Boiling muds and scalding soils: new species and enzymes for the future

David Lloyd

We look to the strangest places on earth to find novelty in microbial form and function. Where the scalding-hot waters of deep-sea volcanic vents (black smokers) meet the icy-cold abyssal sea; that's where you can meet *Pyrolobus fumarii*, the current record-holder for growth at extremely high temperatures. At a depth of 4,000 m (pressure 360 atm) this ecosystem is based on cycles of sulfur reduction and reoxidation, and is completely independent of solar energy. Sites of volcanic activity on land, hot springs, sulfur-rich springs, boiling mud and deep sub-surface strata are filled with hyperthermophilic organisms that like to grow at temperatures higher than 80 °C. All are prokaryotes, although some fungi are known that can survive up to 62 °C, but they are distinctly different from the bacteria of everyday life.

Types of hyperthermophiles

Well known bacterial hyperthermophiles lie in the deepest branches of bacterial genealogy (*Thermotogales* and *Aquificales*). The genome sequences of species, such as *Thermotoga maritima* and '*Aquifex aeolicus*' look as if they have acquired genes from archaeal species. A quarter of *T. maritima* proteins are more similar to archaeal gene products than bacterial ones.

The archaeal hyperthermophiles are much more diverse and fall into three major sub-lineages. The *Crenarchaeota* include those species able to grow at the highest temperatures of all known organisms; they cluster closely together on the phylogenetic tree and have perhaps evolved only slowly from their earliest ancestors on the primitive earth when the oceans were still boiling as a result of bombardment by comets. The *Euryarchaeota* include the methanogenic *Archaea*, and the extremely halophilic halobacteria. A third group, the *Korarchaeota*, branch very near the root of the archaeal tree and were originally discovered as a result of analysis of 16S rRNA sequences from an iron- and sulfur-rich hot spring in Yellowstone National Park, Wyoming, USA. Several cultured examples of these unique organisms are now available and will perhaps provide fascinating new light on early evolution.

Life-based coenzymes, such as ATP and NADP, cannot exist above about 140 °C as this is the upper limit for their stabilities in aqueous solution. So how can it be that these organisms cannot only survive elevated temperatures, but actively carry out the processes for growth? Clearly they must have very special components, and the mechanisms that ensure the stability of their proteins, nucleic acids, lipids and membranes can provide new insights into fundamental questions on the nature of life. More practically, novel fundamental studies frequently provide new applications. Thus, the exploitation of constituents isolated from these robust species may be expected to provide biomolecules in which the exquisitely effective selection processes of at least 3·8 billion years have ensured a remarkable temperature stability. Thus, not only can these hyperthermophile products be exploited directly, but we can use computer-aided molecular modelling to try to improve upon their designs, thereby taking thermal stability into even more impressive domains.

Withstanding the heat

Stability has been built-in by many different strategies. The unique lipids of archaeal membranes are even further modified in the extreme thermophilic methanogen *Methanopyrus kandleri*, providing a good example

ABOVE:
Fig. 1. Boiling mud: New Zealand has a number of geothermal fields with a temperature above 220 °C which are exploited for industrial purposes. Hyperthermophiles thrive in the soils and muds heated by this activity.

RIGHT:
Fig. 2. Steam from Iceland's hydrothermal springs; much of Reykjavik's domestic heating is derived from geothermal activity.

PHOTOS DAVID LLOYD

of where molecular design is radically different from mesophilic organisms. Thus, even the normal ether-linked lipid (dibiphytanyl tetraethyl lipid) common to all *Archaea*, that provides a monolayer rather than a bilayer, contains an unsaturated form of side-chain, geranylgeraniol, so that at the upper temperature limit of 110 °C the membranes can still function. The genome structure of these organisms is protected by possession of a reverse gyrase that introduces positive supercoils (rather than the negative supercoils provided by the normal DNA gyrase). The high ionic strength of the cytosol and the special thermoprotectant potassium cyclic 2,3-diphosphoglycerate prevent chemical degradation that would otherwise occur at high temperatures. The presence of special DNA-binding proteins (Sac7d in crenarchaeotes and basic histone-like proteins in euryarchaeotes) protects at high temperatures.

Intracellular enzymes from hyperthermophiles usually show optimal activities at or near that organism's optimal growth temperature, whereas its extracellular enzymes are optimally active well above this temperature. The efficiencies of these enzymes are similar to those from mesophiles, but their thermotolerance results in their enzyme efficiency matching that of enzymes working at a lower temperature in mesophiles.

Interestingly the primary sequences of the proteins are not profoundly different when comparisons are made between homologous enzymes of mesophiles and hyperthermophiles (typically 40–85 % similar). Their three-dimensional structures are superimposable and their catalytic mechanisms are the same. More than 100 genes from hyperthermophiles have been cloned and expressed in mesophiles during the past 5 years; often strong promoters are necessary because of different codon usage, for example between *Pyrococcus furiosus* and *Escherichia coli*; expression in yeast and complementation of yeast mutations has been demonstrated in some cases.

