MOLECULAR ADAPTATIONS IN THERMOPHILIC EUBACTERIA AND HYPERTHERMOPHILIC ARCHAEA FOR GROWTH AT ELEVATED TEMPERATURES AND THEIR POTENTIAL IN BIOTECHNOLOGY

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ABSTRACT

Thermal niches are widespread throughout the globe and although the environment may at first sight appear inhospitable huge numbers of thermophilic microorganisms inhabit these niches. Thermal niches are not uniform as they vary in pH, in temperature, in degree of oxygen present and in nutrient supply. This has allowed the evolution of microorganisms with different tolerances to temperature, oxygen, pH and with different metabolic mechanisms. Many of the organisms observed in thermal environments are unculturable, however those that have been studied show molecular adaptation to life at high temperatures. Membrane lipids show chemical crosslinking for stability while proteins are intrinsically thermostable and show many alterations to increase stability. DNA often shows a high GC content or is protected with histone like proteins while metabolites which are thermolabile in mesophiles are rarely found in thermophiles., these organisms preferring to alter their metabolic pathways and use different intermediates. In certain thermal niches the organisms present have adapted not only to the temperature but in some cases also to variation in pH and oxygen supply indicating the tremendous versatility within the microbial world and providing biochemists, microbiologists and molecular biologists with a treasure throve of new and exciting experimental systems and biotechnologists with potential new industrial applications.

Key words: Thermophilic microorganisms, thermal niches, molecular biology, biotechnology.

INTRODUCTION

The earth is geologically active with regular movement of the earth's plates giving rise to spectacular terrestrial volcanic and thermal activity. In marine environments, where magma rises to form new oceanic crust, sulphide-rich superheated water emerges from hydrothermal vents, called black smokers, which are characterised by high temperature, high pressure, low oxygen and high salinity. Hot springs form when underground water becomes heated and emerges to form bubbling hot pools or pressurised geysers, as this water passes through the surrounding rocks and clays it may pick up sulphide or sulphur resulting in solfatara fields of boiling acidic muds where as other water systems may remain neutral or be slightly alkaline. In such niches specialised thermophilic organisms abound. Any single niche type may have several parts some only a few millimetres wide showing variations in temperature, oxygen or soluble nutrients.

This diversity of niches with varying properties has resulted in the isolation of a range of different thermophilic organisms, endowed with the ability of growth at elevated temperatures, many with very different metabolic abilities and temperature tolerances. From marine niches organisms with very high temperature, pressure and salt tolerances have been discovered. Although many organisms are capable of some growth at elevated temperatures as the temperature increases the diversity of organisms capable of sustained growth becomes restricted. Eukaryotic thermophiles include some algae, fungi and protozoa but the upper limit is 61°C.

Thermophilic cyanobacteria, such as *Synechococcus lividus*, can grow up to 74°C. At higher temperatures two classes of organism, the thermophilic eubacteria and a more recently discovered group, the thermophilic Archaea which are hyperthermophiles and show molecular characteristics of eukaryotes and prokaryotes, tend to dominate. The discovery of the Archaea has led to much speculation as to the origin of life on earth with many scientists now proposing an origin in high temperature niches. The ability to grow and metabolise at tempera-

tures close to or above 100°C has put considerable stress on their biochemical systems, however many groups thrive at such temperatures. Thus there is much interest in the molecular nature of these organisms which allows them to survive and thrive in such seemingly inhospitable niches.

LOCATION OF THERMAL NICHES

Geothermal areas can be found in many parts of the world including Iceland, Yellowstone National Park in the USA, Rotorua in New Zealand, Japan, Indonesia, Zimbabwe, Italy, the Russian Republic, Hawaii, at mid oceanic ridges and even from geothermal areas near Mount Melbourne in Antartica. Transient thermal environments also exist where thermophiles can be found, these include compost heaps, saunas, warm processing plants, heat treated foods, hot water taps, deep oil field reservoirs flooded with water or desert soils. Indeed thermophiles have also been isolated from normal soils and sediments.

