CURRENT CONCEPTS OF THERMOPHILISM AND THE THERMOPHILIC FUNGI [1]

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A thermophile is defined as an organism that grows at temperatures above those considered to be the maximum limits for most forms of life. The earliest record of thermophilic life is Sonnerat's (**131**) report of fish living in a thermal pool on the Philippine Island of Luzon. Over 50 years later, interest in this curious phenomenon was renewed and additional thermophilic organisms were described (cf. Gaughran, **73**). The first description of a thermophilic fungus is often attributed to Tsiklinsky (**141**) and her discovery of *Thermomyces* (*Humicola*) *lanuginosa* in 1899. Although Fresenius' (**63**) description of *Aspergillus fumigatus* and Lindt's (**95**) description of *Mucor pusillus* were published earlier, it was Tsiklinsky who drew attention to thermophilism in fungi. Cooney and Emerson (**37**) published a comprehensive monograph on the morphology, taxonomy, and biology of the thermophilic fungi. Over 50 fungi that appear to be thermophilic have been reported (TABLE I). Thermophilic yeasts have also been reported (**139**), but the growth temperatures of these organisms do not fall within the generally accepted range of thermophilic fungi and will not be considered as such here (cf. **37**, **39**, **40**, **52**).

The earliest studies of thermophilic organisms were descriptive and consisted of recording the occurrence of various thermophiles from heated habitats. As increased emphasis was placed on the study of the physiology and biochemistry of microorganisms, the importance of temperature as a major factor affecting microbial growth was realized. It became important to understand how thermophiles existed and apparently thrived at temperatures which were lethal for most organisms. Physiological studies have, for the most part, dealt with thermophilic bacteria and, more recently, with thermophilic algae. The thermophilic

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TABLE I

THERMOPHILIC FUNGI AND THEIR CARDINAL TEMPERATURES[a]

	Cardinal temperatures (°C)		
	Minimum	Optimum	Maximum
ZYGOMYCETES			
Absidia corymbifera (Cohn) Sacc. et Trott. (**55**)	14	40	50
Mortierella wolfii Mehrotra et Baijal (**54, 55**)	(14–16)[b]	(40–42)	48
Mucor miehei Cooney et Emerson (**37, 39, 54, 55, 135**)	(24–25)	(35–45)	(55–57)
M. pusillus Lindt (**37, 39, 55, 89**)	(20–27)	(35–55)	(55–60)
Mucor sp. I (**135**)	25	45	56
Mucor sp. II (**135**)	25	(42–45)	55
Rhizomucor sp. (**113**)	(25–30)	(45–53)	(60–61)
Rhizopus cohnii Berl. et de Toni (**55**)	10	42	55
R. microsporus v. Tiegh. (**55**)	12	40	50
Rhizopus sp. I (**135**)	16	(40–45)	55
Rhizopus sp. II (**135**)	16	40	50
Rhizopus sp. III (**135**)	28	(48–50)	60
Rhizopus sp. A (**54, 55**)	25	(45–52)	60
ASCOMYCETES			
Allescheria terrestris Apinis (**4, 55**) stat. conid. *Cephalosporium* sp.	22	(42–45)	55
Chaetomium britannicum Ames (**3**)		n.d.[c]	
Chaetomium sp. A (**54, 55**)	14	40	50
C. thermophile LaTouche (**37, 39, 94**)	(25–27)	50	(58–61)
C. thermophile var. *coprophile* Cooney et Emerson (**37, 39, 55**)	(25–28)	(45–55)	(58–60)
C. thermophile var. *dissitum* Cooney et Emerson (**37, 39, 55**)	(25–28)	(45–50)	(58–60)
C. virginicum Ames (**3**)		n.d.	
Myriococcum albomyces Cooney et Emerson (**37, 39, 55**)	(25–26)	(37–45)	(55–57)
Sphaerospora saccata Evans (**53, 55**) stat. conid. *Botrytis* sp.	12	(35–42)	50
Talaromyces emersonii Stolk (**37, 55, 133**) stat. conid. *Penicillium emersonii* Stolk	(25–30)	(40–45)	(55–60)
T. leycettanus Evans et Stolk (**55, 56**) stat. conid. P. *leycettanum* Evans et Stolk	18	42	55
T. thermophilus Stolk (**6, 37, 39, 55, 133**) stat. conid. *Penicillium dupontii* Griff. et Maubl.	(25–30)	(45–50)	(57–60)
Thermoascus aurantiacus Miehe (**6, 37, 39, 55, 133**)	(20–35)	(40–46)	(55–62)
T. crustaceus (Apinis et Chesters) Stolk (**6, 7, 20, 55, 133**) stat. conid. *Paecilomyces crustaceus* (Apinis et Chesters) Stolk	20	37	55
Thielavia sepedonium Emmons (**53**)		n.d.	
T. thermophila Fergus et Sinden (**62, 78**) stat. conid. *Sporotrichum* sp.	20	45	56
Thielavia sp. A (**54, 55**)	16	40	52
BASIDIOMYCETES			
Coprinus delicatulus Apinis (**5**)		n.d.	
Coprinus sp. (**37**)		n.d.	
Coprinus sp. (**121**)	ca. 20	ca. 45	55

TABLE I—(*Continued*)

