

Life at High Temperatures

Thomas D. Brock

Organisms capable of living at high temperatures, called thermophiles, have long fascinated biologists and earth scientists. Natural high-temperature environments are widely distributed on the earth, being found in association with volcanic activity. Living bacteria are

level. Stetter *et al.* (3) described a bacterial genus, *Pyrodictium*, capable of growth up to 110°C; isolates were obtained from a submarine thermal area off the coast of Italy. However, discovery of the deep-sea hydrothermal systems extended the temperature range available

Summary. Water environments with temperatures up to and above boiling are commonly found in association with geothermal activity. At temperatures above 60°C, only bacteria are found. Bacteria with temperature optima over the range 65° to 105°C have been obtained in pure culture and are the object of many research projects. The upper temperature limit for life in liquid water has not yet been defined, but is likely to be somewhere between 110° and 200°C, since amino acids and nucleotides are destroyed at temperatures over 200°C. Because bacteria capable of growth at high temperatures are found in many phylogenetic groups, it is likely that the ability to grow at high temperature had a polyphyletic origin. The macromolecules of these organisms are inherently more stable to heat than those of conventional organisms, but only small changes in sequence can lead to increases in thermostability. Because of their unique properties, thermophilic organisms and their enzymes have many potential biotechnological uses, and extensive research on industrial applications is under way.

found in most boiling-water environments, where they often reproduce extremely well (1). A question of considerable interest concerns the upper temperature limit for life in liquid water environments. Until a few years ago, the liquid water environments with the highest known naturally occurring temperatures were close to sea level, and hence had boiling points of around 100°C. Over the past decade, however, remarkable habitats with temperatures as high as 350°C have been discovered at the bottom of the oceans, opening up the possibility of organisms living at even higher temperatures than previously imagined. In 1967 I noted, "Bacteria are able to grow . . . at any temperature at which there is liquid water, even in pools which are above the boiling point" (1, p. 1014). In the years since, this statement has been amply confirmed, and many articles on organisms living at or near the sea-level boiling point of water have been published (2).

To extend such observations to higher temperatures, it was necessary to find boiling springs at locations below sea

for biological colonization from 110°C to above 350°C (4). The latter temperature is clearly too hot for life, as peptide and phosphodiester bonds (5) and amino acids (6) are destroyed even at 250°C (7). Thus the upper temperature limit for life is somewhere between 110° and 250°C. Since undersea hydrothermal vents exist at water levels from near sea level to the deep ocean, it should be possible to find high-temperature vents emitting sterile boiling water with thermal gradients in the cooling outflow of such vents where organisms might first appear. This type of habitat would then provide a suitable location for determining the upper temperature limit for life.

Diversity of Thermophilic Organisms

Three broad classes of organisms have been recognized on the basis of temperature optima for growth: psychrophiles, capable of growing at low temperatures; mesophiles, growing in the temperature range 25° to about 45°C; and thermophiles, growing at temperatures from

55°C to the boiling point. As the temperature increases, whole taxonomic groups of organisms are eliminated from the habitat (Table 1) (1, 3). No multicellular animals live at temperatures greater than 50°C, although a few protozoa can live at a slightly higher temperature. Multicellular plants also show upper temperature limits around 50°C. Thus, above 50°C only microorganisms are found. Eukaryotic microorganisms are much more restricted in their distribution than prokaryotic microorganisms; the upper temperature limit for eukaryotes seems to be about 60°C (8). Thus, above 60°C only prokaryotic organisms are found. It seems likely that structural characteristics of eukaryotes, perhaps in nuclear membrane systems, are incompatible with thermostability.

Not all kinds of prokaryotes are able to grow at temperatures greater than above 60°C. Only a few genera are represented, and even among these genera we find that only certain species are capable of high-temperature growth. Furthermore, certain kinds of prokaryotes seem incapable of very high temperature growth. For instance, the photosynthetic prokaryotes show a well-defined upper temperature limit of 70° to 73°C (9). In many parts of the world, photosynthetic prokaryotes are not found even at temperatures as high as 70°C (10, 11).

Although photosynthetic organisms do not live at temperatures greater than 73°C, autotrophic organisms do, but these are autotrophs capable of using inorganic energy sources such as sulfide, elemental sulfur, and ferrous iron. In addition to these chemolithotrophs, heterotrophic bacteria grow rapidly in water at or near the boiling point. The diversity of bacteria living in boiling water is surprisingly high: sulfur bacteria, hydrogen-oxidizing bacteria, elemental sulfur-respiring bacteria, obligate anaerobic heterotrophs, and aerobic heterotrophs. Some of these bacteria are capable of switching from an aerobic to an anaerobic metabolism. Finally, some are not only extremely thermophilic but also acidophilic.

In recent years a number of new and interesting thermophilic bacteria have been isolated and characterized (Table 2). Many of these bacteria are now available from the German Collection of Microorganisms. The organisms can be cultured readily, even in large-scale fermenters, using well-defined culture media.