TEMPERATURE RANGES OF THERMOPHILES

The term thermophile means heat-loving. In most cases organisms capable of growth in excess of 55-60°C are considered to be thermophilic while extreme or hyperthermophilic means growth temperature optima of 88°C or above. The terms T_{max}, the maximum growth temperature, and T_{opt}, the temperature at which the shortest doubling time occurs are useful in distinguishing thermotolerant organisms (organisms which can tolerate heat), from true thermophiles. In general thermotolerant organisms although capable of growth above 45°C have a T_{opt} less than this. Thermophiles have a T_{opt} greater than 45°C whereas hyperthermophiles have a T_{opt} greater than 65°C and certain barothermophiles (pressure resistant species) have T_{opt} greater than 100°C when pressure prevents water from boiling.

Maximum growth temperatures are likely to be limited by the availability of liquid water and the thermostability of energy components, enzymes and cell structures (Wiegel and Ljungdahl 1984). Currently organisms capable of growing with a T_{max} of 115°C such as *Pyrococcus furiosis* have been reported. This may be close to the upper limit for life, although on theoretical grounds organisms may be capable of growing up to 150°C (Brock 1986). In high pressure niches where temperatures may reach 250°C microscopic observations of organisms growing have been made suggesting that there may still be the possibility of even more thermoresistant types.

Table 1 - Some representatives of the thermophilic organisms, data compiled from (Noll 1992; Edwards 1990; Weigel and Lungdahl 1986; Kristjansson 1992).

Microorganism	T_{opt}	Characteristic
Fungi		
Caetonium thermophile	59°C	Heterotrophic
Talaromyces thermophilus	59°C	Heterotroph
Cyanobacteria		
Synechococcus lividus	74°C	Phototroph
Protozoa		
Cercosulcifer hemathensis	56°C	
Archaea		
Pyrodictium occultum	105°C	H_2S autotroph
Pyrococcus furiosis	115°C	Heterotroph accumulates H_2
Thermoplasma acidophilum	60°C	Low pH habitats, no cell wall
Methanothermus fervidus	80°C	Methanogen
Sulfolobus acidocaldarius	75°C	Acidophilic, hetero or autotrophic
Methanococcus jannaschii	85°C	Methanogen
Archaeoglobus fulgidus	83°C	Sulphate reducer and fluorescence
Desulphurococcus mobilis	85°C	Sulphur- respiring
Eubacteria		
Thermus aquaticus	70°C	Oligotroph
Bacillus stearothermophilus	50-65°C	Spore, ubiquitous
Thermomonospora curvata	50	Actinomyces, cellulolytic
Thermotoga maritima	80°C	Sulphate and organic utiliser
Methylococcusthermophilus	56°C	Methylotroph
Hydrogenobacter	72°C	Hydrogen oxidiser
Desulfovibrio thermophilus	65°C	Sulfate reducer
Thermoanaerobacter ethanolicus	69°C	Ethanol producer
Clostridium thermocellum	60°C	Cellulolytic anaerobe

THE ORGANISMS IN THERMAL NICHES

Although many species can grow at slightly elevated temperatures (Edwards 1990), genuine thermophily appears to be restricted to certain eubacterial (true bacteria) and archael species (table 1) . Archaea resemble eubacteria in their general morphology however they possess many characteristics of eukaryotic organisms which has led to the proposal that living organisms be divided into three natural domains, the Archaea, the eubacteria and the eukaryotes (Woese *et al.* 1990). Archaea are clearly distinguishable from other organisms on the basis of their 16S ribosomal RNA sequences, their cell wall contains glycosylated proteins rather than peptidoglycan and they possess membrane lipids that are derivatives of C_{20} -C_{25} ethers. New thinking on the evolution of life has led to many scientists who study Archaea to speculate on a thermophilic origin. As the temperature increases in thermal habitats the species variation decreases with a tendency for the thermophilic organisms to be anaerobic due to the poor solubility of oxygen at high temperatures and above 80°C the Archaea tend to predominate (Edwards 1990).