	Cardinal temperatures (°C)		
	Minimum	Optimum	Maximum
DEUTEROMYCETES			
Acrophialophora fusispora (Saksena) Samson (**54, 55**)	14	40	50
Aspergillus fumigatus Fres. (**39, 54, 55, 89**)	(12–20)	(37–43)	(52–55)
Calcarisporium thermophile Evans (**54, 55**)	16	40	50
Chrysosporium sp. A (**54,55**)	25	(40–45)	55
Geotrichum sp. A (**54, 55**)	10	(35–42)	48
Humicola grisea Traaen var. *thermoidea* Cooney et Emerson (**37, 39, 55**)	(20–24)	(38–46)	(55–56)
H. insolens Cooney et Emerson (**37, 39, 55**)	(20–23)	(35–45)	55
H. lanuginosa (Griff. et Maubl.) Bunce (**37, 39**)	(28–30)	(45–55)	60
H. stellata Bunce (**25, 37**)	<24	40	50
Malbranchea pulchella Sacc. et Penzig var. *sulfurea* (Miehe) Cooney et Emerson (**37, 39, 55**)	(25–30)	(45–46)	(53–57)
Paecilomyces spp. Group b, 7 str. (**89**)	<30	(45–50)	(55–60)
Paecilomyces spp., Group c, 6 str. (**89**)	<30	(45–50)	(55–60)
Paecilomyces spp., Group d, 4 str. (**89**)	<30	(45–50)	(55–60)
Penicillium piceum Raper et Fennell (**55**)	12	(37–40)	48
Penicillium sp. A (**54, 55**)	20	42	55
Scolecobasidium sp. A (**54, 55**) (? = *Diplorhinotrichum galloparum* W. B. Cooke)	(14–16)	40	(50–52)
Sporotrichum thermophile Apinis (**4, 37, 55, 78**)	(18–24)	(40–50)	55
Stilbella thermophila Fergus (**60**)	ca. 25	(35–50)	ca. 55
Thermomyces ibadanesis Apinis et Eggins (**8, 51**)	(31–35)	(42–47)	(60–61)
Torula thermophila Cooney et Emerson (**37, 38**)	23	(35–45)	58
Tritirachium sp. A (**54, 55**)	16	40	55
MYCELIA STERILIA			
Papulospora thermophila Fergus (**61**)	(29–30)	ca. 45	52

[a] Identity presented as published. These fungi appear to fall within the category of thermophilic fungi *sensu* Crisan (**40**), i.e., fungi with growth temperature optima of 40 C or higher. Other parameters for thermophilism in fungi have also been used (**4, 37, 39, 52**).

[b] Temperatures in parentheses indicate the range of a cardinal temperature as derived from values reported by various investigators.

[c] Not determined but other data indicate possible thermophilism.

fungi have been relatively neglected and, beyond our knowledge of their morphological characteristics, little is known about their physiological ability to grow at elevated temperatures. Because of this limited literature on the physiology of thermophilic fungi, it is necessary to draw upon the studies of the bacteria and algae for information concerning the physiological characteristics associated with the thermophilic mode of life. From these studies, certain characteristics may be recognized as being common to all thermophilic microorganisms. Such comparisons of thermophilic organisms lead to a better understanding of the phenome-

non of thermophilism in fungi and may ultimately be used to elucidate the cellular basis for thermophilism.

What is presented here is not an exhaustive review of the literature on thermophiles and thermophilism. Selected information on our present knowledge is presented with a discussion of how it applies to the occurrence of thermophilism in fungi. For a more detailed discussion of the physiological and biochemical characteristics of thermophilic microorganisms and the effects of temperature on living cells, the reader is referred to the reviews by Brock (**18, 19**), Christophersen (**36**), Farrell and Campbell (**58**), Farrell and Rose (**59**), Gaughran (**73**), Koffler (**87**), Langridge and McWilliam (**92**), and Wood (**146**).

CURRENT HYPOTHESES TO EXPLAIN THERMOPHILISM

The primary factor which determines the ultimate temperature limits for the growth of an organism is its genetic constitution. There is evidence that the ability to grow at high temperatures can be transmitted from the thermophilic to mesophilic bacteria through genetic transformation (**104, 126, 127**). Although the role of genes in determining temperature relationships is generally accepted, the present state of our technology does not permit us to ascertain the exact nature of the genetic basis of thermophilism. The additional effects of temperature on specific biochemical and physical events which would permit an organism to grow to the limits of its genetic minimum and maximum have become significant in the study of thermophiles. Consequently, major emphasis has been placed on determining the physiological basis of thermophilism.

Studies comparing thermophilic and mesophilic bacteria are numerous. In practice, when thermophiles exhibit a physiological characteristic which does not occur in mesophiles, the difference is often attributed to the thermophile's intrinsic ability to grow at high temperatures. The possibility that the differences observed may be due to speciation or might lie within the limits of natural variation is not often considered. Unfortunately, the results of this search for the physiological basis of thermophilism have led to numerous hypotheses which are often conflicting. Gaughran (**73**) listed more than 25 hypotheses which have been proposed to explain heat resistance and/or growth at high temperatures. Since Gaughran's review, additional hypotheses have been proposed. Four hypotheses for explaining the ability of thermophiles to grow at high temperatures remain of major interest: (i) lipid solubilization; (ii) rapid resynthesis of essential metabolites; (iii) macromolecular

thermostability; and (iv) ultrastructural thermostability. Ample evidence which supports or disagrees with each of these hypotheses is available.

Hypotheses attempting to explain the inability of most organisms to grow at high temperatures are often based on the concept of a "prime lethal event," i.e., thermal death is due to the interruption of a single metabolic process or destruction of a single component essential for the maintenance of life. The existence of a prime lethal event can also be considered in the context of Blackman's "master reaction" hypothesis (15, 26, 81). Blackman (15) proposed that "when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor." If one component of an essential metabolic process is themolabile, the process and thereby the continued viability of the organism would be limited by the inhibition of this component at an elevated temperature. Death would occur if this component were completely destroyed or became nonfunctional. Death would also occur in the absence of complete destruction if the metabolic process were limited to such an extent to preclude the sustenance of life.

Lipid solubilization or the lipoid hypothesis proposes that thermal death occurs when the cell loses its integrity due to the solubilization, i.e., melting, of the protoplasmic lipids at elevated temperatures (12, 13, 79). It has been shown in a number of plants and animals that cellular lipids become increasingly saturated as the growth temperature is increased (12, 59, 73). At elevated temperatures, lipids contain saturated fatty acids which exhibit higher melting points than their less saturated counterparts. Thermophiles, by virtue of the higher melting point of their more saturated lipids, would be expected to be able to maintain their cellular integrity at higher temperatures than would mesophiles with their less saturated lipids. Gaughran (72) demonstrated that although mesophilic bacilli produced less lipid as the growth temperature was increased, they produced lipid which was more saturated. Within the temperature range studied, thermophilic bacilli did not change the quantitative or qualitative composition of their lipid. The fact that the direct relationship between growth temperature and lipid saturation can be demonstrated in many microorganisms, including mesophiles and thermophiles, has been cited as evidence of its minimal importance in thermophilism (103, 124).

