COMMENTARY

by Bernard Dixon

A CLEVER TWIST ON CONVENTIONAL MICROBIOLOGY

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The novelty-generating activity we call science, coupled with the profit motive of capitalism, should ensure that exploitable discoveries are recognized quickly, and then be harnessed to both immediate and unforeseen practical ends. Witness the staggering variety of uses now being found for monoclonal antibodies. Witness the eager application of new equipment when it first

becomes available commercially, from electron microscopes during the 1950s to DNA sequencers today.

But there are some puzzling failures too. For instance, Felix Franks, writing in *Trends in Biochemical Sciences* (vol 6, p 5), has commented on the imbalance between the use of cryogenics to preserve human fetuses and other animal tissues, and its neglect as a means of conserving irreplaceable plant cells. Here, Franks suggests, there seems to have been an intellectual barrier between the researchers who originated a technique and others, working in an unrelated discipline, who might benefit.

A more specific current example, which I find both exciting and perplexing, is the cunning method for isolating ethanol-tolerant mutants of yeast developed in recent years by Stephen Brown and Stephen Oliver at the University of Kent, near Canterbury. Now continuing in the University of Manchester Institute of Science and Technology, where Oliver's research is being financed by sugar refiners Tate & Lyle, this work promises to improve substantially the economics of alcoholic fermentation. Allied with studies Brown and Oliver are to publish later this year on ethanol production by grande and petite mutants of *Saccharomyces uvarum*, their technique could increase considerably both the work-rate of yeasts and the final yield of their prime product. The puzzle is why biotechnologists seeking quite different categories of mutation—such as resistance to acids or heavy metals—are not yet adopting the Brown-Oliver approach. The initial problem is very similar. The toxicity of ethanol towards its microbial manufacturers is complex, with separate actions on growth rate, fermentation rate and viability. But so too is the inhibitory effect on heavy metals, low pH, and other environmental conditions on many other microorganisms. So, just as efforts to secure ethanol-tolerant mutants by conventional screening and selection are unlikely to succeed, there is little chance of obtaining organisms resistant to these factors by the mutation of single genes.

Brown and Oliver began with a piece of lateral thinking. Contemplating the bleak prospects for orthodox strain improvement, they decided to seek *S. uvarum* mutants by establishing a continuous selection system—one in which the microbial population itself, through a feedback circuit, determined the intensity of selection. Using 1.5 liters of brew, they set up a continuous culture, using infra-red analysis of carbon dioxide in the exit gas to monitor fermentative activity. The rate at which the yeast population generated CO₂ indicated its overall ethanol tolerance.

To establish the desired selective conditions, Oliver and

his colleague added ethanol to the medium (final concentration 2 per cent w/v). They then determined the steady-state of CO₂ evolution and set a potentiometric controller at 20 per cent below this value. Thence to the ingenuity behind this exercise in self-selection. Whenever the CO₂ level in the exit gas equalled or exceeded that pre-set figure on the controller, a relay closed, switching on a pump that introduced 70 per cent ethanol into the culture vessel. This reduced the rate of CO₂ production. But if the amount of CO₂ in the exit gas fell below the pre-set value, the relay operated in reverse, turning off the pump.

Because this was a continuous system, ethanol gradually washed out of the fermentor. So, as the CO₂ level went up again, more alcohol was metered in. In other words, if the yeast population's overall ethanol tolerance increased, the control system would begin to turn on the pump more often. This means that the average concentration of alcohol in the medium rose. The CO₂ trace from the infra-red analyser reflected the operation of the pump—"blips" indicating sudden decreases in CO₂ concentration every time it came on.

Oliver and Brown plotted the time intervals between these blips, but observed nothing remarkable for nearly a month. Then, as they described it in the *European Journal of Applied Microbiology and Biotechnology* (vol 16, p 119), there was a dramatic change. The ethanol pump operated only rarely during the first four weeks, but at around 650 hours the frequency of switching increased considerably. After 710 hours, the rate fell again, declining gradually until the end of the experiment at 1200 hours. Between start-up and completion, the level of alcohol in the fermentor more than doubled to 4.7 per cent.

What precisely had happened? A single, specific mutation at 650 hours would have explained the culture's sudden alteration in behavior, but the investigators think this unlikely. Several mutations would have been required in the same organism because alcohol has at least three separate inhibitory effects on yeast cells. The ethanol-tolerant organisms they isolated also showed a range of phenotypes, suggesting that what had really emerged was a mixture of different mutants. Examined one by one, these included strains with vastly improved fermentative abilities and ethanol tolerance. One of them had a fermentation rate, in the presence of 10 per cent alcohol, which was double that of the wild type and indeed of the most aristocratic lager yeasts.

One notes with interest that while Stephen Oliver's research is now sponsored in part by Tate & Lyle, Stephen Brown has moved to the pharmaceutical company G. D. Searle, where he may well be putting to good use his pioneering experience with this remarkable system. But the continuous selection technique should be applicable to many other industrial processes in which microorganisms yield infuriatingly weak solutions of products that are themselves toxic to their producers. Is this a case where the glamour of gene splicing has distracted attention from a very clever twist on more conventional microbiology?

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