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Effect of Temperature on Species Diversity

The relation between species diversity and temperature for several different single taxonomic groups is illustrated in Fig. 1. As shown, the population structure becomes progressively simpler with increasing temperature. Prokaryotes are currently divided into two major kingdoms, the Eubacteria and the Archaeobacteria (12). One interesting fact, possibly of evolutionary significance, is that at temperatures greater than 90°C, all species capable of reproducing are members of the Archaeobacteria. However, not all Archaeobacteria are thermophiles, and most Archaeobacteria live at mesophilic temperatures. Among one Archaeobacteria group, the methanogens, species are known that live at low mesophilic, moderate thermophilic, and high thermophilic temperatures. Another fact illustrated in Fig. 1 is that the spore-forming bacteria that are thermophilic have generally lower temperature maxima than the species that do not form spores. The Eubacteria living at the highest temperatures (up to 90°C in some cases) almost without exception do not form spores; they are Gram-negative and of uncertain taxonomic affiliation.

The Archaeobacteria were characterized initially from the nucleotide base sequence in the 16S RNA of the ribosome, but have subsequently been shown to differ from other bacteria in a variety of properties (12). On the basis of 16S RNA nucleotide sequences a phylogenetic scheme for all living organisms has been constructed (Fig. 2). The relation of Archaeobacteria to eukaryotes in a number of basic molecular properties suggests that three major phylogenetic lines—Archaeobacteria, Eubacteria, and eukaryotes—diverged early.

Thermoacidophiles

One group of thermophilic bacteria is also acidophilic, living only at low pH. These bacteria are remarkable because of their requirement for two extreme environmental factors at the same time. Most of the thermoacidophiles are Archaeobacteria capable of reducing S° using H₂ as an energy source (sulfur respiration) or of oxidizing elemental sulfur using oxygen or ferric iron as an electron acceptor. It is not surprising that the thermoacidophiles frequently have a sulfur-based energy metabolism, since the low-pH environments are generally sulfur-rich. However, one thermoacido-

phile, *Thermoplasma acidophilum*, does not have a metabolism linked to sulfur. This heterotroph is found in self-heating coal refuse piles. It can live at moderately high temperatures (55° to 60°C) and low pH even though it lacks a cell wall.

Molecular Basis of Thermophily

In conventional organisms macromolecules such as proteins and nucleic acids are inactivated irreversibly by heat, but in thermophiles these components are

stable (13). Supramolecular structures such as ribosomes and membranes are also thermostable in thermophiles, although inactivated by heat in mesophiles. Further, the catalytic activity of thermophilic enzymes is low or absent at moderate temperatures at which conventional enzymes of similar function are optimally active. The temperature optima of thermophilic enzymes are frequently at or above the optima for the growth of the organisms.

The most direct approach to determining the molecular basis of thermostabili-

Table 1. Upper temperature limits for growth (2, 3).

Group	Approximate upper temperature (°C)
Animals	
Fish and other aquatic vertebrates	38°
Insects	45° to 50°
Ostracods (crustaceans)	49° to 50°
Plants	
Vascular plants	45°
Mosses	50°
Eukaryotic microorganisms	
Protozoa	56°
Algae	55° to 60°
Fungi	60° to 62°
Prokaryotic microorganisms	
Blue-green algae (Cyanobacteria)	70° to 73°
Photosynthetic bacteria	70° to 73°
Chemolithotrophic bacteria	>100°
Heterotrophic bacteria	>100°

Table 2. Species and genera of thermophilic bacteria discovered in recent years (25).

Archaeobacteria
Aerobic acidophiles, autotrophs
<i>Sulfolobus acidocaldarius</i> type species
<i>Sulfolobus brierleyi</i>
<i>Sulfolobus solfataricus</i>
Grow at 70° to 90°C (optimum, 75° to 85°C); pH 1 to 4; use organic compounds and S° as energy source; use O ₂ or Fe ³⁺ as electron acceptor
Thermoproteales
<i>Thermoproteus tenax</i>
<i>Desulfurococcus mobilis</i>
<i>Desulfurococcus mucosus</i>
<i>Thermophilum pendens</i>
<i>Thermococcus celer</i>
Grow at 70° to 85°C (optimum, 85°C); anaerobic; acidophilic to neutrophilic; use organic compounds as energy sources; use S° as electron acceptor
Methanothermaceae
<i>Methanothermus fervidus</i>
Grows at 70° to 95°C (optimum, 85°C); anaerobic; uses H ₂ and CO ₂ exclusively
Pyrodictium
<i>Pyrodictium Brockii</i>
<i>Pyrodictium occultum</i>
Grow at 85° to 110°C (optimum, 105°C); anaerobic; use H ₂ and S°; autotrophic
Other new species of thermophiles—all anaerobes
<i>Clostridium thermohydrosulfuricum</i> —optimum, 65°C; produces ethanol
<i>Clostridium thermosulfurogenes</i> —optimum, 60°C; ferments pectin; forms S° from S ₂ O ₃
<i>Thermoanaerobacter ethanolicus</i> —optimum, 65°C; produces ethanol
<i>Thermoanaerobium Brockii</i> —optimum, 65°C; produces ethanol
<i>Thermobacteroides acetoehtylicus</i> —optimum, 65°C
<i>Thermodesulfobacterium commune</i> —optimum, 70°C

