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EDITORIAL

Microbiology: An Endless Frontier

Over the last four decades the molecular revolution in biology has transformed the life sciences, washing away the bridges between many allied disciplines. Many years ago, Arthur Kornberg lamented that the turbulent advance of 'molecular biology' had washed away the bridges to biochemistry and the traditional approach to the molecules of life. Fortunately, the dramatic rise of structural biology, fuelled by X-ray diffraction, NMR spectroscopy, computational methods and more recently a host of powerful new techniques for investigating molecules, assemblies, cells and tissues, has ensured that chemistry and physics are essential for the solution of important problems in biology. In India, the curriculum for students of the mushrooming courses in 'biotechnology', provides little of the background that is needed to savour the excitement of modern biology. Having never been formally schooled in biological subjects, I have watched with amazement the expansion of the field and the deep inroads made by other disciplines into an area that was once the preserve of those who studied botany and zoology. The most exciting and challenging problems of chemistry undoubtedly lie in biology. The question of what constitutes biology has always intrigued me, since I have many times been advised by molecular biologists that the subjects that seem of great interest to an outsider like me are hardly worthy of attention. Years of listening to dismissive judgements have taught me to read the literature, searching for arguments to bolster my case that biological research needs to be enthusiastically pursued on a much broader front in India. Thus, when the latest volume of the *Annual Review of Microbiology* (eds Gottesman, S. and Harwood, C. S., 2009, vol. 63) appeared on my desk for assignment of a reviewer, I spirited the book away hoping to read authoritative perspectives about an area that must surely be viewed as biology's 'endless frontier'. How does one become a microbiologist? This question seemed to find an interesting answer in Lars Ljungdahl's prefatory chapter entitled 'A life with acetogens, thermophiles and cellulolytic anaerobes' (pp. 1–25). The author describes his entry into a four year technical secondary school, on the day World War II broke out, and adds that 'mathematics, physics, chemistry and mechanical engineering' were emphasized; excellent training for an entry into biochemistry and microbiology. Even a cursory glance at prefatory chapters in most

related annual reviews (biochemistry, genetics, immunology, medicine, phytopathology and plant biology) will reveal that many immensely productive scientists in these areas have received training in disciplines other than biology or a specialized sub-discipline.

Idly browsing through the volume of *Annual Reviews* my attention was drawn to an article entitled 'Anaerobic oxidation of methane: progress with an unknown process' (Knittel, K. and Boetius, A., *Annu. Rev. Microbiol.*, 2009, **63**, 311). Could the chemistry of the oxidation of as simple a molecule as methane still be unknown? The abstract provided an equation that surely has no parallel in the laboratory: $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$. While an editorial column is not the place for a chemical equation, I cannot resist the temptation to wonder at nature's amazing chemical virtuosity. Here is an example of the largely unreactive hydrocarbon, that has acquired notoriety as a 'greenhouse gas, which has so far contributed an estimated 20% of postindustrial global warming', being smoothly converted to other substances that seem more benign, by a process that is hardly understood in molecular detail. The authors lament: 'A great deal of biogeochemical research has focussed on the causes and effects of the variation of fluxes of methane throughout earth's history, but the underlying microbial processes and their key agents remain poorly understood. This is a disturbing knowledge gap, because 85% of the annual global methane production and about 60% of its consumption are based on microbial processes'. Geology, chemistry and microbiology appear to intersect in any attempt to understand the anaerobic oxidation of methane in the seabed, which is the home for the anaerobic methanotrophs, the microbes that carry out this remarkable chemistry. The review by Knittel and Boetius draws attention to 'consortia' of microorganisms involved in methane oxidation, suggestive of the role of multiple groups of methanogens in the natural processes that determine methane fluxes. The idea that biogeochemistry may be controlled by communities of organisms is increasingly gaining ground. Indeed there is a growing body of literature that suggests that studies of genes extracted from isolates of microbial communities may provide valuable biological insights; a significant departure from a more traditional view that genomics is best applied to 'pure' organisms that can be grown in culture in the laboratory.

The first large scale study 'to explore environmental bacteria in a culture-independent manner by isolating DNA from environmental samples' appeared a little over five years ago. In a paper entitled 'Environmental genome shotgun sequencing of the Sargasso Sea', Craig Venter and his colleagues 'generated, annotated and analysed' as many as '1,045 billion base pairs of non-redundant sequence' (*Science*, 2004, **304**, 66). These authors estimate that their isolate contained as many as '1800 genomic species ... including 148 previously unknown bacterial phylogenies'. This study threw up as many as '1.2 million previously unknown genes'. I was attracted to this report since the Sargasso Sea has been famously featured in fiction, with Jules Verne's immortal Captain Nemo diving into its waters in his submarine, in *Twenty Thousand Leagues under the Sea*. Verne's description bears retelling: '... cold, quiet immovable ocean called the Sargasso Sea, a perfect lake in the Open Atlantic. ... Such was the region the Nautilus was now visiting, a perfect meadow, a close carpet of seaweed, ficus and tropical berries. So thick and so compact that the stern of a vessel could hardly tear its way through it'.

