COMMENTARY

ANAEROBES AT WORK

by Bernard Dixon

After a study of the microflora existing on maize and other cereals, also those occurring in the soil, he had succeeded in isolating an organism capable of transforming the starch of cereals, particularly maize, into a mixture of acetone and butyl alcohol. Working night and day, he had secured a culture which would enable us to get our acetone from maize...This discovery enabled us to produce very considerable quantities of this vital chemical."

The story, recounted by Britain's World War I Minister of Munitions, David Lloyd George, still bears repetition. Desperately worried about an acute shortage of acetone, which was urgently needed for the manufacture of cordite, Lloyd George had met the exiled Russian-Jewish biochemist Chaim Weizmann, then working at the University of Manchester. A few weeks later, with some help from *Clostridium acetobutylicum*, Weizmann had solved the problem.

In due course, the politician suggested that the scientist might accept an honour in recognition of his unique contribution to the nation. Weizmann demurred, but then mentioned that the Jewish people should once again have a homeland of their own in the Middle East. That was the origin of the Balfour Declaration and of the subsequent creation of the state of Israel in 1949, with Weizmann as its first president.

Whether worldly recognition on this scale will come to Welch microbiologist Gareth Morris is as yet unclear. However, three-quarters of a century after his illustrious predecessor harnessed *C. acetobutylicum* for the industrial production of acetone and butanol, Morris could be on the point of seeing *his* dream come true, too. For several years now, working at the University College of Wales, Aberystwyth, Morris has been arguing that this same organism has considerable potential for the manufacture of a great diversity of high-value products. He sees it, along with other members of the genus, as a much more versatile workhorse than is often supposed, particularly for feats of synthesis based on stereo-specific and stereo-selective reductions.

"If obligately anaerobic bacteria had received the same attention as have aerobic bacteria, Streptomyces, and fungi, it could well be that more of them would now be exploited as sources of valuable small-molecular-weight products such as amino acids, nucleotides, and vitamins," Morris insists. "With a little more imagination than has been displayed hitherto, the unique biochemical potential of some of the anaerobes might be put to good use in effecting highly specific biotransformations." In addition to stereo-specific reductions, hydrogenations, dehydroxylations, and other examples of co-metabolism, applications include complete fermentations—for example, the formation of acrylate from alanine by *C. propionicum*.

As Morris reasons persuasively in *Clostridia* (N. P. Minton and D. J. Clarke, eds. Plenum Press), the Clostridia have many features that should commend them more forcibly to bio-industry. Although categorised as obligate anaerobes, they can survive occasional encounters with oxygen perfectly well in culture or washed cell suspension. Yet they are highly reducing organisms, capable of developing and sustaining low redox potentials in the environment, their fermentative metabolism generating the required reducing power. Clostridia acquire free energy by fermenting different substrates via a variety of pathways. Even their capacities to form spores and to germinate readily are advantageous properties—facilitating the selection, storage, and transport of cultures, and providing a convenient mode in which the organisms can be immobilised for use in bioconversion.

In addition to quirks of fashion and historical accident, two factors account for past failures to capitalise on these characteristics. Firstly, microbiologists have concentrated on bacterial genera which they believed were easier to handle on the bench and in the factory. Secondly (and rather more seriously), *C. acetobutylicum* has been far less amenable than many other bugs to genetic manipulation. But times are changing. Over the past few years, two or three methods of introducing plasmids into the organism have been described, albeit with limitations such as low rates of plasmid transfer. Now three of Gareth Morris's colleagues in Aberystwyth—Ross Williams, Danielle Young, and Michael Young—have made a much more substantial step forward.

As with recent progress in the genetic manipulation of Streptomyces (*Bio/Technology* **7**:398, May '89), the Aberystwyth work has its roots in the studies by Patrick Trieu-Cuot and his co-workers at the Pasteur Institute in Paris on a broad-host-range conjugation system encoded by *inc*P plasmids such as RK2, which can be transferred to bacteria of many different genera. This mechanism can be harnessed to mobilise small non-conjugative plasmids from *Escherichia coli* to a wide range of different organisms. Ross Williams and his co-workers have now adopted this strategy for *C. acetobutylicum*. As reported in the current issue of the *Journal of General Microbiology* (**136**:819, 1990), they have constructed shuttle vectors containing replicons from pAMβ1 (*Enterococcus faecalis*), pCB101 (*Clostridium butylicum*), or pWV01 (*Streptococcus cremoris*), together with the *cis*-acting *ori*T region of RK2, and succeeded in transferring them to *C. acetobutylicum*, where they became established.

These conjunctive transfers from *E. coli* occurred under strictly anaerobic conditions. The Aberystwyth group believes, therefore, that *inc*P may also prove useful for the genetic manipulation of other anaerobes, such as methanogens, into which conventionally armed genetic engineers have failed to insert novel plasmids. For the moment, however, the work with *C. acetobutylicum* is causing most excitement. This could be the step that allows the Clostridia to take their full and deserving place in biotechnology—one that truly matches their striking metabolic versatility.