ty is to examine the amino acid sequence of a protein formed by a temperature-sensitive mutant and to compare this sequence to that of the wild-type parent. Such a study was done by Grütter *et al.* (14) for the lysozyme of bacteriophage T4. The three-dimensional structure of the temperature-sensitive mutant lysozyme was identical to that of the moderately thermostable wild-type enzyme. The authors concluded that "differences in thermostability of proteins are, in general, due to subtle changes in hydrophobic interactions, hydrogen bonds and so on and not to a single determinant such as metal binding or changes in secondary structure" (14, p. 668), and also that "the net free energy of stabilization of proteins is small, and derives from a delicate balance between large stabilizing forces, principally due to hydrophobic interactions, and large destabilizing ones, principally due to chain entropy" (14, p. 668).

Perutz (15) analyzed the factors involved in the thermostability of proteins. Comparisons were made of amino acid sequences and the three-dimensional structures of ferredoxins, hemoglobins, and glyceraldehyde phosphate dehydrogenases exhibiting different degrees of thermostability. The results showed that the greater heat stability of the thermostable proteins was due to extra salt bridges between portions of the folded molecules. Perutz asked, "Why are mesophile enzymes unstable when it would be so cheap, in structural terms, to stabilize them?" (15, p. 1191). He suggested that they become denatured and are then broken down by proteases while fresh ones are being synthesized, an apparently wasteful process that may be an essential part of metabolic regulation. Another explanation for the thermolability of mesophilic proteins is that "interactions between proteins and small molecules require a certain flexibility of structure which is incompatible with a highly cross-linked, rigid, and hence heat-stable protein" (1, p. 1016).

Salt bridges may not fully explain protein thermostability. Yutani *et al.* (16) studied the effect of single amino acid substitutions on the stability of the alpha subunit of the tryptophan synthetase of *Escherichia coli*. They found that a single amino acid change increased the stability of the molecule without a gross change in conformation. The stability of the enzyme was greatly increased by an increase in hydrophobicity brought about by the substitution of a few suitable amino acid residues.

Matsumura *et al.* (17) determined the nucleotide sequences of the gene en-

coded by the plasmid carrying a kanamycin-inactivating enzyme (kanamycin nucleotidyltransferase) in a thermophilic bacterium (*Bacillus stearothermophilus*) as compared with the same enzyme in a

mesophilic bacterium (*Staphylococcus aureus*). Despite the fact that the plasmids coding these two enzymes had completely separate origins, the nucleotide sequences were identical except for only one base in the midst of the structural gene (Fig. 3). This base change resulted in the substitution of a threonine residue with a lysine residue. The position of this lysine substituent was such that the protein surface could acquire increased electrostatic bridging without any significant change in three-dimensional structure, a situation consistent with the conclusion of Perutz (15). The effect of this substitution on thermostability is shown in Fig. 4.

In all these studies, enzymes of similar function differed in thermostability by only 5° to 10°C. It seems likely that an enzyme capable of remaining active at 90° to 100°C would differ in a number of ways from an enzyme of similar function active at 30° to 40°C. It is difficult to compare in a meaningful way enzymes of similar function with such broadly different temperature stabilities, because the enzymes are perforce derived from taxonomically unrelated organisms and hence may (probably do) differ, as a result of evolutionary changes, in many ways not related to temperature stability. However, it appears that thermostability is an inherent function of the structure of a macromolecule and is not due to the presence of stabilizing substances. It also seems that the protein-synthesizing machinery of thermophiles is more thermostable than that of mesophiles (18).

Genetics and Evolution of Thermophily

The question of the origin of thermophilic microorganisms has intrigued scientists for over 100 years. Two major hypotheses have been advanced: (i) the first organisms arose in high-temperature environments, so that thermophilic organisms were primordial and subsequent organisms were derived from them by evolution and (ii) the first organisms were not thermophiles but were adapted to moderate temperature conditions, and thermophilic organisms have had a secondary origin from psychrophilic or mesophilic types.