The diversity in thermal niches tends to be a function of the nature of the nutrients present and the physico-chemical properties of the niche with certain types of organisms being distinguished such as thermoacidophiles, found in acid muds or springs, barothermophiles in pressurised environments such as the deep sea and thermoneutrophiles in neutral pH environments.

THE MOLECULAR AND BIOCHEMICAL BASIS OF THERMOTOLERANCE

Living at elevated temperatures puts many stresses on biological components which is not evident in mesophilic counterparts. The ability to reproduce, grow and metabolise at elevated temperatures has meant that thermophiles have had to evolve significantly differently to mesophilic counterparts and indeed the differing thermotolerances observed in eubacteria thermophiles and hyperthermophilic Archaea mean that there are significant differences in the molecular mechanisms that both groups have used to survive at elevated temperatures.

THE NATURE OF PROTEINS FROM THERMOPHILES

Life at elevated temperatures requires proteins with high thermostabilities for transport of nutrients, as catalysts and as structural components. Cloning of thermophilic eubacterial and archael genes encoding proteins into mesophiles has revealed that when expressed these proteins are intrinsically stable, meaning that the protein itself contains all the information for stability encoded within the polypeptide backbone. Sequencing of such proteins has revealed that only the common amino acids occur in thermostable proteins thus it is the complex interactions within secondary, tertiary or quaternary structure coupled to the unique amino acid sequence that confers thermostability.

Thermodynamically the difference between a mesostable and a thermostable protein in terms of their tendency to denature is quite small accounting for the introduction of only a few hydrogen bonds. Many studies have compared sequences of thermostable proteins with their mesostable counterpart but few definitive changes have been observed that result in thermostability. Rather it now appears that small changes resulting in several larger changes may result in thermostabilisation.

Surface hydrophobicity tends to be reduced, internal volume changes make thermostable proteins more compact and many thermostable proteins have an increased tendency to form ion pairs. There is currently not enough information on the 3D structure of thermostable proteins to draw firm conclusions but it appears that many small changes are more common than any single change.

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E.coli    MAIDENKQKALAAALGQIEKQFGKGSIMRLGEDRSMDVETISTGSLSLDIALGAGGLPMG
Thermus   --MDESKRKALENALKAIEKEFGKGAVMRLGEMPKQQVDVIPTGSLALDLALGIGGIPRG

E.coli    RIVEIYGPESSGKTTLTLQVIAAAQREGKTCAFIDAEHALDPIYARKLGVDIDNLLC
Thermus   RIVEIYGPESGGKTTLALTIIAQAQRRGGVAAFVDAEHALDPLYAQRLGVQVEDLLV

E.coli    SQPDTGEQALEICDALARSGAVDVIVVDSVAALTPKAEIEGEIGDSHMGLAARMMS
Thermus   SQPDTGEQALEIVELLARSGAVDVIVVDSVAALVPGAEIEGEMGDQHVGLQARLMS

E.coli    QAMRKLAGNLKQSNTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRI
Thermus   QALRKLTAVLAKSNTAAIFINQVREKVGVTYGNPETTPGGRALKFYASVRLDVRKS

E.coli    G-AVKEGENVVGSETRVKVVKNKIAAPFKQAEFQILYGEGINFYGELVDLGVKEKL
Thermus   GQPIKVGNEAVGVKVRVKVVKNKLAPPFREAELEIYFGRGLDPVADLVNVAVAAGV

E.coli    IEKAGAWYSYKGEKIGQGKANATAWLKDNPETAKEIEKKVRELLLSNPNSTPDFSV
Thermus   IEKAGSWFSYGELRLGQGKEKAAEALRERPELLEEIRAKVLERSD--Q---VVLAA