It is often asserted that thermostability requires extra 'rigidity', but the methods used for measurement (frequency domain fluorimetry, anisotropy decay, hydrogen–deuterium exchange rates, tryptophan phosphorescence) give information on different time-scales and a protein may be rigid on a nanosecond scale, but flexible over milliseconds. Even femto- and atto-second time scales are now experimentally accessible, and it has been shown recently that some protein functions depend on such ultrafast atomic motions. The principal components of hyperthermophilic stability of proteins as we currently understand them may be listed as follows:

- the hydrophilic effect is believed to be the major driving force of protein folding and hydrophobility is a major force required for stability
- charged-neutral H bonding (i.e. between a side-chain atom of a charged residue and either a main-chain atom of any residue or a side-chain atom of a neutral residue) is especially favourable
- ion pairing, though not an important factor in protein folding, may also be a strong stabilizing mechanism for hyperthermophilic proteins
- intersubunit interactions are also likely to be important in multimeric enzymes and many hyperthermophilic enzymes have a higher oligomerization state than their mesophilic homologues

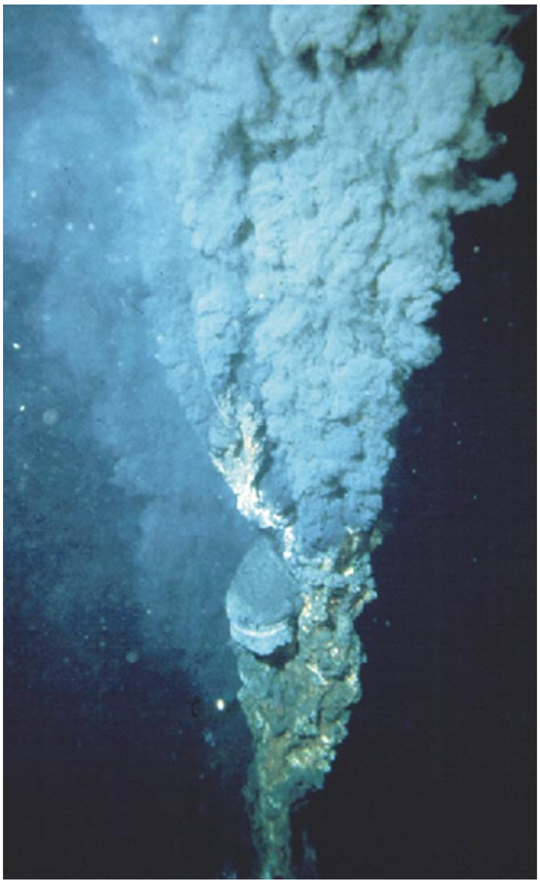
Other factors include:

- release of conformational strain
- anchoring of N- and C-terminal ends
- metal binding, especially Ca^{2+}, Mg^{2+} and Mn^{2+}
- stabilization by salts, substrates or by post-translational modifications, especially glycosylation

Commercial applications

There are already several examples of thermophilic and hyperthermophilic enzymes of commercial importance. In the fermentation industry, starch processing represents an enormously important starting point for the production of fermentation syrups prior to their conversion to ethanol, organic acids or amino acids. Thermostable amylases (from *Bacillus licheniformis* or *Bacillus stearothermophilus*), followed by pullulanase and glucoamylase in combination, are used to produce glucose syrups. Xylose isomerases, the first large-scale immobilized enzymes used for an industrial process, convert glucose into fructose. Several hyperthermophilic enzymes show promise for applications in this process. Protein engineering (e.g. by site-directed mutagenesis and directed evolution) is already being employed in attempts to modify their already thermostable properties to match the exacting conditions traditionally in use.

The widespread use of *Thermus aquaticus* (*Taq*) DNA polymerase cloned in *E. coli* provides the basis of PCR technology where it survives exposure to 95 °C and functions repeatedly above 65 °C. Other polymerases with proof-reading 3′–5′ exonuclease capability, such as Vent DNA polymerase (from *Thermococcus litoralis*) or Deep Vent DNA polymerase (from *Pyrococcus furiosus*), have been developed and marketed for use where high fidelity is necessary. Molecular biology now uses a variety



RIGHT:
Fig. 3. A black smoker: hydrothermal vents harbour unique ecosystems which include hyperthermophilic bacteria.
PHOTO PROFESSOR D. RICHARDS

of thermostable DNA ligases, aminopeptidases and carboxypeptidases, alkaline phosphatase and restriction endonucleases. Cyclodextrins are used to encapsulate hydrophilic molecules in pharmaceutical, cosmetic and food products; cyclomaltodextrin glycosyltransferases are used to produce these compounds, and are now available from a *Thermococcus* species.

Thermostable enzymes may also be used (sometimes in organic solvents) at low water activity to catalyse key biotransformation steps in difficult organic syntheses. There are many laboratory projects to develop processes that can produce specific stereoisomers by exploitation of enzyme-catalysed syntheses. The only industrial synthetic process currently using a thermophilic enzyme is the synthesis of the artificial sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester) with thermolysin. Lignocellulose degradation to ethanol or other bacterial fermentation products represents a huge, and so far only moderately successful, achievement. The economically widespread utilization of the most abundant non-fossil carbon source on the planet may await the further development of this line of enquiry and its practical applications. Perhaps one day hydrogen, the fuel of the future, will be commercially manufactured using the ingenuity of a hyperthermophile!

