Microbial diversity beyond *E. coli*: new microbial worlds, new concepts in biology

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Microbial diversity explores the universe of microorganisms beyond classical models such as *Escherichia coli*, influenza virus, or *Saccharomyces cerevisiae*. Exploring such new microbial worlds is essential for a microbiology which needs to learn about all the scientific and practical possibilities offered by billions of years of microbial evolution. Here we illustrate some examples of how studying a wide range of microbial diversity can assist microbiology as a fundamental and a practical science.

Australian microbiologist 'Vic' Skerman long ago opened our eyes to a microbial world beyond *E. coli*'s relatively flat land – a truly biospherical world including predatory *Bdellovibrio*, stalked iron-encrusted *Gallionella*, and wonderful fruiting myxobacteria with their microbonsai tree-forming *Chondromyces* and *Stigmatella*[1]. Since then, with application of rRNA gene sequencing and genomics, knowledge of microbial diversity has exploded to more than 60 phyla of Bacteria, perhaps at least 10 of Archaea, many uncultured[2]. So far we have only perhaps 9000 species of bacteria formally described[3], suspecting that there are at least a thousand-fold as many, since there seem to be at least 4000 species-level genomes in a single gram of soil and possibly more than a million in just a 10 gram sample of some soils[4-6], and even in a litre of seawater there may be at least 38,000 different kinds of bacteria, with many millions probable in the whole ocean[7,8]. Even among the published species there is a wonderful variety of cell structure, shape, size as well as physiology and ecology. And then there are all the viruses and the eukaryote microbes comprising the major diversity of eukaryote trees. Why should we study this rich baroque ornamentation at the micro scale of life? And how does it inform our science of microbiology?

The reasons come under several categories – most profoundly, microbial diversity is essential to understanding biology itself and solving major biological problems. To understand ecosystem functions of bacteria and to understand bacterial evolution clearly requires us to understand whole phyla not yet cultured. The central role of viruses in microbial communities has only been revealed recently[9,10]. Diversity itself contributes to ecosystem function. New applications similar to the *Thermus aquaticus*-derived Taq polymerase[11] will derive from the full panoply of bacteria. And of course the true microbiologist is not likely to admit that their favourite microbe is any less fascinating than a dolphin or a sugar glider.

Planctomycetes as a divergent phylum with major biological implications

Concepts of prokaryote and eukaryote have been important not only for microbiology but for biology in general, but have recently been subject to considerable controversy[12-16], with implications for microbiological education. The planctomycetes challenge our classical notions of what a bacterium is, of prokaryotic cell organisation and relationships of bacteria to eukaryotes[17,18]. They share a remarkable cell plan in which internal membranes divide the cell into compartments[19]. In *Gemmata obscuriglobus*, a radiation-resistant planctomycete[20] isolated from a freshwater dam in Queensland[21], this includes a double-membrane coat around the nucleoid DNA resembling the nuclear envelope, a defining feature of eukaryotes (Figure 1). The implications for understanding how the eukaryote nucleus evolved are major, and have connected microbiology once again with the rest of biology. There may even be implications for human health, since genomics now suggests that the intracellular pathogenic chlamydias may be some of the closest relatives of the free-living planctomycetes among the bacteria[22]. Recently, a functional process resembling eukaryote-specific endocytosis mechanisms for uptake of macromolecules has been discovered in *Gemmata obscuriglobus*, suggesting functional as well as structural homology of planctomycetes with eukaryote cells, and giving insights into possible origins of eukaryote endomembranes[23]. But planctomycetes have another, more biotechnological, surprise.

Anammox planctomycetes – new functions discovered in a divergent bacterial group

In 2002, an industrial-scale plant was cleaning Rotterdam's wastewater[24,25] using a process unknown to microbiology before
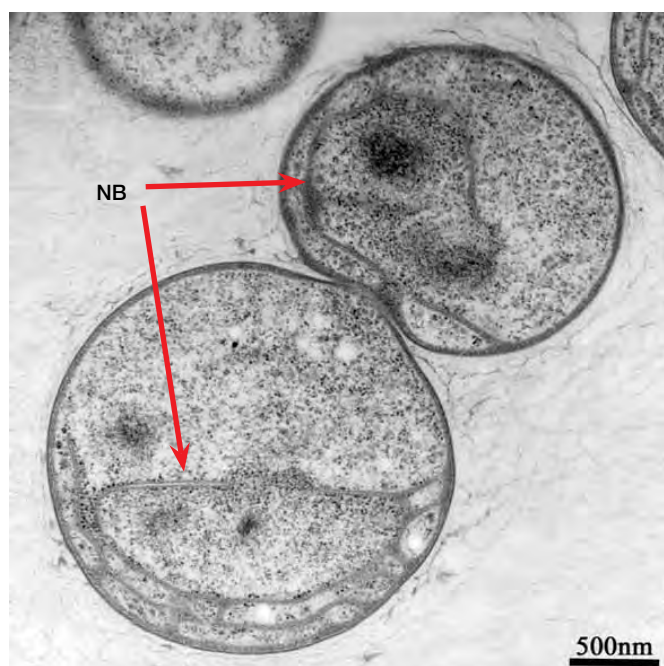
Figure 1. Transmission electron micrograph of sectioned budding cell of planctomycete *Gemmata obscuriglobus* prepared by cryosubstitution showing membrane envelopes surrounding nuclear bodies (NB) containing nucleoid DNA.