E.coli    DDSEGVAETNEDF 353
Thermus   GEDEGE------- 340
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Figure 1 - *Alignment of the protein sequences for the functionally conserved recA proteins from* E. coli *and* Thermus thermophilus. *The alignment was carried out using ClustalW and displayed using Boxshade sequence analysis programmes.*

Figure 1 illustrates the protein sequences of *E. coli* and *Thermus* recA proteins aligned using the ClustalW alignment programme. This protein is functionally conserved amongst species having several functions such as ATP binding and hydrolysis, protease activity, single strand DNA binding capacity and promotion of recombination. It provides a useful tool for studying proteins with similar function but with different thermostabilities. Figure 2 illustrates the 3D structure of the *E.coli* recA protein and a recA protein from Thermus thermophilus which we have recently modelled. Although there are alterations in amino acid sequences the 3D structures are remarkably similar. Many of the proteins derived from thermophiles have half lives of several hours at the growth temperature of the organism from which the protein was derived.

Given that organisms such as Pyrodictium has a T_{opt} of 105°C there is much interest in how thermostability is achieved and in potential biotechnological applications. Occasionally proteins purified from certain thermophiles do no posses the degree of thermostability that might be expected. In such cases other stabilising factors such as ions, highly charged macromolecules, such as polyamines, or compatible solutes, such as 2,3-diphosphoglycerate, may play a role in stabilisation.

THE PROTEIN SYNTHESIS MACHINERY IN THERMOPHILES

Studies on the thermostability of tRNA's from the eubacterium Thermus have demonstrated that replacements in the tRNA structure particularly G-U with G-C, coupled to modifications such as thiolation to give thioribothymidine all result in a more stable tRNA with elevated melting temperatures. Thermophilic archael ribosomes are 70S in size with two subunits of 50S and 30S similar to those of bacteria containing three rRNA species of 23S, 16S and 5S. Protein sequence comparisons between eubacterial and archael ribosomal subunits has revealed much sequence similarity, however additional ribosomal subunits appear to be present in thermophilic Archaea and these may play a role in increasing the thermostability of the entire ribosomal complex.

THE DNA OF THERMOPHILES

The genomes of many thermophiles including the eubacterium, Thermus, and the Archaea, *Sulpholobus, Pyrococcus* and *Thermococcus* have been mapped and sized. Genome size varies from 1.9Mb in Thermococcus to 3Mb in *Sulpholobus* with the chromosomes being circular. Indeed the complete nucleotide sequence of *Pyrococcus* has

