HIGH YIELDS LURK IN LOW-TEMPERATURE MICROBES

There's no longer anything *outré* about the suggestion that industry could benefit by tooling up with thermostable enzymes from exotic organisms living in torrid environments. Bang goes that old antithesis between the belching chimneys and extreme parameters of traditional chemical engineering, and the nice, moderate working conditions preferred by "nature's own little catalysts."

But what of the notion that life from the bottom end of the mercury column can also find applications in bio-industry? Almost exactly a century after the discovery that some microorganisms thrive at zero degrees centigrade, I sense that their skills too are being recognized as worthy of encouragement. Both psychrophilic bacteria, with optimal growth temperatures of 15° C or lower, and merely psychrotrophic (cold-tolerant) bugs are beginning to commend themselves as rewarding targets for genetic manipulation.

Candidate organisms are not hard to find. The last decade has seen some remarkable discoveries from the world's frozen wastes—habitats not only lacking warmth but also desperately short of requirements such as nutrients that are *de rigueur* for regular, mesophilic life. Lichens and other microbes grow in the extreme cold and dessication of sandstone in the Ross desert of Antarctica. Algae photosynthesise with extraordinary efficiency in the freezing waters of the high Arctic, where they receive only 0.01 percent of the light reaching the surface. Mats of *Phormidium frigidum*, resembling the stromatolites of the Precambrian Era, grow in rich profusion at the bottom of dimly lit, permanently ice-covered lakes in the Antarctic dry valleys. And rich "cold seep" communities of chemosynthetic bacteria thrive deep in the freezing depths of the Canadian Arctic Archipelago.

While some time may elapse before the members of this catalogue of characters find their places in bioindustry, certain applications of psychrophiles and psychrotrophs are much closer to realisation. Writing in the current *Journal of Applied Bacteriology* (**71**: 386, 1991), Anne-Monique Gounot of the Universitie Lyon (1-Claude Bernard in Villeurbanne Cedex, France), draws attention to several recent manipulations that point to the use of psychrotrophic bacteria as candidates for genetic bioengineering. The toluate-degrading TOLpWWO plasmid from *Pseudomonas putida*, for example, has been transferred and expressed at zero° C in a psychrotrophic strain of the same species. A transconjugant of one strain of a psychrotrophic species of *Moraxella* has been shown to express three antibiotic resistance genes carried by plasmid RP4 at both 4° and 25° C. Three lipase genes of this strain have been cloned in *Escherichia coli*. And psychrophilic bacteriophages have been isolated from psychrophilic bacteria.

But why should we get excited about organisms that prefer or tolerate cool conditions? Three thoughts indicate the diverse portfolio of potential applications for their cells or genes. Firstly, the unique adaptability of psychrotrophic bacteria to changes in temperature should help us to understand more about the genetic basis of heat shock proteins—which, induced in response to heat and other stresses such as starvation, confer transient resistance to otherwise lethal conditions. Greater knowledge of this system could help in manipulating the robustness of organisms for both industrial fermentations and for release into the environment.

Secondly, the dairy industry could benefit by using organisms working at low temperatures, minimising contamination by mesophiles and arresting remaining activity by pasteurisation. When, for example, *Aspergillus* or *Kluyveromyces* enzymes are used to hydrolyse lactose, to increase the digestibility and sweetness of milk, incubation has to take place at 30-40° C for 4 h. These are ideal conditions for the growth of unwanted mesophiles. At 5-10° C, on the other hand, it takes at least four times longer to achieve three quarters of the same hydrolysis. Psychrophilic enzymes may offer a perfect solution to this problem.

The third idea, and one of the most appealing, is to exploit bacteria living at low temperatures and possessing low proteolytic activities as tools for producing useful molecules that are vulnerable to proteolysis when made in other microorganisms at higher temperatures. Alan Hipkiss, at King's College London, has found that when the human interferon alpha-2 gene is spliced into and expressed in *E. coli* or *Methylophylus methylotrophus*, and the organisms are reared at their favoured 37° C, they generate proteases that rapidly degrade the interferon. But the protein is not broken down when produced by the same organisms cultured at 29° C. It is quite possible that other substances of commercial and medical interest will undergo similar stabilisation, but at much lower temperatures.

Together with Anne-Monique Gounot and other colleagues at Villeurbanne, Hipkiss has taken a particular interest in *Arthrobacter globiformis* S155, which can grow down to -5° C and up to 32° C but thrives best at 20-25° C. Originally isolated from a Scandanavian Arctic glacial region where surface temperatures vary considerably, this bacterium might well be expected to be highly adaptable in response to prevailing conditions. Hipkiss and his coworkers have found that cell-free extracts of the organism cultured at 32° C have considerably greater proteolytic activity (against casein and insulin) than extracts of the same organism grown at 20° or 10° C. Proteolysis also increases rapidly when the cells are grown at 10° C and then transferred to 32° C.

Interesting stuff. The only puzzling feature of this pregnant scenario is that while the work carries on apace in the French laboratory, severe pressures currently afflicting academic research in the U.K. have left Alan Hipkiss without funding to pursue his ideas further. Maybe some enlightened benefactor will now bring him in from the cold? ///