With the development of improved and more sophisticated analytical techniques, interest in this temperature-lipid relationship has been renewed. Studies have been made to determine the changes which occur

in the proportions of specific fatty acids produced by an organism as the growth temperature is increased. In addition to producing lipids which contain fatty acids which are more saturated, thermophilic bacteria produce a larger proportion of long-chain fatty acids some of which contain branched chains (**30, 42, 80, 119, 120, 125, 143**). A complex relationship exists between the melting point of a fatty acid and molecular characteristics such as the number of double bonds, the length of the carbon chain, and the presence of branched carbon chains. The increase of one or more of these molecular factors increases the melting point of a fatty acid (**42, 119, 125**).

The rapid resynthesis hypothesis proposes that thermophilic growth is not due to the presence of unusually thermostable metabolites such as enzymes (see below) but is due to a particularly active metabolism which replaces thermolabile metabolites at a rate equal to or greater than the rate at which they are being destroyed (**1, 72, 73**). A thermophile thus maintains a surplus of these essential metabolites which permits metabolic processes to remain operative at temperatures usually causing denaturation. This concept has not been widely accepted because studies of the metabolism of thermophiles indicate that their biosynthetic rates are not significantly greater than those of mesophiles (**87**). More recent studies, however, may necessitate a reexamination of this hypothesis. Bubela and Holdsworth (**24**) found that a more rapid turnover of labile ribonucleic acid (RNA) occurs in *Bacillus stearothermophilus* Donk than occurs in *Escherichia coli* (Migula) Castel. et Chalmers. They suggested that this may be a mechanism by which the thermophile utilizes a higher rate of protein synthesis to replace labile cellular constituents. In studying the extreme thermophile *Thermus aquaticus* Brock et Freeze, McFeters and Ulrich (**105**) determined that the rate of oxygen uptake was four times greater at 70 C than at 50 C. They suggested that this increased respiratory rate indicated a difference between the electron transport system of this thermophile and that of mesophiles.

The third major hypothesis suggests that thermophiles are capable of producing essential macromolecules such as enzymes and other proteins which exhibit an unusual degree of thermostability. As the growth temperature is increased, these macromolecules retain their functionality and the organism can continue to grow at temperatures above the maximum limits for mesophiles. The enzymes of several thermophilic bacteria have been shown to be unusually thermostable (**2, 58, 87**). Certain structural proteins of thermophilic bacteria also exhibit an intrinsic thermostability (**88, 128**).

The nucleic acids of thermophiles do not appear to be unusually thermostable. Comparisons of the base compositions and melting profiles of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from thermophiles and mesophiles indicate that these nucleic acids do not differ significantly in thermostability regardless of their source (**58, 64, 102, 132**). Friedman and Weinstein (**67, 68**) suggested that coding errors which occur in messenger RNA at high temperatures may play a role in determining the maximum temperature limit for growth of bacteria. Eucaryotic organisms are less susceptible to this type of miscoding (**66, 144, 145**).

The fourth and most recently developed hypothesis suggests that thermophiles contain ultrastructural elements or organelles which exhibit a greater degree of thermostability than do similar components of mesophiles. This hypothesis is an amalgamation of several proposals since authors differ as to the identity of these thermostable structures. Rahn (**117**) considered the single unit subject to thermal inactivation to be the gene. Death would follow an irreversible change which makes a specific gene nonfunctional or lethal. An increasing number of workers suggest that the thermostability of cell structures, in particular, plays a role in maintaining the integrity of the cell and thereby its functionality at high temperatures (**18, 19, 20, 21, 23, 31, 75, 82, 118, 119, 120, 136**). The fine structure of the ribosome may also be a factor in thermostability. Although the component ribosomal RNA of thermophiles does not exhibit extraordinary thermostability, intact ribosomes and ribosomal subunits of thermophiles are unusually thermostable (**28, 65, 122, 132**).

The effects of other factors on thermal resistance have also been studied. The limited amount of growth occurring at high temperatures appears to be an effect of the decreased amount of dissolved oxygen available in the medium. Downey (**49**) found that equivalent amounts of growth occurred in bacterial cultures at normal and high temperatures if the same amount of dissolved oxygen were available in the medium.

Setchell (**123**) noted a yet unexplained relationship between the greater thermal resistance of algae growing in thermal pools containing siliceous water and the lesser resistance of the same species in calcareous pools. Cations such as Mg^{++} and Ca^{++} have been reported to increase the thermostability of enzymes, ribosomes, membranes, and other cellular components (**73, 74, 97, 98, 106, 118, 132, 137**). Thermophilic bacteria appear to have a higher requirement for Ca^{++} than do mesophiles (**10**).

Oppenheimer and Drost-Hansen (**50, 114**) suggest that thermal anomalies in the structural properties of water occur within ±2° of 15, 30, 45, and 60 C. Physiological activities such as growth occur optimally near the mid-point between any two of the consecutive temperatures where structural changes occur. Growth of some bacteria occurs in cycles with minimal physiological activity occurring at temperatures which coincide with the changes in the structure of water (**43, 44**). The water content of a cell may have other effects on the thermostability of protoplasm (**14, 16, 23, 73, 76, 90**). The thermostability of protein molecules varies inversely with the degree of molecular hydration; this may be due to conformational changes which occur in protein structure in the presence of water (**16, 96, 140**). Water provides the medium for the movement of metabolites through cellular membranes, and may affect the rate of proton interchange along the surface of membranes by interacting with membrane lipoproteins (**32**). Protection against thermal denaturation has been attributed to the presence of various substances such as sugars, salts, amino acids, proteins, and certain elusive "protective factors" (**23**).

