news and views

Rhizobium as a free-living nitrogen fixer

from John Postgate

SCIENTISTS interested in biological nitrogen fixation have for many years been perplexed by the apparently complete dependence of Rhizobium, the bacterial partner in the well-known legume symbiosis, on its host plant. Despite frequent attempts nobody, until about six months ago, had successfully obtained growth and fixation by rhizobia in the absence of living plant material (either as a nodulated plant or an association between rhizobium and a tissue culture of plant callus). The plant, indeed, contributes genetic information important to the nodule symbiosis, so Dilworth and Parker (*J. theoret. Biol.*, **25**, 208; 1969) earlier suggested that some of the genes determining rhizobium's ability to fix nitrogen (*nif* genes in geneticists' shorthand) were actually 'banked' with the host plant. More recently evidence had been accumulating to support the contrary view: that rhizobia possess the complete quota of *nif* genes themselves but that the genes are silent in free-living cells.

One line of evidence stemmed from the work of Dunican and Tierney (*Biochem. biophys. Res. Commun.*, **57**, 62; 1974) who took a strain of *Klebsiella aerogenes* which did not normally fix nitrogen, transferred genetic material from *Rhizobium trifolii* to it, and obtained progeny which fixed. The presumption was that the *K. aerogenes* Nif^+ progeny were using rhizobial *nif* genes but, as the authors recognised, ability to fix nitrogen is common among klebsiellae and the possibility that *K. aerogenes* possessed cryptic (latent) *nif* genes, and that some activator had been transferred, was not excluded. Circumstantial evidence came also from H. J. Evans's group, who showed that *R. japonicum* grown in conventional laboratory culture, in the absence of plant material, possess not only the electron transport factor associated with nitrogenase (*Plant Physiol.*, **51**, 136; 1973) but also a protein immunologically similar to the molybdenum–iron protein of nitrogenase (*Biochim. biophys. Acta*, **381**, 248; 1975). Even stronger evidence was provided by the experiments published in *Nature* a few months ago (see *Nature*, **253**, 305; 1975) in which strains of the slow-growing cowpea type of rhizobium were shown not only to fix nitrogen in the presence of callus from non-leguminous plants, but also to continue fixing nitrogen for up to twelve hours after the callus was removed. Callus was clearly providing a diffusible material which permitted nitrogen fixation by the rhizobia; it might be a genetic activator, but actual gene transfer was hardly likely, particularly since non-leguminous callus functioned as well as legume callus in the artificial symbioses.

In the event, the situation has proved remarkably simple. In this issue of *Nature* three groups, two from Australia and one from Canada (pages 406, 407 and 409) simultaneously report successful cultivation of nitrogen-fixing rhizobia in the complete absence of plant material. As with the earlier experiments using callus culture associations, the possibility that the rhizobial cultures might be contaminated by free-living nitrogen-fixing bacteria had to be rigidly excluded, because rhizobia are difficult to sustain as pure cultures, and the slow growth of the cowpea group makes them even more awkward in this respect. In addition, positive acetylene tests needed to be backed up with $^{15}N_2$.

These things have been done and the key to the question proves to be the carbon source: for fixation, a pentose such as arabinose or xylose as well as a dicarboxylic acid such as succinate seem to be essential. Note that both classes of carbon source are common plant constituents. A relatively small amount of fixed nitrogen (such as glutamine, glutamate or nitrate) seems to be helpful; in this respect the cowpea rhizobia seem to resemble free-living aerobic nitrogen-fixing bacteria such as *Derxia gummosa* or *Mycobacterium flavum* which also fix nitrogen best when 'kicked off' with a little pre-fixed nitrogen. In *D. gummosa*, the need for fixed nitrogen can be simply interpreted: nitrogen fixation is an oxygen-sensitive process, so a little pre-fixed nitrogen permits colonies or cultures to grow to a density such that, within the colony, the cooperative respiration of the bacteria lowers the oxygen tension to a level at which fixation can occur (Hill, *J. gen. Microbiol.*, **67**, 77; 1971).

So far, all the successful experiments with rhizobia have made use of media set with agar, on the surface of which the colonies of rhizobia grow and within which colonies the oxygen tension may range from zero to atmospheric. Since fixation by rhizobia has long been known to involve aerobic metabolism, it is tempting to assume that the cowpea rhizobia, like *D. gummosa* and *M. flavum*, are bacteria which become microaerophilic when fixing nitrogen. There is an apparent paradox, brought out by the Canadian group, that nitrate or ammonia, which repress nitrogen fixation in liquid cultures of ordinary fixers such as clostridia, azotobacters or klebsiellae, actually promote fixation by free-living rhizobia, but the local ammonia or nitrate concentration near an agar colony cannot readily be assessed and it would be premature to conclude that regulation of *nif* in these rhizobia is unusual. Resolution of both of these problems must await the successful culture of nitrogen-fixing rhizobia in homogenous liquid media, where both oxygen and fixed nitrogen concentrations can be measured. No doubt this is only a matter of time.

The substantial advance represented by this work is not only the final proof that cowpea and some other rhizobia carry the complete complement of *nif* genes; it is also the fact that many strains and species of rhizobia now join the ranks of free-living nitrogen-fixing bacteria, with revolutionary consequences for the study of their biochemistry and genetics—for one thing, the host plant can be by-passed in the laboratory. A well established obligate symbiosis is crumbling; in what does the host specificity of the traditional legume symbioses reside? And is the nodule nothing more than a compartment to restrict access of oxygen to rhizobia? If only a pentose and a dicarboxyclic acid are needed for rhizobial fixation, how readily can this information be used to set up new associations with plants and forage crops? And how many other free-living nitrogen-fixing bacteria have been missed by microbiologists because two carbon sources are needed?