





have been found in many studies throughout the world's oceans, but have never been cultured. Conversely, many examples of the other common marine  $\alpha$ -*Proteobacteria* group, the *Roseobacter* cluster, have recently been isolated as colonies on simple nutrient media from coastal water and their physiology has just started to be studied. This indicates that whereas some novel uncultured bacteria will be difficult to isolate, others will be easy. We must try to culture the most abundant bacteria in all environments to study their physiology, to understand their roles and to tap this large biotechnological resource.

● **Expanding knowledge of bacterial biodiversity**

In 1990 about 10 divisions of the domain *Bacteria* were known. Now 40 have been described and this remarkable expansion in our knowledge of bacterial biodiversity has occurred entirely due to the recent explosive growth of molecular approaches (Fig. 1). Furthermore, 13 of these divisions are currently known only from sequences and have no cultured representatives. Some of these divisions of uncultured bacteria are phylogenetically extremely varied, for example WS6 and OP11 are the most diverse divisions known, showing 26 and 33% sequence divergence, respectively.

These observations further indicate the need for greater effort in growing these organisms and will be illustrated by consideration of three bacterial divisions known to be abundant from their 16S rRNA sequences but with few cultured representatives.

● **The *Nitrospina* division**

In 1994 Erko Stackebrandt's group reported that a newly isolated Gram-negative, obligately anaerobic, heterotrophic bacterium belonged to a new bacterial group. They called this organism *Holophaga foetida* as it originated from smelly, anoxic sediment and suggested it could belong to the  $\delta$ -*Proteobacteria*. At a similar time my research group in Cardiff obtained several bacterial 16S rDNA sequences from very deep, Japan Sea sediment, which also seemed to be deep branching  $\delta$ -*Proteobacteria*, called the JAP504 cluster. Collections



of further 16S rDNA sequences have now revealed that these bacteria belong to a new division, named the *Nitrospina* division after *Nitrospina gracilis*, which is also in this group. This division has representatives from a wide variety of marine, freshwater and terrestrial habitats and consists of 12 subgroups made up of 188 sequences, about 94% of which are from uncultured organisms. The two named cultures in this division are physiologically very different; *H. foetida* is an anaerobic heterotroph degrading a plethora of complex organic compounds and *N. gracilis* is an aerobic, nitrifying chemolithotroph. Such diversity indicates that this division is not only widespread in nature, but also as physiologically diverse as the *Proteobacteria*.

● **The WS6 division**

The WS6 division is one with no cultured representatives. Earlier this year Norman Pace and colleagues designed PCR primers to amplify the 16S rDNA of members of this division from 12 diverse environments. They found WS6 members to be most abundant in anaerobic environments but were also present in some aerobic habitats. They found 57 different 16S rDNA clone types that increased the number in this division to 60. These clones were isolated from marine, freshwater and hot-spring sediments, contaminated aquifers and one from topsoil. They surmise that members of this division might be anaerobic, but until they are cultured this will not be confirmed. This study convincingly shows that the undiscovered bacterial diversity in the environment, from even widely distributed habitats, is almost certain to be enormous.

ABOVE LEFT: Phase contrast micrograph of an agar-coated slide enrichment of bacteria from freshwater sediment. There are filamentous (probably *Beggiatoa* spp.) and non-filamentous bacteria present, probably representing culturable and non-cultured species. COURTESY J.C. FRY

ABOVE RIGHT: A sub-tropical river with sediment that is a likely source of many uncultured bacteria known only from their 16S rRNA gene sequences. COURTESY H.G. WILLIAMS, CARDIFF

## Further reading

**Barnes, S.M., Takala, S.L. & Kuske, C.R. (1999).** Wide distribution and diversity of members of the bacterial kingdom *Acidobacterium* in the environment. *Appl Environ Micro* **65**, 1731–1737.

**Dojka, M.A., Harris, J.K. & Pace, N.R. (2000).** Expanding the known diversity and environmental distribution of an uncultured phylogenetic division of *Bacteria*. *Appl Environ Microbiol* **66**, 1617–1621.

**Giovannoni, S.J., Britschgi, T.B., Moyer, C.L. & Field, K.G. (1990).** Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345**, 60–63.

**Hugenholtz, P., Goebel, B.M. & Pace, N.R. (1998).** Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* **180**, 4765–4774.

For access to the current range of aligned sequences of 16S rRNA genes and associated information for cultured organisms and environmental isolates go to the Ribosomal Database Project website at <http://www.cme.msu.edu/RDP/html/index.html>

## ● The *Acidobacterium* division

The distribution and diversity of the *Acidobacterium* division, which has only one cultured member, has been investigated by similar methods. These bacteria are very widespread, they are present in many different soil types, marine and freshwater sediments, as well as in hot-spring mats and sediment. Furthermore, they sometimes form the dominant group in a habitat. In one set of arid soils they were the dominant phylogenetic group making up 51% of the 16S rDNA clones isolated, but were entirely absent from culture plates from the same habitat. Despite this, culturing some of the group might not be difficult because the one cultured member of the division, *Acidobacterium capsulatum*, is an aerobic, mesophilic, chemo-organotroph able to use a variety of carbon sources and to grow up to pH 6.0. However, other members of the division might well need specialized approaches for their culture.

## ● Future challenges

These examples indicate that culturing many of these 'unculturable' bacteria will be an enormous task. However, the following arguments suggest that if more effort were put into growing these bacteria more of them would prove culturable. Many bacteria that are grown on plates do not match existing cultured bacteria. When effort is put into growing novel aquatic bacteria they are sometimes grown relatively easily once suitable media are developed (e.g. *Legionella* spp.). Little research effort is expended in studying unspecialized, aerobic, heterotrophic bacteria, whilst studies of specialist groups abound, even when they are hard to cultivate (e.g. methanogens, sulphate-reducing and nitrifying bacteria). In 1995 Karl Stetter's research group was the first to successfully culture a prokaryote only previously identified from a 16S rRNA gene sequence. In this case the organism was a hyper-

thermophile from a hydrothermal vent and isolation was by selective enrichment, *in situ* hybridization with a 16S rRNA probe and micromanipulation. Similarly, a *Thermus aquatilis*-like strain and *Synechococcus lividus* strains that were prevalent as gene sequences, but not yet isolated in culture, have been isolated from hot-spring mats by David Ward's group. So I believe that the time is right to attempt to obtain further cultures of some major groups of 'unculturable' marine bacteria. Furthermore, the *Roseobacter* and *H. foetida* examples described above add to the arguments for cultivating the uncultureables.

● **John C. Fry** is Professor of Microbial Ecology and Deputy Director of Cardiff School of Biosciences, Cardiff University, Main Building, Museum Avenue, Cathays Park, Cardiff CF10 3TL. Tel. 029 2087 4190; Fax 029 2087 4305; email [fry@cardiff.ac.uk](mailto:fry@cardiff.ac.uk)



LEFT: Part of an activated sludge aeration tank from a sewage treatment works showing inflows of settled sewage (lighter coloured inflow) and re-circulated sludge (darker coloured inflow). Once again this is a likely source of many uncultured bacteria known only from their 16S rRNA gene sequences.

COURTESY J.C. FRY