The nutritional requirements of some organisms change during growth at sub- or supraoptimal temperatures (**9**). Nutrient supplementation is required to overcome a low thermal tolerance in some organisms, whereas in others, growth at elevated temperatures is accompanied by an increased nutritional sufficiency (**29, 91**). Thermophilic bacteria appear to require less supplementation at high temperatures than do mesophiles (**10**).

TEMPERATURE EFFECTS IN FUNGI

Limited information on the physiological characteristics of thermophilic fungi is available. Only recently has enough research data accumulated on these microorganisms to provide some insight into their relationship to other thermophilic microorganisms. It is necessary to again supplement our limited knowledge of the thermophilic fungi by drawing upon other organisms, in this case, the mesophilic and psychrophilic fungi.

Definitive studies on the composition of fungal lipids in relation to growth temperature only recently have included thermophilic fungi. It is difficult to compare the results of these investigations owing to the variety of species studied and the variation in cultural conditions are methods of analysis used. Several inferences, however, can be derived from the available data.

Fungi, like most poikilothermic organisms, produce increased amounts of unsaturated fatty acids at lower growth temperatures. Linoleic acid

is the major fatty acid occurring at lower temperatures while oleic acid predominates at higher temperatures. Linolenic acid, although not found in large quantities at any temperature, rarely occurs at high temperatures (**85, 108, 109**). Sumner et al. (**134, 135**) compared the lipid composition of psychrophilic, mesophilic, thermotolerant, and thermophilic species of *Mucor* and *Rhizopus*. Although all species contained a greater quantity of unsaturated lipid at lower growth temperatures, the lipids of the psychrophiles and mesophiles were similarly unsaturated. The thermophiles produced lipid that was significantly more saturated at both high and low growth temperatures. The lipid composition of the thermotolerant species resembled that of psychrophiles and mesophiles at lower cultural temperatures and that of thermophiles at higher cultural temperatures. In a comparison of the lipid composition of sporangiospores and mycelium, spores contained less lipid than the mycelium but the spore lipid was always more highly saturated (**134**).

Sumner and his coworkers (**134**) also found that the degree of saturation of the lipid was influenced by the oxygen concentration of the medium, suggesting that molecular oxygen was an essential cofactor in the desaturation of fatty acid molecules. They related their findings to the specific activities of both fatty acid desaturating and saturating enzymes (desaturases and saturases). Saturases require carbon dioxide for the synthesis of saturated fatty acids. These enzymes are more active at higher temperatures where an oxygen deficit is created by the relative increase in respiratory carbon dioxide in the presence of a reduced oxygen tension. At lower temperatures, an increased oxygen tension favors the activity of desaturases which specifically require molecular oxygen for the synthesis of unsaturated fatty acids. In photosynthetic tissues, a similar relationship has been reported between the activity of desaturases and the amount of dissolved oxygen (**77**).

Meyer and Bloch (**107**) proposed that two desaturating enzyme systems were operative in *Torulopsis* (*Candida*) *utilis* (Henneberg) Lodder. Using cell-free extracts, these investigators showed that at least two factors were involved in the conversion of steryl-Co A to oleate and oleyl-Co A to linoleate. The first reaction occurred in the presence of the particulate fraction of the extract whereas the subsequent conversion to linoleate required the addition of the supernatant portion of the extract. Since the supernatant factor was more active at 19 C than at 30 C, it is either less thermostable than the particulate fraction or is only activated at low temperatures. Both conversions require the presence of molecular oxygen and reduced nicotinamide adenide di-

nucleotide phosphate (**85, 107**). Fulco (**70, 71**) found that bacilli also contain two distinct fatty acid desaturating systems only one of which is affected by growth temperature. Brown and Rose (**22**) concluded that two distinct processes are involved in the synthesis of unsaturated fatty acids in *Candida utilis* (Henneberg) Lodder et Kreger-van Rij; at low temperatures, they observed an increase in the activity of desaturating enzymes and a decrease in the activity of chain-lengthening enzymes. They did not consider these effects to be interrelated.

In a recent study, Chang and Matson (**31**) showed that *Saccharomyces cerevisiae* Hansen also produced more highly saturated fatty acids at 40 C than at 26 C; unsaturated fatty acids were virtually absent from the high temperature cultures. Arrhenius plots of temperature against the electrical conductance of cell suspensions indicated transition points at 50 C and 60 C in the 26 C and 40 C cultures, respectively. These changes in cell conductance were interpreted as indications of changes in the integrity of the cellular membranes and, therefore, the membranes' thermostability.

Mumma et al. (**108, 109**) compared the lipid compositions of nine species of thermophilic fungi with an equal number of mesophilic species of the same genera. In general, the thermophilic species contained more lipid than their mesophilic counterparts. In the thermophiles, the polar lipids were significantly more saturated than were the neutral lipids of the thermophiles or the polar or neutral lipids of the mesophiles. In mesophilic species, the predominant fatty acids were palmitic, linoleic, and linolenic, as compared with oleic and other highly saturated fatty acids in the thermophiles. Mesophiles preferentially incorporated palmitic and linoleic acids into their polar lipids while thermophiles incorporated saturated fatty acids. Phosphatidic acid was a major component of the phospholipid of *Humicola grisea* var. *thermoidea* and occurred in a greater concentration than had been previously reported in fungi (**110**). Sterols and their derivatives also comprised an unusually large fraction of the lipid of this thermophile, suggesting a possible role in thermostability.

