

CHAPTER 56

Thermal Stability of Ribosomes and Nucleic Acids from Thermophilic and Psychrophilic Fungi

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A comparative study was carried out on the physical and chemical properties of deoxyribonucleic acid (DNA), ribosomes (80S), ribosomal subunits (60S and 40S), ribosomal RNA (rRNA), and transfer RNA (tRNA) isolated from two thermophilic (*Mucor pusillus*, *M. miehei*), and three psychrophilic (*M. strictus*, *M. sphaerosporus*, *Zygorynchus psychrophilus*) phycomycetes. The thermophilic melting temperatures (T_m) of DNA and ribosomes were 4-5 degrees and 12-15 degrees higher than were the corresponding psychrophile T_m values. Chemical analysis revealed no major differences in the protein (44-45%) and RNA (55-56%) contents of thermophile and psychrophile ribosomes. The T_m of thermophile rRNA was 3-4 degrees higher, and the T_m of tRNA was up to 9 degrees higher than the T_m values for the corresponding subcellular fractions from psychrophilic fungi. The rRNA and tRNA of thermophiles were higher in guanine plus cytosine than similar components of the psychrophiles. Ribosomes and the activating enzyme fraction (S-100) from the psychrophile *M. sphaerosporus* were adversely affected by heat and were rendered nonfunctional after exposure to 45 C for 10 min. Heat-treated ribosomes had a reduced capacity to bind phenylalanyl-tRNA. Elevated temperatures also impaired the protein-synthesizing system of *M. pusillus*, a thermophilic member of the same genus.

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INTRODUCTION

The molecular determinants responsible for the maximum growth temperatures of thermophilic, mesophilic, and psychrophilic microorganisms have been sought by many investigators. In general, the ability of thermophilic microorganisms to grow at high temperatures (about 55 to 90 C), and the inability of psychrophilic and mesophilic microorganisms to grow above the maximum growth temperature have been ascribed to chemical and physical differences of their macromolecules and subcellular components. For example, it has been reported that the ribosomes of thermophilic bacteria are more heat-stable than the ribosomes of mesophilic and psychrophilic bacteria (Stenes and Yang 1967; Campbell and Pace 1968; Zeikus et al. 1970; Friedman 1971; Irwin et al. 1973). The temperature at which 50% of polyuridylic acid-directed ^{14}C -phenylalanine-incorporating activity is lost was reported to be substantially higher for thermophiles (72 C) than for either mesophiles (57 C) or psychrophiles (53 C), and thermophile activating enzyme fractions were also more thermostable than mesophile or psychrophile S-100 fractions (Irwin et al. 1973).

Thermolabile enzymes that play key roles in metabolism also have been characterized in psychrophiles, including malate dehydrogenase (Langridge and Morita 1966), cytochrome *c* reductase (Purohit and Stokes 1967), prolyl-tRNA and glutamyl-tRNA synthetases (Malcolm 1969), and triose phosphate isomerase (Shing et al. 1972). In addition, it was found that exposure of psychrophiles to temperatures greater than the maximum growth temperature damaged the cell membranes resulting in the loss of cytoplasmic components (Haight and Morita 1966; Madeley et al. 1967).

Most studies seeking to identify the molecular determinants of thermophily and psychrophily have been carried out with procaryotic systems, and several reviews have been published on these topics (Farrell and Rose 1967; Brock 1969; Singleton and Amelunxen 1973; Innis 1975; Morita 1975). On the other hand, information pertaining to the thermal stability of macromolecules and subcellular components of thermophilic and psychrophilic eucaryotes is relatively limited (Crisan 1973). For example, membrane damage induced by heat was reported to occur in the psychrophilic yeast *Candida nivalis* (Nash and Sinclair 1968). The presence of a heat-sensitive pyruvate decarboxylase in another psychrophilic yeast, *C. gelida*, was described by Grant et al. (1968), and later, Nash et al. (1969) characterized in the same yeast the thermolability of specific aminoacyl-tRNA synthetases and activating enzymes. Ribosomes of psychrophilic yeasts had a lower T_m value (49 C) than the ribosomes of mesophilic yeasts (52 C), and the ribosomes of the psychrophile *C. gelida* lost most of their phenylalanine-incorporating capacity after exposure to 40 C for 5 min (Nash and Grant 1969). In another study carried out with fungi, Miller and Shepherd (1973) found that ribosomes and ribosomal subunits from the thermophilic mold *Penicillium duponti* were more heat-stable than the ribosomal particles from the mesophile *P. notatum*.

This report presents the results of a comparative study on the relative heat stability of ribosomes, ribosomal subunits, and nucleic acids that were isolated from thermophilic (*Mucor pusillus*, *M. Miehei*), and psychrophilic (*M. strictus*, *M. sphaerosporus*, *Zygorynchus psychrophilus*) phycomyces. In addition, data are presented on the thermola-

bility of the protein-synthesizing system in thermophilic (*M. pusillus*) and psychrophilic (*M. sphaerosporus*) fungi that are members of the same genus.