The oceans cover approximately 70% of the earth's surface. Much of the methane, that I began this column with, originates below the ocean floor. However over 90% of the methane produced is consumed by bacteria that inhabit the 'sulfate-penetrated seafloor zones' by the process of anaerobic oxidation (*Annu. Rev. Microbiol.*, 2009, **63**, 312–313). Clearly, marine microorganisms are a key element in controlling methane fluxes; even more interestingly, the potential for carrying out unusual chemistry by marine organisms is both vast and untapped. The 2004 paper by Venter *et al.* launched a new approach to studying microbial communities and diversity in the oceans, demonstrating that the techniques of shotgun DNA sequencing may allow rapid progress in our understanding of microbial evolution, ecology and chemistry. Indeed the initial report in *Science* in 2004 discusses the 'photobiology of the Sargasso Sea', noting that this global gene-fishing approach yielded 'more than 782 new rhodopsin-like photoreceptors'. Such studies provide an extraordinary wealth of genomic data. When translated into protein sequences they add an entirely new dimension to studies of sequence evolution. The competing imperatives of random mutations and selective pressures for maintaining biochemical function find reflection in this large body of sequence data. 'Data mining' (a particularly infelicitous term) may present great opportunities for gaining useful biochemical and biological insights, although this may require thoughtful analysis, which is not blinded by the brute power of computational methodology.

There is a spirit of romance and adventure in exploring the oceans. Just over two years ago the reports of the *Sorcerer II* expedition appeared in *PLoS Biology*, grouped as a part of the 'Oceanic metagenomics collection'. An engaging commentary provides the background to this fascinating study (Gross, L., 'Untapped bounty: Sampling

the seas to survey microbial diversity', *PLoS Biol.*, 2007, **5**, e85). The expedition drew inspiration from the 19th century *Challenger* expedition (1872–76), mounted by the British, which Gross notes, 'discovered hundreds of new genera and nearly 5000 new marine species, its gun stations replaced with research stations. ...'. The 21st century expedition traversed 9000 kilometers, periodically pumping seawater 'through a series of increasingly fine filters' which were then frozen and stored for DNA isolation. The sequences, referred to as Global Ocean Sampling (GOS) sequences, must turn out to be a treasure trove for computational biologists, who revel in handling thousands of sequences, looking for patterns that might reveal the hidden hand of evolution fashioning the relationships between protein structure and biological function. The *Sorcerer II* expedition has added dramatically to the number of predicted sequences of proteins, many of which do not have a known biological function. In describing an 'expanding universe of protein families', Venter and his colleagues note 'that new (protein) families are being discovered at a rate that is linear or almost linear with the addition of new sequences, implying that we are still far from discovering all protein families in nature' (Yooseph, S., *PLoS Biol.*, 2007, **5**, e16).

Microbiology is being transformed as environmental ('meta') genomics gains ground. A great deal of current interest in microbial ecosystems stems from the realization that the fluxes of the two major gases that contribute to global warming, carbon dioxide and methane, may be controlled by microbial metabolism. Microbes which remained hidden from the prying eyes of scientists, until Leeuwenhoek invented the microscope, coexist comfortably with insects, plants and animals; the symbiotic relationships are often mutually beneficial. Microorganisms can provide complementary and essential biochemistry to facilitate survival of hosts; the gut is a comfortable habitat for many bacteria. Biology is full of examples of cooperation and coexistence. 'Metagenomics' has been applied to bacterial communities inhabiting the hindgut of the wood-feeding termite. The ability of termites to degrade wood is 'important both for their roles in carbon turnover in the environment and as sources for biochemical catalysts for efforts aimed at converting wood into biofuels' (Warnecke, F. *et al.*, *Nature*, 2007, **450**, 560). The metagenomic analysis revealed 'a large diverse set of bacterial genes for cellulose and xylan hydrolysis'. Intriguingly, genes for hydrogen (H₂) metabolism, CO₂ conversion and N₂ fixation were also found, emphasizing the versatility of microbial chemistry. The authors note that 'termites may be considered to be rich reservoirs of bacterial enzymes' adding that their 'study illustrates how complex a 1 µl (microliter) environment can be'. The millions of microorganisms that inhabit both terrestrial and marine environments must surely be regarded as an endless frontier for chemical biology.

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