In a similar study, I determined the lipid content of *H. lanuginosa* cultured at 37 C and 52 C (unpublished data). This thermophile produced almost nine times more lipid at the lower temperature. On a dry-weight basis, the mycelial lipid content decreased from 75.1% at 37 C to 8.5% at 52 C (Table II). Mumma et al. (**108**) found that the same organism produced 17% lipid in cultures at 45 C. The unusually high lipid content at 37 C might represent the production of a larger amount of storage lipid at the suboptimal temperature. At both 37 C

TABLE II

NEUTRAL LIPIDS OF HUMICOLA LANUGINOSA

Neutral lipid component	37 C		52 C	
	mg/g dry wt	% total wt	mg/g dry wt	% total wt
Free fatty acids	259.4	41.0	4.2	5.8
Diglycerides	34.7	5.5	22.6	31.3
Triglycerides	288.7	45.6	32.5	45.0
Sterols	19.1	3.0	5.6	7.7
Sterol esters	31.3	4.9	7.4	10.2
Total mycelial lipid	750.5	75.1	85.2	8.5
Total neutral lipid	633.2	84.4	72.3	84.9
Total phospholipid	117.3	15.6	12.9	15.1

[a] Cultures grown in liquid Emerson's YpSs medium for 72 hr. Extraction, separation, and identification of lipids using methods described by Jack (**83, 84**).

and 52 C, the ratio of neutral lipid to phospholipid remained essentially the same, but the proportion of specific types of neutral lipids did change with temperature. At 52 C, there was a significant decrease in the free fatty acids while the amount of diglycerides, sterols, and sterol esters increased.

The thermostability of enzymes and other proteins has not been studied exensively in thermophilic fungi. In my laboratory, electrophoretic analyses of cellular extracts of *Penicillium dupontii* and *H. lanuginosa* indicated that the production of thermostable proteins in thermophilic fungi may be an exception rather than the rule (**41** and unpublished data). Cultures of *P. dupontii* grown at 35, 42, and 50 C were extracted with sucrose-Tris buffer and the extracts were resolved using acrylamide gel disc electrophoresis as described by Ornstein and Davis (**45, 115**). Duplicate portions of each extract were exposed to 60 C for periods of 30, 60, and 120 min to determine the thermostability of individual protein components. The resulting electrophoretograms are shown in FIG. 1. All extracts exhibited a significant loss of protein after 30 min at 60 C. Upon further exposure to 60 C, additional bands were lost; 35 C and 42 C extracts, however, lost more of their original protein bands after 120 min than did 50 C extracts. Although the proteins of *P. dupontii* do not appear to be unusually thermostable, these results suggest that the fungus produces a greater number of thermostable proteins at higher incubation temperatures.

An electrophoretic analysis of *H. lanuginosa* gave similar results. Cultures grown at 35, 45, and 55 C were extracted and subjected to

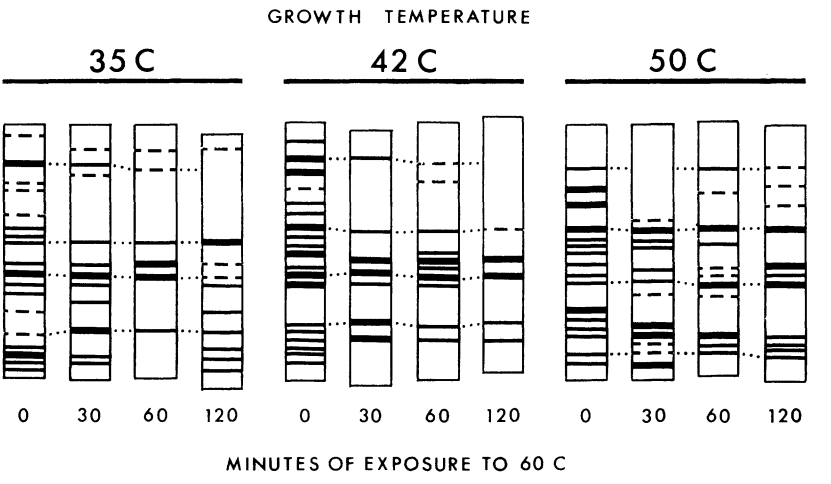

FIG. 1. Disc electrophoretograms of cellular proteins produced by *Penicillium dupontii* NRRL 2155. Cell-free extracts were prepared from submerged cultures grown in liquid Emerson's YpSs medium for 72 hr at 35, 42, and 50 C. Duplicate portions of each extract were incubated at 60 C for 30, 60, and 120 min before electrophoretic analysis to determine the thermostability of individual proteins. Relative concentrations of individual proteins are reflected by the naphthol blue black dye-binding capacity of each band. Dotted lines between adjacent electrophoretograms indicate bands of apparent similarity.

electrophoresis as described above. Duplicate portions of each extract were exposed to 50 C, the optimum growth temperature for this thermophile, for 60 min before analysis. Very little intrinsic thermostability was observed (FIG. 2). Two proteins (bands 2 and 9) were produced in greater concentrations at lower temperatures but were intrinsically thermostable. Four proteins (bands 3–5, 7) were more stable in extracts from 55 C cultures than the same proteins in extracts from low temperature cultures. Only one protein (band 11) showed a direct relationship between the amount of protein produced and thermostability as the growth temperature was increased.

In a comparison of the cellulolytic enzymes produced by mesophilic and thermotolerant strains of *Aspergillus fumigatus,* Loginova and Tashpulatov (101) found the C_1, C_x, and cellobiase (β-glucosidase) of the thermotolerant strain exhibited optimum enzymatic activity at 60 C, 15° above the optimum growth temperature of 45 C; the same enzymes in the mesophilic strain exhibited maxima at 55 C, 25° above the optimum growth temperature of 30 C. The relative thermostability of the mesophilic enzymes appears to be more significant than that of the thermo-