Let us consider the second hypothesis first and imagine the processes that might be involved in deriving a thermophilic organism from a mesophilic one. As we have indicated, all macromolecules of thermophiles are stable at the high temperatures at which these organisms live, whereas functionally similar macromolecules from mesophiles are de-

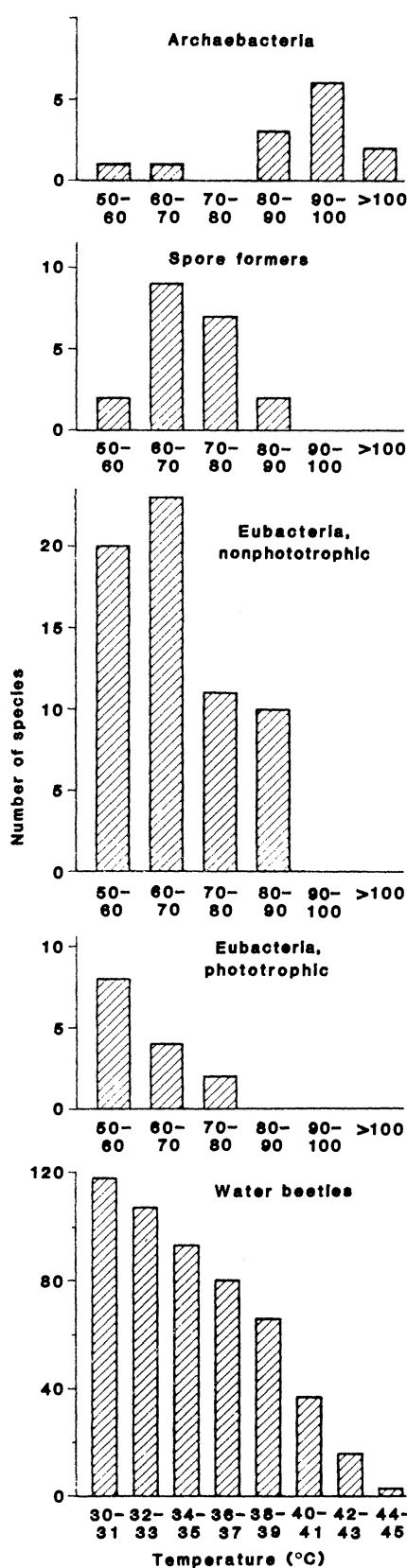


Fig. 1. Effect of temperature on species diversity of various groups of organisms (2).

stroyed under the same conditions. Therefore, many genetic changes must take place for a thermophilic organism to be derived from a mesophile. On the other hand, if the first organism were a thermophile, it is quite easy to imagine a temperature-sensitive mutant arising, incapable of growing at higher temperatures. However, one difficulty with the hypothesis of a primordial origin of thermophily is that thermophily is not restricted to a single phylogenetic line of bacteria, but is found in both Archaeobacteria and Eubacteria (Fig. 2). Thus the common ancestral organism of all life forms postulated in Fig. 2, even the eukaryotes, would have had to have been a thermophile. Without additional evidence, further speculation about this point is probably fruitless.

These evolutionary considerations point to the importance of careful genetic studies of thermophilic organisms. Our knowledge of this important area is poor (13). Systems for genetic analysis were recently developed for *Bacillus stearothermophilus* and *Thermus thermophilus* (19). Plasmids for use in transformation and molecular cloning have made possible genetic analysis of *B. stearothermophilus*, and a system that produces efficient gene transfer has been developed for *T. thermophilus*. To date none of these systems has been applied to the genetics of the thermophilic property, although molecular cloning was used in the studies of the kanamycin nucleotidyltransferase protein. In another approach, Oshima (18) is inserting genes from thermophilic microorganisms into *E. coli* in order to explore the molecular basis of thermostability. Restriction enzyme mapping and base sequence analysis is permitting a more facile determination of amino acid sequences of proteins. The gene for thermostable 3-isopropylmalate dehydrogenase has been cloned into *E. coli* and the amino acid sequence deduced from the base sequence. The codon usage of the *T. thermophilus* gene was quite different than that optimally used in *E. coli*, but despite this the gene was efficiently expressed in *E. coli*. The tryptophan synthetase gene from *T. thermophilus* has also been transferred to and expressed in *E. coli*. Other genes cloned into *E. coli* from thermophiles include a malate dehydrogenase from *Thermus flavus* and a 5S RNA gene from *T. thermophilus*. Attempts have also been made to clone genes from the thermoacidophilic Archaeobacteria into *E. coli*, but it appears that these genes cannot be translated by the *E. coli* protein-synthesizing machinery. Thus, only ribosomal RNA's and transfer RNA's from

thermoacidophiles have been studied in *E. coli* by molecular cloning techniques.

The most striking observation relating to the genes of the thermoacidophilic Archaeobacteria is the discovery of introns. The genes coding for two transfer RNA molecules in *Sulfolobus solfataricus* contain an extra oligonucleotide fragment immediately following the anticodon sequence that is excised when the transfer RNA molecules mature (20).

Although little solid information on the origin of thermophily is available, the new genetic techniques that are now being applied should ultimately permit a more sophisticated understanding.

