions about this work. Most important, perhaps, is to investigate how efficiently genes from different bacteria are expressed in BACs. Clearly the efficiency of expression will depend on the size of inserts, on how completely environmental genomic DNA is sampled in the library and on how similar the protein translation, modification and export machinery is between *E. coli* and the source of the cloned DNA. These are all open questions, but we can be cautiously optimistic given that previous results from these authors suggest that a wide range of *Bacillus cereus* genes are successfully expressed from a BAC library. Also important is the phylogenetic range of DNA captured in the libraries. This is only reported from 16S rRNA genes from the smaller library, which are likely to reveal only a small fraction of the total diversity covered. It would be interesting to see what diversity of rRNA and other genes are present in the larger library reported here.

In principle, at least, we may one day see the complete genome sequence for organisms that have never been kept in pure culture, and perhaps understand both why microbiologists have failed to culture them, and what part they play in microbial ecosystems. The sort of 'metagenomic library' described here should have an important role in understanding the microbiology of many environments and in learning about the biology of currently uncultured bacteria. By allowing traditional and functional genomics of uncultured microorganisms, the method used in this paper should also be of use to biochemical prospectors searching for useful industrial and medical proteins.

Table of links

Applied and Environmental Microbiology

References

1. Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, et al: Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol. 2000, 66: 2541-2547. 0099-2240