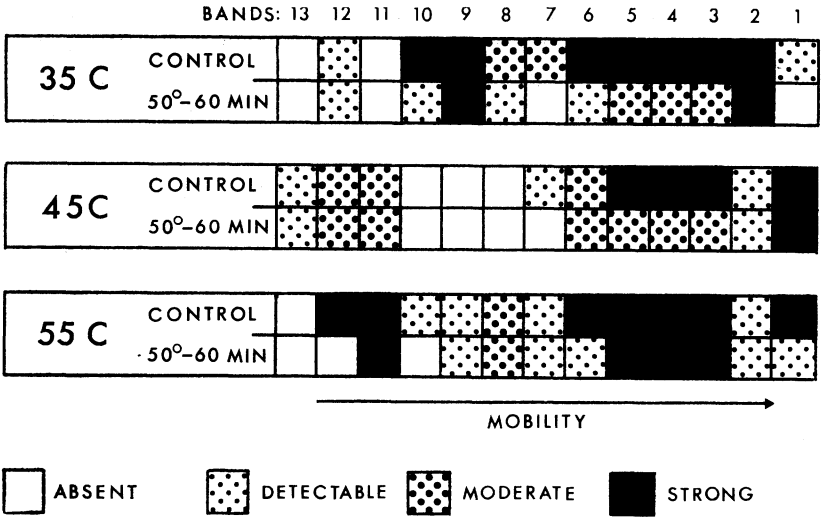

FIG. 2. Diagrammatic electrophoretograms of cellular proteins produced by *Humicola lanuginosa* strain 109. Cell-free extracts were prepared from submerged cultures grown in liquid Emerson's YpSs medium for 72 hr at 35 C, 45 C, and 55 C. Duplicate portions of each extract were incubated at 50 C for 60 min before electrophoretic analysis to determine thermostability of individual proteins. Relative intensity of stained protein bands were recorded as indicated.

tolerant enzymes, if one considers the respective growth optima of the organisms. Henry and Tansey found *Chaetomium thermophile* cellulases to exhibit optimal activity at ca. 70 C (M. R. Tansey, personal communication).

Craveri and Colla (**38**) studied the ribonucleases of eight mesophilic and eight thermophilic fungi. The average temperature for optimum enzymatic activity was 55 C in the mesophiles and 60.5 C in the thermophiles. Three of the thermophilic cultures produced ribonucleases exhibiting dual optima: two had optima at 55 C and 60 C and one had optima at 55 C and 70 C. *Mucor pusillus* acid protease exhibits an optimum temperature for hydrolysis at 55 C; 90% of its activity is lost after 15 min at 65 C (**129**). Lipase from *M. pusillus* exhibits a slightly lower optimum temperature at 50 C; approximately 50% of its activity is lost after 120 min at 60 C (**130**). The enzymes of *Talaromyces dupontii* Apinis (= *T. thermophilus*, cf. TABLE I) also differ in their thermostability. An aminopeptidase produced by this fungus is activated at a temperature of 55 C while a higher rate of activation at 65 C is tempered by an increased rate of thermal denaturation (**35**).

A carboxypeptidase is active at 60 to 70 C (**148**). A glucose-6-phosphate dehydrogenase, on the other hand, loses 55% of its activity after 5 min at 55 C and all of its activity after 5 min at 70 C (**17**). The acid phosphatase of *H. lanuginosa* is not significantly more thermostable than that of mesophilic organisms (**41**).

Nutritional supplementation affects the thermal resistance of fungi also. Fries (**69**) found that a strain of *Coprinus fimetarius* Fr. having a temperature optimum for growth between 35 C and 40 C, could continue to grow at 44 C if supplemented with methionine or, to a lesser extent, with homocysteine. The growth of a thermotolerant strain of *Saccharomyces cerevisiae* at 40 C was accelerated after supplementation with Tween 80, ergosterol, or oleic acid (**99**).

The leakage of cellular constituents from cells exposed to supraoptimal or lethal temperatures has been observed repeatedly in microorganisms (**23, 75, 138**). An increase in the concentration of amino acids, polypeptides, nucleic acids, and other essential metabolites in the growth medium occurs after cells are exposed to elevated temperatures. In *Merulius lacrymans* Jacq. ex Fr., there is a direct relationship between the amount of leaked metabolites and the increase in temperature above 22 C (**93**). In the psychrophilic yeast, *Candida nivalis* di Menna, a rapid loss in viability accompanied a significant loss of essential cellular constituents at temperatures above 20 C (**111**). Damaged cells recovered if transferred to complex media at lower temperatures, but not in simple media. Supplementation of the growth medium with cysteine, glutathione, and thioglycollate promoted complete recovery. A low-temperature basidiomycete exihibited an immediate leakage of UV-asorbing materials after exposure to a supraoptimal temperature of 30 C (**142**). Although the loss of cellular materials slowed considerably after 1 hr, viability of the mycelium was maintained and, after 7 days at the elevated temperature, the cultures were still capable of resuming growth after transfer to 15 C.

Evison and Rose (**57**) considered the inactivation of respiratory metabolism to be a major factor in the thermal death of *Candida utilis*. Langvad and Goksøyr (**93**), on the other hand, detected significant respiratory activity in *M. lacrymans* for at least 2 hr after cellular death occurred at 37.5 C; the thermal death time is 4 hr at this temperature. The respiratory activity of thermophilic fungi appears to be higher but not significantly different than that of mesophiles (**112**). This is also true for a high temperature strain of *Saccharomyces fragilis* Jörgensen (**100**).

DISCUSSION

Physiological adaptation to a thermal environment has not been considered as a major source of thermophilic organisms. Attempts to increase the thermal tolerance of mesophilic microorganisms have been relatively unsuccessful. This lack of success, however, can be attributed to the nature of the adaptive process itself. If a sufficient number of mutations have occurred to endow an organism with the genetic constitution of a thermophile, thermophilic growth will be possible. Where the mutational process has been limited and few mutations have occurred, the organism will remain a mesophile or, at most, become thermotolerant.

Natural selection must play a role in establishing the capability for growth at high temperatures. In this selection process, the organism as a whole must be capable of functioning at extreme temperatures. Although the thermostability of individual macromolecules is important in extending the range of thermal tolerance, thermophilism per se can only exist in organisms capable of functioning as integrated entities under conditions of thermal stress. It then follows that as the complexity of an organism increases, the probability of maintaining its metabolic integrity at higher temperatures decreases.

One of the most critical yet most difficult distinctions to be made is that between thermophilism and simple thermal stability. Thermophilism indicates a stability of the entire functioning organism. Any individual cellular component or process, however, may exhibit simple thermal stability independent of other components or processes. It is in this distinction that the confusion in understanding thermophilism originates. Thermophilism is based on an organism's genetic constitution whereby the existence of fixed phenotypes permits normal growth at high temperatures. These genes are responsible for establishing and maintaining homeostasis in the thermophile at temperatures which surpass the limits of the homeostatic mechanisms of mesophiles and psychrophiles. In contrast, thermal stability of an individual component or process may result from genetic adaptibility wherein a limited phenotypic flexibility permits changes to meet temporary environmental fluctuations. This adaptive flexibility may manifest itself in the thermostability of one or a few cellular components which may permit the continuance of life or, more probably, limit the destruction of life at lethal temperatures. A thermostable component need not be found only in a thermophile.