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Further reading

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SGM 150th meeting in the news

Not only was this the biggest meeting the Society has had in recent years in terms of the number of delegates, but slowly and surely we are beginning to see increased media coverage of our meetings as we work hard to raise our profile.

A full two-page conference report appeared in *New Scientist* (13 April 2002, pp 12–13). This resulted in Michael Hudson from CAMR being interviewed on the TV programme *South Today* (22 April) about his team's work on a possible new vaccine against group B meningitis. *New Scientist* also ran two stories the following week (20 April 2002, pp 5 & 14) featuring an article on the possible link between cot death and the presence of bacteria on soiled mattresses, and another on the similarities between camelpox and smallpox.

Several stories appeared on *BBC On-line* over the course of the week, and the website *BioMedNet news* featured five papers. We also had interest from further afield. One researcher appeared on Radio Singapore, and another on the South African Broadcasting Corporation!

Microbiology remains constantly in the headlines, mainly as a consequence of large disease outbreaks such as foot-and-mouth or BSE. Many people in the scientific community are concerned that such stories can be hyped up or portrayed inaccurately. But this is all the more reason why scientists need to be more responsive to media enquiries to get a balanced message across. One way that you can help the SGM to do this is to include your details on the Society's media relations database. If you are an expert in a particular research field and would be willing to respond to media enquiries, please email Tracey Duncombe at pa@sgm.ac.uk


society for general Microbiology
Embargo: Not for use before 1415 hrs on Monday 08 April 2002
'Seek and destroy' vaccines for meningitis outdated
The ability of meningococci bacteria to change their cell surface proteins could reduce the effectiveness of the current meningitis C vaccine. Now scientists are working on vaccines that would allow us to co-exist happily with these microbes, according to research presented today (Monday 08 April 2002) at the spring meeting of the Society for General Microbiology at the University of Warwick.
"We have identified several proteins secreted by meningococci, which are involved in causing disease but which do not prevent the bacteria from living and reproducing in the body. By including secreted proteins in a vaccine we may avoid the strong selective pressures that cause meningococci to mutate so quickly," says Dr Del Ala'Aldeen of the University Hospital of Nottingham.
Dr Ala'Aldeen explains, "We have shown that meningococci disguise themselves by changing their coat proteins while carried in the throat, which allows them to avoid being detected by the immune system. There is genuine concern that such escape among Group C meningococci may result from the strong selective pressure applied as a result of mass vaccination."
"It is possible that we may never be able to … meningococci or even reduce their circulat… on targeting antigens that are largely secre… disease) but do not affect colonization. Th… invasive disease, and accept an eternal co… says Dr Ala'Aldeen.
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NEWS RELEASE

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Helping the aged gut replace good bacteria may reduce cancer risk
Eating certain foods can increase the number of protective microbes in the gut. These bacteria help prevent food poisoning and can reduce levels of some toxic chemicals that may cause cancer, scientists heard today (Wednesday 10 April 2002) at the spring meeting of the Society for General Microbiology at the University of Warwick.
…ung and elderly volunteers. What …f beneficial gut microbes are reduced …se. Eating certain non-digestabl… …his trend," says Emma Woodmansey
… delicately balanced ecosystem …body's immune system, and …n-beneficial bacteria such as …on of ammonia and other toxic …rmation.
…alactoligosaccharides (GOS) and …l gut bacteria such as bifidobacte… …protection against food poisoning …rease the immune system reactivity."
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NEWS RELEASE

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Bacteria change 'fingerprints' and baffle detectives
Tracing the source of a campylobacter food poisoning outbreak can be very difficult even with modern DNA fingerprinting methods. There is now evidence that campylobacters can rearrange their DNA, disguising their fingerprint, and confusing such detective work, scientists heard today (Tuesday 09 April 2002) at the spring meeting of the Society for General Microbiology at the University of Warwick.
Professor Diane Newell and her colleague Dr Anne Ridley of the Veterinary Laboratories Agency say, "We can only track campylobacter strains, for example from food to human infection, using a DNA fingerprinting method if the strain type stays stable over time and geographical distance. DNA changes can have an observable effect on the type that is identified. We call this genotypic instability."
Genotypic instability may have a big advantage for the campylobacter. DNA rearrangements may be involved in helping the bacteria survive in hostile environments and providing strain variants that can colonise chickens and other animals better.
Professor Newell explains, "We've found that chicks are campylobacter-free when they are born but they become infected within 2-3 weeks of life. In order to control infection in poultry flocks, methods are required to track infecting strains all over the farm to the finished poultry product sitting in the fridge at home."
"The most effective way to control campylobacter food poisoning would be to stop chicks becoming infected. Our research has shown that good hygiene is not enough to keep campylobacters out of farms and broiler houses. An alternative approach would be to use "friendly" campylobacters that can infect chicks but not cause disease in humans, which would exclude any harmful campylobacters," says Professor Newell.
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