Biotechnological Applications of Thermophiles

Thermophilic microorganisms appear to offer some major advantages for microbial technology, and a considerable amount of research on thermophiles is motivated by these potential applications. The most attractive attribute of thermophiles is that they produce enzymes capable of catalyzing biochemical reactions at temperatures markedly higher than those of conventional organisms. In addition, enzymes from thermophiles are more stable at conventional temperatures than are enzymes from mesophiles, prolonging the shelf life of commercial

products. An increase in temperature results in an increase in the diffusion rate and in the solubility of most nongaseous compounds. An increase in temperature also reduces the viscosity and surface tension of water, which has some positive effects for microbial fermentations. The decrease in gas solubility at high temperature is of little consequence if a process is being carried out under anaerobic conditions, although aerobic processes may be seriously limited. Large-scale reactions are often limited by physical processes, and thermophilic enzymes are stable and very active under these high-temperature conditions. Therefore, an industrial enzymatic process should occur much more rapidly if it can be carried out with a thermophilic enzyme (21).

Metabolic activity results in heat production, an especially serious problem in large-scale fermentations. With conventional heat-sensitive microorganisms, extensive effort must be given to cooling the fermentation process, and as much as 10 percent of the energy cost of a microbial fermentation may be for heat transfer. Thermophilic fermentations need not be cooled, with a consequent saving in energy.

There has been considerable interest in the use of thermophiles for the production of fuel and bulk chemicals such as alcohols and acids. The main attrac-

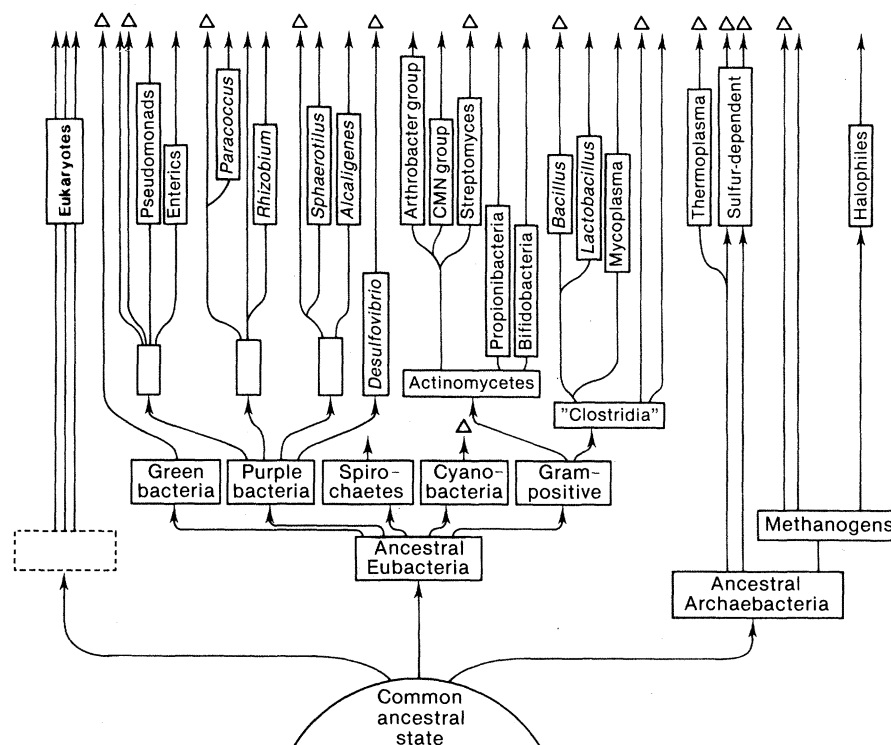


Fig. 2. Phylogeny of living organisms, showing the presence of thermophiles in both the Eubacteria and the Archaeobacteria lines (12). Lines with thermophilic members (defined by the ability to grow above 55°C) are indicated by the triangles. The CMN group contains the genera *Corynebacterium*, *Mycobacterium*, and *Nocardia*.

tion of microorganisms for the production of fuels and commodity chemicals is that they permit the use of organic materials derived from plant biomass. Cellulosic biomass from the agricultural and forest industries is available in vast amounts and is a potentially inexhaustible source of fermentable carbohydrate. Another widely available carbohydrate is starch, derived primarily from corn. Corn syrup (hydrolyzed corn starch) is produced in enormous amounts by the corn wet-milling industry and can be readily utilized in microbial fermentations. Another fermentable carbohydrate is cane sugar.

A potentially major microbial process is the production of ethanol, primarily for use as a fuel additive in motor cars. However, ethanol also has a major industrial use as a feedstock for the production of ethylene. Ethylene is the largest-volume organic chemical produced in the United States and is used both as a starting material for the production of other chemicals and as the building block of polymers such as polyethylene.