1995 – anaerobic oxidation of ammonium. This process not only can clean up wastewater nitrogen, but since the process is chemoautotrophic and uses no organic substrate, it actually uses up CO_2, so is a carbon-debt reducer. The bacterium responsible was only identified in 1999 as a member of the phylum Planctomycetes[26], and these bacteria are also compartmentalised. Cells of these anammox (anaerobic ammonium oxidation) Planctomycetes have a unique membrane-bounded organelle, the anammoxosome, where enzymes for ammonium oxidation are housed[27].

Through study of a divergent bacterial group, we have not only found the basis for a new environmental remediation program and an essential missing link in the global nitrogen cycle[28] – in the anammoxosome we have revealed a new type of bacterial organelle[27]. This is a compartment bounded by a single membrane and containing enzymes needed for ammonium oxidation to N_2 using nitrite as electron acceptor (Figure 2). It is now known that ATP synthase is lodged in this membrane, consistent with a model where a proton motive force generated across the membrane during oxidation is used to generate ATP within cells, a process we are more familiar with from eukaryote mitochondria[29]. This biochemistry may form the basis for new systems of generating energy from sewage as well as remediating wastewater nitrogen[30].

Marine actinobacteria – new products from going beyond *Streptomyces*

Microorganisms have contributed immensely to biodiscovery

efforts to reveal sources of new chemical compounds to act as antibiotics, anti-cancer chemotherapeutics and other biopharmaceuticals. The actinobacteria from soil, especially species of genus *Streptomyces*, have been immensely useful, and their microdiversity has meant a rich source of chemical diversity can be tapped even within one genus – the source of vancomycin, rifampicin, clavulanic acid and daunomycin among other important biopharmaceuticals[31]. However, understanding the true extent of actinobacterial diversity and exploring new habitats for such bacteria can yield new chemical diversity with pharmaceutical potential. This is illustrated by the amazing potential revealed within just one genus of marine actinobacteria, *Salinispora*[32] – the anti-TB antibiotic rifamycin[33], the anti-MRSA agent arenimycin[34], as well as the proteasome-inhibiting anti-cancer agent salinosporamide A and many other diverse compounds of potential pharmaceutical use are all produced by members of this one genus. *Salinispora* can be cultured from sponges of the Great Barrier Reef among other habitats[35]. Thanks to unsuspected microbial diversity, natural product screening programs by pharmaceutical companies are again exploiting microbial screening for new natural products. Salinosporamide A (now known as Marizomib) from *Salinispora tropica* is
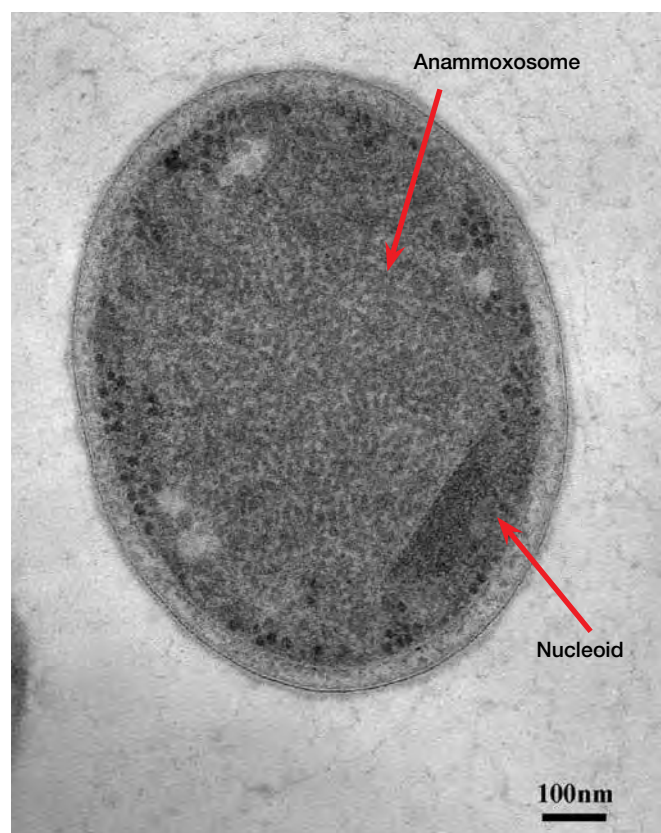