Thermal death appears to be a unified process. The deterioration of life at supraoptimal temperatures is accompanied by certain physiologi-

cal and structural changes which are similar regardless of whether the organism is a psychrophile, mesophile, or thermophile. Thermal death in microorganisms appears to be logarithmic and follows first order kinetics. This implies that a single event, a prime lethal event, is responsible for initiating the chain of events leading to death. The goal of most studies of the physiological basis for thermophilism has been to identify this single temperature-sensitive event.

Wood (**146**) suggested three possible mechanisms to explain cellular inactivation in terms of a single lethal event. First, a change in a single molecule may occur which initiates an autocatalytic chain reaction leading to death. Second, a spontaneous internal change in cellular organization may occur which exposes the cell to the action of an injurious agent; or last, a single molecular change within a cell may bring about the inactivation of a single unit which is essential for cellular function.

Within the context of the current hypotheses for thermophilism, the death of mesophilic organisms due to the thermal inactivation of the genetic apparatus would be explained by the first or third mechanism, whereas thermophiles would exhibit a higher thermal resistance to such changes. Lipid solubilization and ultrastructural stability fall within the second mechanism and macromolecular stability within the third mechanism. Wood favors the third mechanism because he considers it the most plausible and simplest to explain in chemical and physical terms.

Some of the deleterious effects of high temperature on living organisms are climatic lesions, i.e., metabolic defects resulting from exposure to a temperature which is detrimental but not necessarily lethal to the normal growth of an organism. If the affected process is essential, a climatic lesion may be lethal. In most cases, however, the lesion is not lethal or its potential lethality is circumvented either physiologically by use of alternate metabolic pathways, or nutritionally by supplying the organism with the end products of the interrupted process (**86**). Climatic lesions may also result in the production of toxic products in cells exposed to high temperatures. In addition to being active at the site of production, in multicellular organisms, these toxins may be translocated to other sensitive sites (**147**).

The rapid resynthesis hypothesis for thermophilism is somewhat ambiguous. While it proposes that thermostable cellular components and metabolites are not necessary for growth of thermophiles, the fact that a dynamic metabolic system capable of replacing thermolabile metabolites at high temperatures must itself be composed of thermostable components is ignored. Observations that the majority of thermophilic

microorganisms do not exhibit an unusually active metabolic apparatus further discount the importance of rapid resynthesis as a major factor in determining cellular thermostability.

The hypothesis of macromolecular thermostability has engendered the most support among students of thermophilism. The isolation of numerous thermostable enzymes from thermophilic microorganisms has been offered as evidence in support of this hypothesis. Under the prime lethal event concept, the destruction of one key thermolabile enzyme is responsible for the death of a given organism. Although thermostable enzymes do occur in thermophiles, it is difficult to envision the existence of one specific enzyme, common to all thermophiles, which determines the maximum growth temperature of all organisms. This key enzyme concept is subject to further question when one notes that the same enzymes are not thermostable in all thermophiles. Many important enzymes isolated from thermophiles are, in fact, thermolabile. An alternative to accepting the existence of a common key enzyme is to suggest that different organisms may contain different key enzymes. The enzyme most susceptible to thermal denaturation would be the key enzyme of a given organism. Accepting this, it then becomes difficult to explain why groups of related organisms exhibit a very definite, narrow range of maximum temperatures, e.g., the maxima for most thermophilic fungi fall between 50 C and 60 C. If different enzymes serve as key enzymes in different organisms, one would expect that a wide range of temperature maxima would occur and there would not be a significant break between the maxima of mesophiles and those of thermophiles. Bausum and Matney (**11**) observed that a temperature boundary between bacterial mesophilism and thermophilism occurred at 44–52 C.

The existence of thermostable enzymes in microorganisms can be explained genetically. The mutability of the genes responsible for the synthesis of individual enzymes has been established and, under conditions of elevated temperatures, the frequency of mutation can be expected to increase (**92, 116**). The theory of natural selection suggests that an organism capable of producing one or more thermostable enzymes would be better equipped to function at elevated temperatures depending on the metabolic importance of the particular enzyme. As noted for several bacteria and fungi, in addition to producing thermostable macromolecules continuously at all growth temperatures, organisms may be capable of producing them in response to exposure at high temperatures, possibly as a result of a temperature related form of induction. Mecha-

nisms similar to those operating in the synthesis of saturated and unsaturated fatty acids may be involved.

The successful transformation of mesophilic bacteria into thermophilic forms involves an unusually small number of genes (104). This would indicate that the transfer of this limited amount of genetic information would only affect one or a very few enzymes. Although this lends some support to the key enzyme concept, the transferred genes could also carry other factors, such as those controlling the stability of critical cellular structures, which would influence the thermostability of the recipient cell.

In discussing the lipid solubilization hypothesis, Heilbrunn (79) suggested that cellular lipids per se were responsible for maintaining the functional integrity of the cell by protecting proteins from denaturation in the presence of deleterious ions such as Ca^{++}. At that time, it was difficult to analyze for the various classes of lipid compounds produced by cells and to determine the specific functions of each in cellular metabolism. Studies of the relationship between temperature and lipid composition were often misleading and a definitive explanation of this relationship was not developed. With the development of the electron microscope and subsequent elucidation of the ultrastructural components of cells, it has become possible to determine the biochemical composition of specific cellular components. The lipid of the cell is distributed between two distinct locations. The major concentration of lipid occurs as mono-, di-, and triglycerides in the cytoplasm. Although exhibiting compositional changes as growth temperature varies, a major function of the cytoplasmic lipid is the storage of energy and, as such, probably has minimal effect on the thermal stability of the cell. Due to the high concentration of cytoplasmic lipid in the cell, most quantitative and qualitative studies of "cellular" lipid are, in fact, a reflection of the cytoplasmic lipid composition.