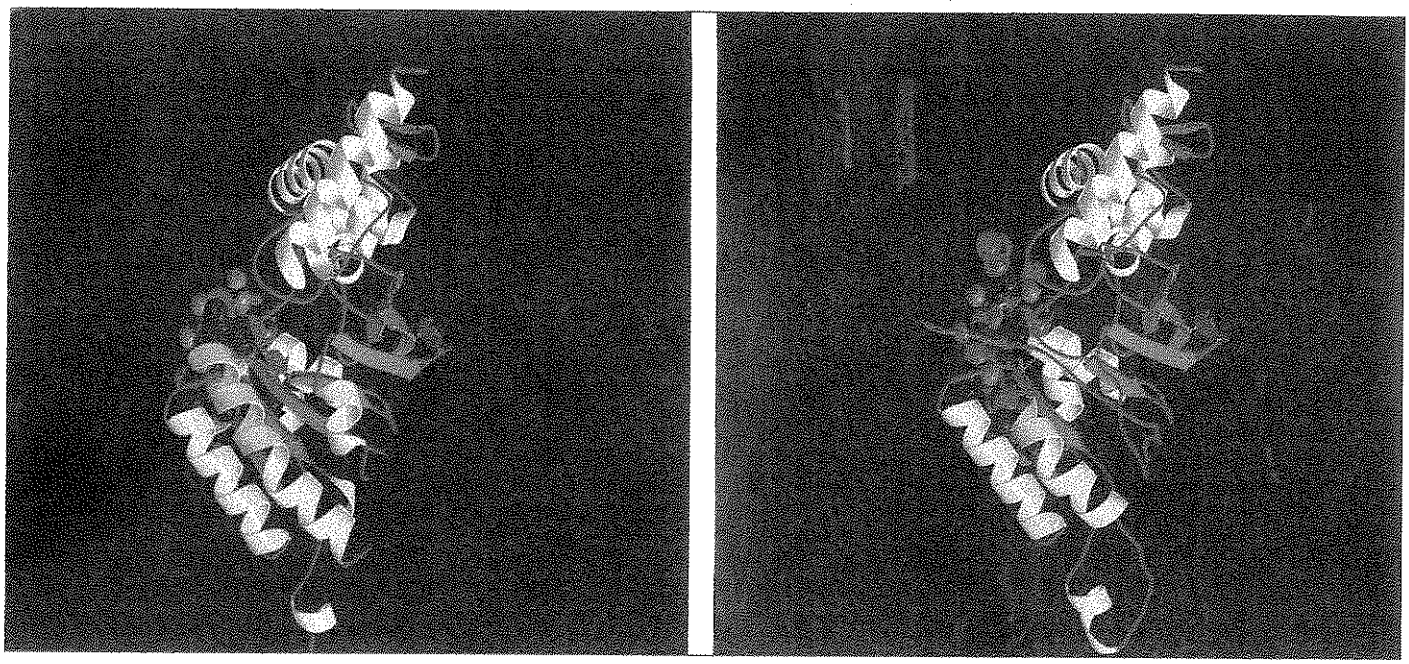

Figure 2 - *Comparison of the 3D structure of a functionally similar protein from the mesophile* E. coli *and the thermophile* Thermus.

been determined and can be viewed at *http:// www. ncgr.org /microbe/ pyrococcusfurtxt.html*, entry to the genome data can be had at ncgr. There appears to be little difference between eubacterial and archael genomes. In general in eubacteria as the temperature tolerance of the organism increases so to does the %GC content-Thermus has a %GC of 67-70% giving a more stable genome with increased interchain hydrogen bonding between GC residues.

However as the temperature increases further DNA tends to denature and other strategies are required and used to maintain genome integrity as in *Sulpholobus* which has a %GC of 37%. Several histone like proteins have been isolated from thermophilic Archaea. The archael HMf histone like protein resembles the eukaryotic histones and may be involved in the formation of nucleosome like particles, indeed HMf and HTa have been shown to increase the melting temperature of DNA *'in vitro'*. Thermal degradation of DNA can also occur at elevated temperatures with cleavage of phosphodiester linkage, depurination and deamination occurring. Monovalent and divalent salts may play a role in protection and in reduction of such thermodegradation, indeed many thermophiles possess elevated levels of Mg^{++}. Reverse gyrase, a type I topoisomerase, was first discovered in *Sulpholobus*, its function in putting positive supercoils in DNA. It has been suggested that positively supercoiling around histone like particles plays a major role in thermostabilising DNA. Because methylated cytosine residues tend to deaminate quite rapidly there is a tendency for thermophiles not to methylate their DNA as occurs in mesophiles where as much as 60% of the DNA may be methylated tn give 5-methyl cytosine. Where methylation occurs in thermophiles it is often to give 4-methyl cytosine, which is not susceptible to deamination.