Figure 2. Sectioned cell of cryosubstituted anammox bacterium *Candidatus* Brocadia showing anammoxosome compartment containing tubules and the crescent-shaped fibrillar DNA nucleoid in a major compartment outside the anammoxosome. Micrograph generated with assistance of Marc Strous, Radboud University of Nijmegen and Rick Webb, The University of Queensland.

already in Phase 1 clinical trials as an anti-cancer agent[36]. We do not need to rely on soil actinobacteria alone for new bacterial pharmaceuticals[37], and now can open a whole new sea chest of microbial diversity.

Mimivirus – a virus with a virus and a giant genome with evolutionary lessons

Microbial diversity is so extensive that sometimes it becomes hard to determine at first whether a microorganism is a cellular life form or not. This was the case with the discovery of the exceptionally large Mimivirus which infects amoebae, where during a study of potential new pathogens in cooling systems, an organism was detected that appeared to be a Gram-positive bacterial coccus[38]. It turned out to be a very large virus in the bacterial size order up to ca. 0.8 μm diameter and with a genome size of 1.2 Mb overlapping with that of the smallest bacteria[39,40] (Figure 3). An X-ray laser has been successfully used to study single Mimivirus virions[41]. The complexity of its genome, with genes even for such cellular processes as translation, challenges our concepts of a 'virus'[42], and thus contributes to our deep knowledge of microbiology. The genome of Mimivirus has even resulted in the hypothesis of a fourth domain of life connecting the NCDLV (nucleocytoplasmic large DNA viruses) to an unsuspected ancestral form of life[43], albeit a controversial one[44]. Even for classical medical microbiology, discovery of Mimivirus has meant insights into unsuspected pathogens which may exist in the microbial world[45,46]. Remarkably, the first instance of a virophage[47] – a virus infecting a virus – has been found with the Mimivirus model, opening a new vista in our concepts in microbial parasitism.

Diverse futures from diverse microbes

Microbial diversity is where the future of microbiology is – beyond *E. coli* there is new cell biology and physiology, and new insights into that ancient past we share with the other cellular forms
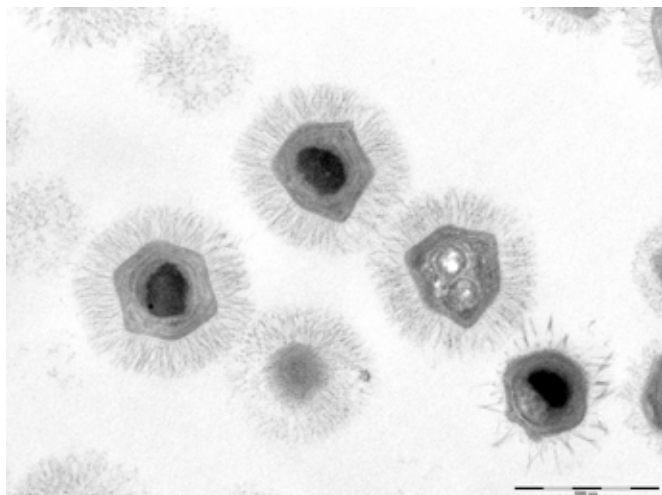

Figure 3. Electron micrograph of Mimivirus (courtesy of Didier Raoult, Unité des Rickettsies, IRD-CNRS, Marseilles).

of life. Whether giant Archaea in tropical mangrove waters[48], multicellular magnetotactic sphere-forming cells[49], *Shewanella* bacteria with their own electrically conducting nanowires[50] or a new bacterial phylum with anaerobic cells generating their own oxygen[51], the environmental microbial world has much more than we have dreamt of. So too the diversity of bacterial life colonising humans may be more diverse, as shown by bacterial species in cystic fibrosis[52] – beyond *Pseudomonas aeruginosa* many different bacterial phyla are relevant to lung infection in this important disease, but until application of modern approaches to microbial diversity they were invisible to us.

Understanding the evolution of microorganisms will form the basis for understanding microbiology through understanding the full range of microbial diversity. In hospitals and human cities, and the oceans, coral reefs and soils impacted by climate change[53,54], such diversity may still be evolving, and understanding it and how to work with it will be important for our survival individually and as a global community.

Acknowledgements

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