Bacterial physiology

New kingdom for nitrogen fixation

from John R. Postgate

THE report[1] by Murray and Zinder on page 284 of nitrogen fixation by a strain of methane-producing bacteria may seem to be just another addition to the list of nitrogen-fixing bacteria. In fact, it is something of a landmark in bacterial physiology, as it provides the first example of diazotrophy — the ability to 'fix' atmospheric nitrogen by converting it to a metabolically available form — outside the kingdom of 'regular' bacteria.

The methane-producing bacteria or methanogens, which form 'marsh gas' from decaying vegetation and which inhabit sewage sludge, the rumens of ruminants and the inner depths of aqueous sediments, are a difficult group of bacteria to study. They are very strict anaerobes, completely inactivated by exposure to air; they require complex media, with fixed nitrogen plus reducing agents, to grow in the laboratory; and they are difficult to obtain in pure culture because they often cohabit with other anaerobes, particularly sulphate-reducing bacteria[2]. Indeed, a report thirty years ago[3] of nitrogen fixation by the methanogen *Methanobacillus omelianskii*, soundly based on uptake of $^{15}N_2$ by the bacteria, collapsed when the 'species' was shown to be a consortium of two different bacteria[4]. There was no evidence to show whether the methanogen rather than its consort was responsible for the diazotrophy.

There the matter rested for many years; though new diazotrophs were reported among other bacterial groups, the sheer awkwardness of the methanogens, and perhaps the relative dullness of adding yet another diazotroph to the list, seems to have inhibited further examination of methanogens for diazotrophy.

The matter became interesting again around 1980, when the methanogens, together with certain halobacteria and sulphur-metabolizing bacteria, were shown to belong to an entirely new group of living things: the archaebacteria[5]. Early scepticism has been dispelled, and we now know that the archaebacteria comprise a separate kingdom, which differs from the other two kingdoms, eubacteria (regular bacteria) and eukaryotes (multicellular organisms), more than those kingdoms differ from each other. The separateness of the archaebacteria was first established from 'fingerprints' of their ribosomal RNA and is reflected in a variety of biochemical properties unique to the group[6].

Murray and Zinder's report of diazotrophy in *Methanosarcina barkeri* is the first definitive demonstration that nitrogen fixation occurs among the archaebacteria. It is definitive because it includes two important controls. The first is that uptake of $^{15}N_2$ is shown to accompany growth, so there is no possibility of simulated diazotrophy arising from growth on nitrogenous impurities in the gases or reagents, a problem which has led to many false reports of diazotrophy[7]. The second and perhaps more important control is that the authors have shown to the best of their ability that their culture is pure and, in particular, have excluded sulphate-reducing bacteria, which are known to be diazotrophic[7].

Genetic and biochemical studies of nitrogen fixation among the eubacteria are converging to suggest that the genetic determinants for diazotrophy, the *nif* genes, are highly conserved and that their products, nitrogenase in particular, are much the same whatever their genetic background. The *nif* genes are fairly readily transferred among bacteria by experimental means — wholly new diazotrophs have been generated in this way — and there are compelling reasons to believe that they represent a genetic linkage group that has spread naturally among the eubacteria[8]. It has not spread to the eukaryotes — as far as we know — and all eukaryotic nitrogen-fixing systems are in fact biocoenoses involving mutualisms with diazotrophic eubacteria, the legume-rhizobium symbioses being the most familiar example. It is fascinating, then, that diazotrophy should now be found among the archaebacteria.

Did it spread to the archaebacteria from the eubacteria or, rather, originate in the archaebacteria and spread to the eubacteria? Could it be so ancient a property that it originated before the archaebacteria and eubacteria diverged, very early in the history of life on Earth[6]? Or could diazotrophy have originated separately in the two kingdoms? A study of *nif* gene organization and the biochemical nature of nitrogenase in a methanogen ought to be most instructive, for the possibility exists of finding nitrogenase enzymes that differ substantially from those of eubacteria.

A hint that the archaebacterial nitrogenase system is different comes from the report[9] on page 286 of this issue by Belay, Sparling and Daniels that a presumptive diazotrophic methanogen, *Methanococcus thermolithotrophicus*, functions at 64°C. The temperature maximum for diazotrophy in eubacteria is generally 35–40°C (although the cyanobacterium *Mastigocladus* can just about manage at 60°C). The reason appears to be that, although nitrogenase itself is reasonably thermostable, the genetic activator for *nif* expression (the *nifA* product) is thermolabile[8]. Belay, Sparling and Daniels's finding could imply that archaebacterial *nif* systems can be unusually temperature-tolerant, so it is regrettable that their report omitted the meticulous controls used by Murray and Zinder. Thus, absence of sulphate-reducing (or other) eubacterial thermophiles was apparently not checked and, secondly, error due to inadvertent introduction of fixed nitrogen into the experimental system was not rigidly excluded by using $^{15}N_2$. Their success in obtaining acetylene reduction, a test avoided by Murray and Zinder in this context because of its obliquity, tends to support their claim for thermophilic diazotrophy. But some doubt must linger about whether the organism responsible is an archaebacterium and not some diazotrophic commensal eubacterium. □

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Atmospheric chemistry

Ozone fears revisited

from H.I. Schiff

THE threat to the Earth's ozone layer promises to be a saga with as many sequels as *Star Wars*. Just as we have become assured that this protective shield is more robust than had been feared, M.J. Prather, M.B. McElroy and S.C. Wofsey warn that the Empire might strike back. In a paper on page 227 of this issue, they show that large decreases in ozone can occur if the emission of chlorine compounds into the stratosphere is allowed to increase by a factor of 5 or more.

The ozone layer is located in the stratosphere, between about 10–50 km above the Earth's surface. Although more than 95% of atmospheric ozone is contained in the stratosphere, its concentration never amounts to more than a few parts per million of air. And yet its presence is essential for life on this planet, since it prevents biologically-damaging solar ultraviolet radiation from reaching the surface. It also plays an important role in determining the climate of the planet.