A smaller but more significant concentration of lipid occurs in the various cellular membranes. The major components of these membrane lipids are phospholipids which, in association with proteins, determine some of the critical properties of the membrane matrix (32, 48). Compositional changes which can occur in the fatty acid moieties of the membrane phospholipids can preserve metabolic homeostasis by maintaining the functionality of the membrane during growth at various temperatures. Lipid solubilization, in this sense, does not fall within the parameters of the hypothesis proposed by Heilbrunn (79) and Bělehrádek (12, 13), but rather in the area of ultrastructural thermostability.

The hypothesis that ultrastructural thermostability determines the thermal resistance of an organism appears to be the only hypothesis compatible with the occurrence of thermophilism in a variety of different organisms. The concept that structural complexity may play a role in a cell's thermal characteristics is not new. In 1897, Davis (46) postulated that the ability of an organism to grow at high temperatures might reside in the degree of protoplasmic organization inherent to the cell. In comparing the existence of thermophilic species in various groups of organisms, Brock (18) found that the number of thermophiles decreased significantly among more highly evolved organisms and thermophilism is essentially unknown among the higher plants and animals. In this regard, Sonnerat's (131) observation of fish growing at elevated temperatures may be open to question.

Only three major structural components are common to the cells of all organisms regardless of their evolutionary position: the genes (or an equivalent genetic mechanism), the ribosomes, and the intracellular membranes. The thermal sensitivity of the genes and ribosomes has been discussed. The membranes offer a third, and very important, potential site of thermal inactivation. The critical interfaces within a cell are composed of and protected by membranes. Individual organelles in which vital cellular processes occur are, for the most part, bounded by membranes. The passage of essential metabolites into and toxic products out of the cell is controlled by the membranes. The function of the membranes is among the first of the vital activities to be lost when death occurs at supraoptimal temperatures.

Studies of synthetic membranes and membranes of living cells have shown that many of their structural and functional properties are determined primarily by the nature of the phospholipid component of the membrane (32, 33, 34, 48). Phospholipids exhibit multiphasic properties and, for a membrane to function properly, its phospholipid must be at or near the point of transition between a crystalline and liquid phase (27, 33, 34). Only at this transitional point does the membrane exhibit the necessary degree of flexibility to permit reactions to occur at its interface and to control the selective passage of molecules into and out of the cell. Phase transition in a phospholipid is a function of the melting point of the lipid moiety as determined by the molecular characteristics of its component fatty acids (34, 47).

Proteins, the second major component of the membrane lipoprotein, have already been shown to exihibit a degree of thermostability in some thermophiles and might, within the membrane, exert an additional effect on cellular thermostability. Other factors such as ionic species, osmolal-

ity, acidity, and the degree of cytoplasmic hydration also affect or are controlled by the characteristics of the cell membranes and the thermal limits of the organism (**33, 34**).

Only two of the hypotheses proposed for explaining thermophilism still appear to be of major importance. The importance of macromolecular thermostability is questionable since the existence of a single, thermostable, essential macromolecule, common to all thermophiles, has not been demonstrated. Thermophilic fungi, although not exhibiting as high a degree of thermal stability as do some bacteria and blue green algae, do not appear to contain proteins of unusual thermostability yet have the capacity to grow at high temperatures.

Ultrastructural thermostability, on the other hand, appears to be a more promising hypothesis especially in view of the apparent inverse relationship between the occurrence of thermophilism and the level of ultrastructural complexity. The observations which led to both the lipid solubilization and rapid resynthesis hypotheses can be also explained in terms of ultrastructural thermostability. Based on our increasing knowledge of membranes and their possible function in determining the effect of temperature on living organisms, it appears that ultrastructural thermostability is the only hypothesis, at present, which can explain the existence of thermophilic fungi.

Within the context of the hypothesis of ultrastructural thermostability, the optimum temperature of an organism can be defined as that temperature which is optimal for achieving an integration of all essential metabolic activities. Although some individual processes may be capable of more efficient operation at higher or lower temperatures, the overall physiology of the cell is controlled by the most rate-limited reaction in the integrated metabolic system.

At minimal temperatures for growth, the lipoidal constituents of one or more of the cellular membranes reach a point of minimal fluidity and flexibility. Passage of essential metabolites into and toxic products out of the cell are inhibited and metabolic reactions on the membrane surface or within the membrane-bounded organelles are limited. If the temperature is raised above this minimum, fluidity and flexibility are restored and the physiological function of the cell can be resumed.

As the maximum temperature for growth is approached, the excessive fluidity and flexibility of the same lipoidal constituents affect the physical integrity of the membranes and subsequently their function. This disruptive process decreases the membrane's ability to serve as permeability barriers or as sites for enzymatic activity. As the maximum temperature for growth is exceeded, disorganization of the mem-

branes reaches irreparable proportions and constituents essential for membrane resynthesis are lost from the immediate vicinity of the membranes. With the increased permeability, toxic molecules from outside the protoplast gain access to sensitive metabolic sites within the cell, promoting an autocatalytic destructive process. Once this point has been reached, a change to a lower temperature cannot restore the functional capacity of the cell, due to the loss of molecules essential for membrane repair and the cell's subsequent inability to initiate such structural repairs.

Microbiologists have exercised a certain degree of professional provincialism in studying the growth of microorganisms at extreme temperatures. Few investigators have studied and compared both psychrophiles and thermophiles. If the effect of temperature on all organisms is the same and thermal inactivation is truly a unified process, it should be possible to formulate one hypothesis which explains thermal stability and thermal death in psychrophiles, mesophiles, and thermophiles.

The variety of fungal species available for investigation include numerous examples of psychrophilic, mesophilic, and thermophilic forms. In some cases, representatives of all three physiological types can be found in the same or related genera. Fungi, occupying an intermediate position between the less organized procaryotic bacteria and the complex, tissue-producing eucaryotic plants and animals, may provide the ideal tools for studying the physiological basis for growth at extreme temperatures.

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