MEMBRANE LIPIDS IN THERMOPHILES

Membranes function in providing a barrier between the internal and external environments of cells. Membranes contain complex mixtures of molecules which undergo various transitions as temperature increases. The main role of the lipid composition of the membrane is to provide a fluid barrier at the growth temperature not a solid or not a complete liquid. As the temperature increases changes to the lipid membrane must occur to maintain this membrane fluidity. Such changes involve decreasing the degree of unsaturation of fatty acids, increasing the chain length of fatty acids and increasing the proportion of methyl branch chains. However as the temperatures increase further as with the growth temperatures of thermophilic Archaea more radical changes are required to maintain the same degree of membrane fluidity. From the point of view of thermophilic eubacterial membranes the best studied organisms are *Thermus* and *Bacillus* stereothermophilus. In general iso and ante iso fatty acyl chains predominate with iso C17 and iso C15 being most significant in Thermus. In addition *Thermus* and *Thermomicrobrium* have glycolipids replaced by a series of straight chain or methyl branched 1,2 diols of C_{16} - C_{23} In thermophilic anaerobic *Clostridia* and *Thermotoga* condensation of two C_{15}. Fatty acids occurs, forming a dicarboxylic acid of C_{30}, suggesting the presence of diglycerol tetra esters. In general adaptation to temperature occurs by altering chain length and degree of unsaturation and increasing iso and anteiso branched chain fatty acids which increase the liquid crystalline gel phase transition allowing thermophiles to maintain the same membrane fluidity as mesophiles at their respective optimum growth temperatures.

Archaea lack fatty acyl chains in their lipids, thermophilic archael lipids (Langworthy 1985) consist of C_{20} -C_{25} diacylglycerol diether: sn-2,3 diphytanylglycerol diether and its dimer the tetraether in which the diether moieties are linked head to head. Figure 3 illustrates some of the core structures of archael lipids from which many others are derived. A number of

thermoacidophilic Archaea such as *Sulpholobus, Thermoplasma, Thermoproteus* and *Desulfurococcus* have been examined for lipids.

The core lipid consists of caldarchaeol or nonitolcaldarchaeol with zero to four cyclopentane rings per C_{40} biphytanyl chain. *Thermoplasma* contains at least six different glycolipids and seven different phosphorus containing lipids all based on caldarchaeol with different degrees of cyclization. In *Sulfolobus* the biosynthesis of these lipids has been studied using ^{12}C and ^{3}H-glycerol indicating that the biosynthetic pathways are unique. The fact that all archael lipids are derived from diphytanylglycerol ether or its dimer suggests that these lipids have a common function with the structure of archaeol and caldarchaeol and the covalent linkage at the ends of the chain together with the introduction of pentacyclic rings ensures that fluidity is maintained as temperature increases. The unique structures are also resistant to many common lipolytic enzymes, such as phospholipases, thus imparting a survival mechanism in mixed communities where such enzymes may be released. Many thermophilic archael membranes also possess a high negative charge on the inside of the membrane which may be shielded by amino groups of membrane proteins and may impose some stabilising feature to the membrane.