At present, all the ethanol produced microbially in the United States is made by the fermentation of sugar with yeast. Because a number of anaerobic thermophilic bacteria produce ethanol as a metabolic product, the use of thermophilic bacteria for ethanol production has frequently been proposed. There are three advantages: (i) the elevated incubation temperature makes distillation of the ethanol product more efficient; (ii) the cooling requirement (needed in the yeast fermentation) is obviated; and (iii) some thermophilic bacteria can carry out a direct fermentation of polysaccharides to ethanol, whereas yeast is incapable of hydrolyzing polysaccharide polymers. The most interesting bacterium of this kind is *Clostridium thermocellum*, an organism capable of fermenting cellulose to ethanol. However, ethanol is only one of the fermentation products produced by this organism and considerable amounts of the fermentable carbohydrate end up in organic acids. Also, the bacterium is fairly sensitive to the ethanol it produces, and the self-inhibitory process markedly re-

duces the amount of ethanol that can be formed (a strain has been isolated that will tolerate 5 percent ethanol, whereas the parental strain stops growing in the presence of 3 percent ethanol). Finally, most of the research with *C. thermocellum* has been done with purified polysaccharides as starting materials; crude biomass polysaccharide is fermented considerably more poorly.

Acetic acid produced by thermophilic microorganisms has many industrial uses (in addition to its use in vinegar). *Clostridium thermoaceticum* and *Clostridium thermoautotrophicum* carry out an acetic acid fermentation, but, because these thermophiles are moderately sensitive to the acid they produce, the fermentation must be done at pH values at or above the pK_a of acetic acid, which is 4.80 at 60°C. Economical recovery of acetic acid from such fermentation broths at such high pH is not possible. Until an economic method of product recovery and a more efficient, higher yielding production of acetic acid is obtained, it is unlikely that this process will be used.

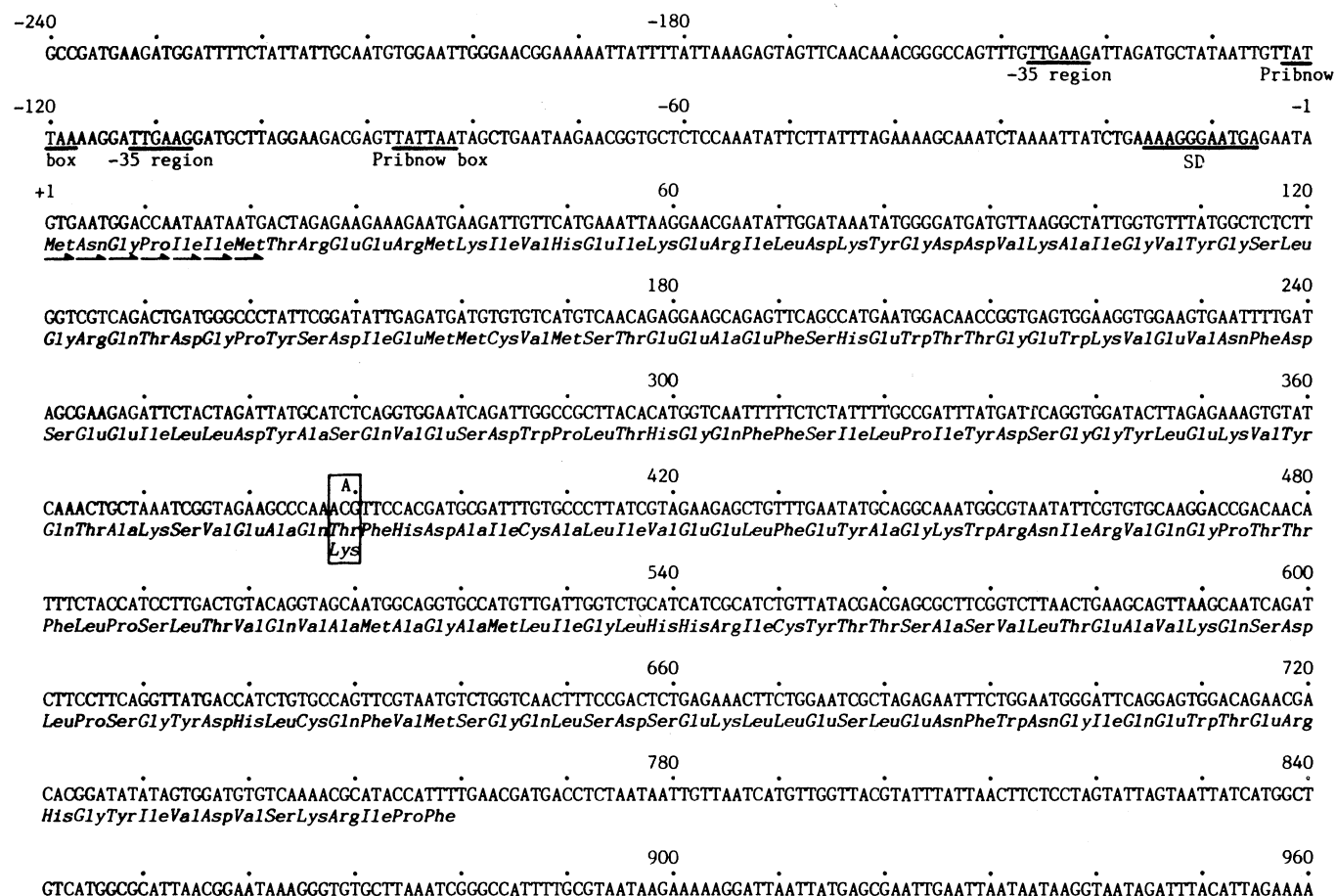


Fig. 3. Comparison of the nucleotide and amino acid sequences of kanamycin nucleotidyltransferase from a mesophilic bacterium with that of the thermophile *Bacillus stearothermophilus*. The nucleotide sequence is equivalent to the messenger RNA sequence except for the replacement of T for U. The flanking regions are also shown. The open reading frame begins at the base labeled +1 and continues through base +759. The only difference from the mesophile gene is at position +389, where the C of the mesophile is an A, resulting in replacement of the amino acid threonine of the mesophile with a lysine in the thermophile. [From Matsumura *et al.* (17)]