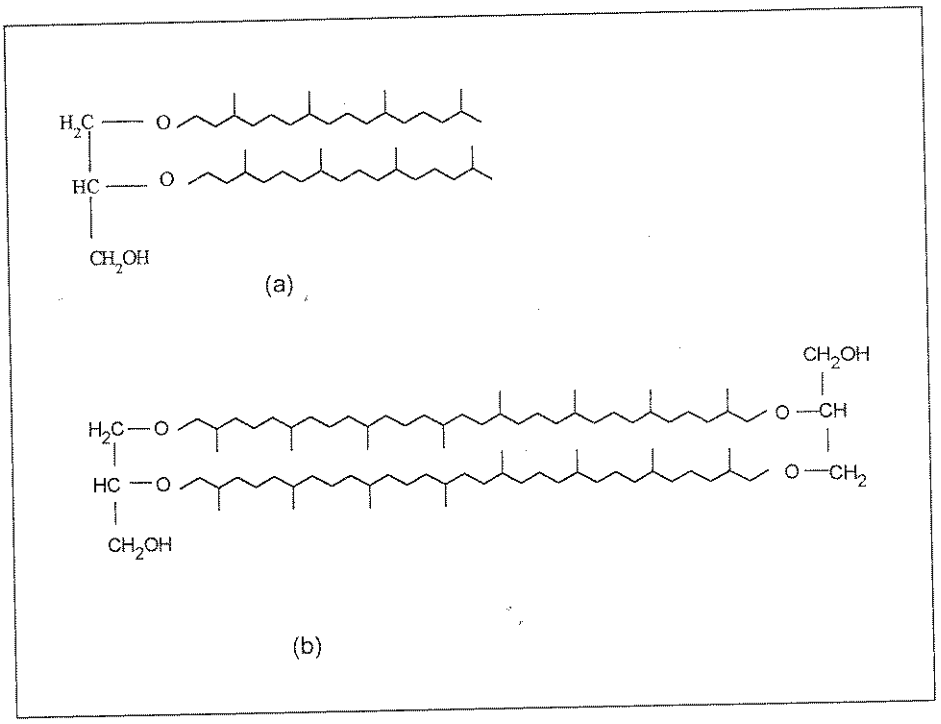

Figure 3 - *Structural lipids found in the membranes of thermophilic Archaea. Structure of (a) diphytanylglycerol ether (archaeol) and (b) dibiphytanyldiglyceroltetraether (caldarchaeol) which from the core lipids from which many other are derived in thermoacidophiles and methanogens.*

METABOLISM IN THERMOPHILES

In thermophilic Archaea such as *Sulpholobus* as with other Archaea the pathways of central metabolism differ from those normally found in mesophiles. The enzyme phosphofructokinase has not been detected thus the central glycolytic pathway would not appear to be used as in eukaryotes or eubacteria. Variations of the Entner-Doudoroff pathway in which glucose is oxidised to gluconate and then dehydrated to 2-keto-3deoxygluconate is observed. In *Sulfolobus* this is followed by direct aldol cleavage to pyruvate and glyceraldehyde without phosphorylation. Glyceraldehyde is then converted to a second molecule of pyruvate. In *Pyrococcus* glucose is converted to gluconate via a ferrodoxin linked glucose oxidoreductase and a glyceraldehyde oxidoreductase which converts glyceraldehyde to glycerate. Thus these organisms have evolved novel pathways using ferrodoxin instead of NAD or NADP which is normally used as a reductant in mesophiles but which is thermolabile. There is no net yield of ATP via this pathway although ATP could be generated from acetate via acetyl CoA. Since many thermophiles are anaerobic many grow autotrophically such as the sulphate dependent Sulfolobus. Here CO_2 appears to be fixed via a reductive citric acid cycle. In Archeoglobus acetyl CoA oxidation occurs via a carbon monoxide dehydrogenase indicating the use of different enzymology in many of the pathways. Many Archaea are chemolithotrophs deriving their energy from reduction of sulphate or oxosulphur compounds or indeed elemental sulphur to hydrogen sulphide with growth yields being quite low.

BIOTECHNOLOGICAL APPLICATIONS WITH THERMOPHILES

Currently there is much interest in possible biotechnological applications of thermophiles. Some early applications involved the use of thermostable restriction enzymes from *Thermus aquaticus*, Taq1, which is widely used to cut and analyse DNA for cloning and DNA manipulation. With the development of PCR (the polymerase chain reaction) as a tool for the amplification of DNA the need arose for a thermostable DNA polymerase which could faithfully copy DNA and withstand DNA denaturing temperatures so that the copied strand could be denatured and be copied again. Again *Thermus aquaticus* was used as a source of Taq polymerase. More recently this enzyme has been replaced with Pfu polymerase from *Pyrococcus furiosis* which is an Archaea and whose enzymes are more stable. Indeed in general enzymes from thermophiles are not only more thermostable but also show significant resistance to other denaturing agents such as detergents and organic solvents and as such these properties offer tremendous potential for the development of new biotechnological applications. Over 70% of the global use of enzymes, estimated at close to $1.5 billion, is limited to a few industries such as brewing and starch hydrolysis, utilising amylases, the detergent and dairy industries utilising proteases, and industries utilising diagnostic enzymes. Counterparts in thermophilic organisms have been discovered such as amylases and proteases (table 2) in *Pyrococcus* and *Sulfolobus*, β-galactosidase in *Sulfolobus* and *Thermus* and a variety of potential diagnostic enzymes in numerous thermophiles. In certain circumstances direct substitution may be feasible, however many processes are optimised for low temperatures while others such as starch processing or lactose hydrolysis in whey could be made more efficient using thermostable enzymes if the pro-

ducing organism were GRAS (Generally Regarded as Safe) listed. Biotransformations, the use of enzymes in fine chemicals and pharmaceuticals synthesis, is a potential area for the use of thermostable enzymes. Currently processes such as the production of acrylamide via nitrile hydratase and production of optically pure amino acids via racemases have illustrated the tremendous potential of using specific enzymes. One of the major limitations to expanded usage tends to be the lack of stability and function in organic solvents or organic-water mixtures (Govardhan and Margolin 1995). Thermostable enzymes, because of their innate stability often function in such solvents allowing the potential to utilise such enzymes in processes which might in other circumstances not be feasible. Examples include the use of alcohol dehydrogenase to reduce aldehydes and ketones to chiral alcohols or the use of esterases to form alcohols or acids in organic solvents. The potential to utilise thermophiles in waste treatment is being examined particularly in anaerobic digestion processes where production of methane offers an economic fuel source from waste. Whole cells of the thermophilic fungus *Paecilomyces varioti* have been grown, in a process known as the Pekilo Process, on sulfite waste from paper processing for single cell protein and animal feed supplementation, while *Clostridium thermocellum* has been studied for the production of ethanol as a 'gasahol' from waste cellulose. Yet another possibility is the use of thermophiles in microbial leaching where exothermic oxidations produce heat, producing an environment ideal for thermophiles. In addition the low pH used in leaching would suit thermoacidophiles. Currently ores being investigated include nickel, iron, copper and uranium utilising *Sulfolobus* which offers cleaner alternatives to smelting and its associated air pollution. There is also the possibility of removal of organic sulphur from coal allowing the production of cleaner fuels.

Thermophiles also offer the possibility of producing biologically active substances such as antibiotics as from the thermophilic actinomyces *Thermoactinopolyspora* spp. Given that relatively few of the microscopically observed organisms present in thermal niches have ever been cultured it is to be expected that in the near future new biologically active substances will be discovered amongst inhabiting communities once suitable culture techniques have been developed.

Table 2 - Some potential and real biotechnological applications of thermophilic microorganisms.

Biotechnological Product	Application
Biotransformation	
Racemases from *Thermus*	Optical resolution of amino acids
Alcohol dehydrogenase	Acid and ester formation
Reduction of 3-keto groups (*Sulfolobus*)	Progesterone modification
Speciality Enzymes	
Thermostable DNA polymerases	PCR for DNA amplification
Thermostable restriction enzymes (*Taq, Pfu*)	Gene cloning and mapping
Industrial enzymes	
Proteases from Bacillus	Stable detergent enzymes
β-galactosidase	Whey processing
Amylase *Bacillus lichenformis*	Starch Processing
Fuels	
Liquid fuels	Ethanol, butanol and methanol
Methane from thermophilic methanogens	Methane
Waste Treatment	
Waste management	Composting
Anaerobic digestion	BOD removal
Coal desulphurisation	Sulphur free/reduced coal
Microbial Leaching	
Ore recovery with Sulfolobus	Metal leaching
Organic Acids	
Lactic, acetic, malic, fumaric	Fine chemicals
Amino acids	Animal feed supplimentation
Speciality Chemicals	
Antibiotics-thermorubrin, thermoviridin	Microbial control
Bacterocins as in *Bacillus stereothermophilus*	Thermocin microbial control
Stabilising factors in *Methanothermus*	Stabilising enzymes
Stable co-factors	Biosensor devices

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