Several other products might be produced by using thermophilic microorganisms (21). Among these are lactic acid, produced by bacteria of the genera *Clostridium*, *Thermoanaerobium*, *Thermoanaerobacter*, and *Bacillus coagulans*; carotenoids, produced by *Thermus aquaticus*; amino acids, produced by *Bacillus coagulans*; antibiotics, produced by thermophilic streptomycetes of the genus *Thermoactinomyces*; some alkaloid-like products produced by *Thermoactinomyces*; some uncharacterized bioactive products produced by thermophilic cyanobacteria; some unusual lipids produced by the thermophilic Archaeobacteria; and some nonlipid structural components, such as cell wall macromolecules, which may have some unique properties suitable for commercial exploitation.

Thermophilic microorganisms can carry out biotransformation reactions, converting in a stereospecific manner one starting material into a more valuable product. For instance, Lamed and Zeikus (22) showed that *Thermoanaerobium brockii* is able to carry out a stereospecific reduction of several ketones to the corresponding secondary alcohols. Several other potential reactions of this sort were reviewed by Weimer (21).

As noted, thermostable enzymes have considerable industrial importance (21). Among these is the protease from *Bacillus thermoproteolyticus*, called thermolysin, and the α -amylase of *Bacillus stearothermophilus*, which converts starch into glucose. Glucose isomerase would be of considerable utility, because the production of fructose from glucose is carried out enzymatically in the manufacture of high-fructose syrup. No glucose isomerase from an extreme thermophile has been reported, possibly because of the lack of an effective screening method. The enzyme used commercially to produce high-fructose syrup is actually a xylose isomerase produced by the moderate thermophile *Bacillus coagulans*. The *B. coagulans* enzyme is reported to be most active at 75°C, although the organism does not grow at temperatures nearly that high.

Thermophilic microorganisms may also be of value in the waste treatment process. Anaerobic waste treatment processes leading to the production of methane are used in almost all sewage treatment systems, as well as in many industrial waste treatment systems. Typically, anaerobic waste treatment is carried out in the mesophilic temperature range, but treatment processes operating in the range 45° to 65°C have been known for a

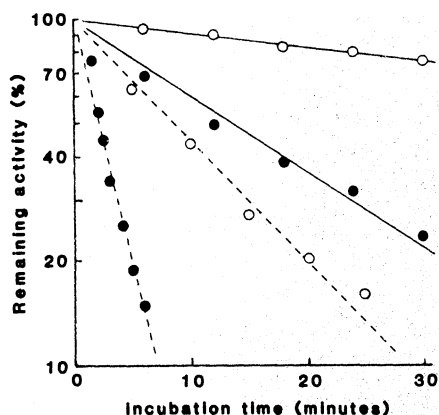
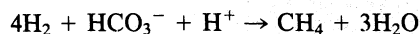
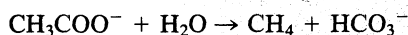


Fig. 4. Thermostability of kanamycin nucleotidyltransferase for the two proteins illustrated in Fig. 3. Symbols: solid circle, mesophile; open circles, thermophile; dashed lines, enzyme heated at 50°C; continuous lines, enzyme heated at 55°C. After heating for the times indicated, the samples were cooled and assayed for enzymatic activity at 37°C. [From Matsumura *et al.* (17)]

long time. One major advantage of anaerobic digestion processes in general is that the complex and insoluble organic materials of the waste are converted into methane gas, a clean, energy-rich fuel, which, because it is water-insoluble, is readily separated from the fermentation process. Thermophilic methanogenic bacteria are capable of carrying out the two major methanogenic reactions:



and



Several full-scale thermophilic anaerobic waste digestion processes have been operated in various cities around the world at temperatures ranging from 50° to 60°C. It has not been possible to develop a stable process that operates at a temperature greater than 60°C. It should be noted, however, that the substrates of the methanogenic bacteria, hydrogen gas and acetate, must be produced from the complex organic wastes by other thermophilic organisms present in the system, so that limitations on the thermophilic digestion process could be due either to the methanogens or to these other organisms. Even in carefully controlled laboratory-scale bioreactors, it has not been possible to maintain a methanogenic process at temperatures greater than 65°C (23). The advantages of thermophilic anaerobic digestion over the mesophilic process are (i) increased reaction rate and decreased retention time; (ii) destruction of pathogenic microorganisms that might be present in the sewage; (iii) lower viscosity so that less

energy is required for mixing; and (iv) easier dewatering of the resulting sludge. However, there are also some disadvantages of a thermophilic anaerobic digestion process: (i) the high energy requirement for heating (insufficient heat is generated by the process itself); (ii) the difficulty of maintaining the process under a stable condition, since the process frequently fails for unknown reasons; and (iii) the quality of the liquid effluent is often poor because of the presence of high levels of organic acids and other soluble materials.

Another potential practical use of thermophilic microorganisms is in microbial mining, that is, the use of microorganisms in the recovery of metals from ores and mineral wastes. This process, called leaching, results from the action of bacteria on insoluble metal-containing ores, primarily sulfide minerals. The most extensive use of bacterial leaching has been in the processing of low-grade copper ores, but microbial leaching is also used in an indirect way to extract uranium from ore. All bacterial leaching processes currently take place at low pH values, where the solubilized metal ions do not precipitate. A major advantage of bacterial leaching over conventional recovery of ore by smelting is the low operating cost and the elimination of air pollution. However, bacterial leaching is of economic value only when a low-grade ore is used. Bacterial leaching has been done at mesophilic temperatures for a long time; it is only in recent years that it has become evident that in many commercial leaching operations high temperatures develop in parts of the leach piles and that thermophilic microorganisms might be present.

Two organisms, the archaeobacterium *Sulfolobus acidocaldarius* and the eubacterium *Thiobacillus ferrooxidans* (a thermophilic strain designated TH-1) have been used in laboratory leaching studies. Strain TH-1 grows at temperatures up to about 55°C and *Sulfolobus* up to about 90°C. Although no detailed studies have been reported, inside the large leaching piles used commercially temperatures greater than 50°C frequently develop as a result of the self-heating process. Thus it seems reasonable that in a large leaching pile the leaching process could be limited by the unavailability of a thermophilic organism, so that an inoculation of the pile would be of value. However, inoculation may not always be necessary if the thermophilic organisms become dispersed naturally to a leaching pile.

Brierley and Brierley (24) indicate that

leaching with thermophilic organisms occurs much more rapidly than with mesophilic organisms. However, they also indicate that there are considerable problems in the use of thermophilic organisms for leaching of metals in a practical situation because little is known about the factors necessary for encouraging the growth and development of these organisms.

Conclusion

Although biotechnological applications of thermophiles seem promising, there is no extensive large-scale use. Many thermophiles have only been discovered recently, and extensive research on thermophily has not been carried out. It seems almost certain that something of real value may eventually develop. To date, the most useful process involves the use of a thermostable xylose isomerase for the production of high-fructose syrup for the soft-drink industry. This enzyme, however, has been obtained from a moderate thermophilic microor-

ganism incapable of growing at the temperature at which the enzyme functions. Thus, if a suitable screening method were developed for detecting the production of glucose isomerase, it might be possible to find such enzymes from more typical thermophiles. It should be emphasized that the industrial potential of microorganisms has not yet been realized primarily because the requisite biotechnological research is far from complete.

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What Makes a Good Computer Device?

Robert W. Keyes

The realization of the large digital computer in the 1940's began a continuing search for devices that are better than those in existing machines. The invention of the transistor quickly led to a revolution in digital device technology. Germanium transistors were used in the earliest transistorized computers, but were soon supplanted by silicon devices and silicon transistors embodied in silicon integrated circuits. The integrated silicon chip has been remarkably successful. Increasing integration has rapidly reduced the cost and power dissipation of modern microelectronics and en-

dowed it with high reliability and high speed of operation.

Nevertheless, suggestions continue for alternative computer devices judged to be in some way superior to silicon transistors. Indeed, ever since the introduction of the transistor, major research and development efforts to find a better device have persisted. These efforts have, however, been notably unsuccessful. It behooves us, therefore, to try to identify the reasons for the remarkable achievements of silicon transistor technology and for the failure of the alternatives. Some of the reasons are rooted in chemistry and material science, but others are to be found in the nature of digital computation.

General Purpose Computers

The word "computer" is used here to mean "general purpose computer." Some of the important features that distinguish the general purpose computer are as follows. It can accept many types of problems. The nature and length of the input that defines the problem is not fixed or known in advance. Nor is the length of the calculation known very well; it is often determined by application of a test within the computation to decide if the calculation is finished. In fact, there may be many branch points, at each of which the direction to be taken depends on the results obtained up to that point, embedded in a procedure. Frequent references to random locations in memory must be possible. The computer is constructed from individual logic circuits or gates, entities that perform elementary functions.

Great depth in the handling of information may be involved; that is, the result of an elementary operation is used in a succeeding operation, the result is used again, and so on, through thousands or even millions of steps. For example, in simulating the evolution of a system through time the outputs are recycled to become inputs many times. Information must not be allowed to deteriorate during such long series of operations.

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