MATERIALS AND METHODS

Organisms and culture conditions. The thermophilic phycomycetes *Mucor pusillus* (from the Purdue University Culture Collection) and *M. miehei* (from D. J. Cooney, University of Nevada), and the psychrophilic phycomycetes *M. strictus*, *M. sphaerosporus*, and *Zygorynchus psychrophilus* (from M. A. A. Schipper, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands) were maintained on potato dextrose agar (Difco) slants at 4 C. The medium of Gordon et al. (1971), supplemented with 1% (w/v) neopeptone and 0.5% (w/v) yeast extract (Difco), was used for growing fungi in liquid culture. Thermophilic fungi (*M. pusillus* *M. miehei*) were cultivated at 40 C for 24 h for the purpose of preparing ribosomal particles and nucleic acids. Incubation conditions were different for psychrophilic fungi: *M. strictus* was cultivated at 23 C for 48 h, *M. sphaerosporus* at 20 C for 48 h, and *Z. psychrophilus* at 15 C for 72 h. In all cases, 4 liters of medium (500 ml per 2-liter Erlenmeyer flask) were inoculated to 10^5 spores/ml, and flasks were agitated on a Psychrotherm shaker (New Brunswick Scientific Company, Edison, NJ) set at 200 rpm.

Isolation of DNA. Mycelia were rapidly filtered by suction on Whatman filter papers (no. 42), and the mycelial mats were immediately washed two times with 200 ml of cold 0.15 M NaCl and 0.1 M ethylenediaminetetraacetate (EDTA), pH 8.0. The mycelia were homogenized by grinding to a fine white powder with crushed dry ice in a mortar. The powder was then allowed to warm to 0 C in an ice bath. The DNA was prepared by the method of Marmur (1961). After the last purification step, the DNA was dissolved in 0.015 M sodium citrate (pH 7.0) containing 0.15 M NaCl. After perchloric acid hydrolysis and paper chromatography according to Wyatt (1951), uracil was absent indicating that the DNA preparations were free of RNA. Likewise, protein was not detectable in the DNA preparations.

Isolation and purification of ribosomes (80S) and ribosomal subunits (60S and 40S). The homogenized mycelial powder was extracted with TMK buffer (10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 10 mM $MgCl_2$, 10 mM KCl, 5 mM β -mercaptoethanol, pH 7.2), using 2-ml amounts of buffer for each gram of powder. The resulting slurry was centrifuged at 10,000 g at 4 C for 10 min to remove cell debris. The low-speed supernatant fraction was centrifuged twice, each time at 30,000 g for 20 min. The final 30,000 g supernatant fraction was centrifuged at 105,000 g for 90 min. The upper one-half of the 105,000 g supernatant fractions (S-100) was dialyzed against 100 vol of TMK buffer at 4 C for 5 h and used as the source of activating enzymes for amino-acid incorporation. The ribosomal pellet was resuspended in 10 mM Tris-hydrochloride buffer at pH 7.0, containing 10 mM $MgCl_2$, 500 mM KCl, and 5 mM β -mercaptoethanol; the suspension was centrifuged at 20,000 g for 15 min to remove denatured material and then centrifuged into a 1.0-ml cushion of 2 M sucrose (in TMK) at 150,000 g at 4 C for

60 min. The final ribosome pellet was clear and colorless.

Ribosomal 60S and 40S subunits were prepared by centrifuging through 10 to 30% linear sucrose gradients as described by Orłowski and Sypherd (1978). Particles were sedimented for 18 h at 20,000 rpm in a Spinco SW40 rotor at 4 C. The gradients were scanned at 260 nm. Fractions corresponding to 60S and 40S ribosomal subunits were pooled separately and recovered from the sucrose by sedimentation at 45,000 rpm in a Beckman 50Ti rotor. The final preparations were resuspended in TMK buffer and stored at -70 C.

Isolation and purification of rRNA. Ribosomal RNA (rRNA) was isolated from the frozen 80S ribosomal particles by precipitation with 2-chloroethanol, according to the method of Fogel and Sypherd (1978). The precipitated rRNA was removed by centrifugation at 10,000 *g* for 10 min. The pellet was resuspended in 0.15 *M* NaCl and dialyzed against 100 vol of 0.15 *M* NaCl to remove traces of 2-chloroethanol. The final rRNA contained 0.6% contaminating protein.

Transfer RNA (tRNA) was prepared from mycelia according to the technique of Schmoyer and Lovett (1969). Attached amino acids were hydrolyzed from the aminoacyl-tRNA by incubation in 0.5 *M* Tris-hydrochloride buffer at pH 8.8. Purified tRNA was precipitated with 2 vol of cold 95% ethanol, dissolved in TMK buffer and stored at -70 C.

Determination of melting temperature (T_m). The hyperchromic increase of absorbance of DNA, ribosomes, ribosomal subunits, rRNA, and tRNA during heating was followed in a Cary 15 recording spectrophotometer equipped with a Lauda K-2/R temperature-controlled circulator geared to give temperature increments of 0.5 C per min. All samples had initial absorbances at 260 nm of 0.30-0.50. The temperature at which 50% increase in relative absorbance was observed at 260 nm was defined as the T_m value (Marmur and Doty 1962). The melting of DNA samples was carried out in 0.015 *M* sodium citrate (pH 7.0) containing 0.15 *M* NaCl. The guanosine plus cytosine (G + C) content of DNA preparations was estimated from the following relationship: $T_m = 69.3 + 0.41 (\%G + C)$, according to Marmur and Doty (1962).

The T_m values of 80S ribosomes, 60S and 40S ribosomal subunits, and tRNA were determined in TMK buffer, pH 7.2. The thermal denaturation profile of rRNA was determined in 0.15 *M* NaCl.

Functional stability of ribosomes and activating enzymes. Polyuridylic acid (polyU)-dependent phenylalanine incorporation was used to test the functional stability of ribosomes isolated from psychrophilic and thermophilic fungi, and the same system was used to determine the heat-induced impairment of the activating enzyme fractions (S-100). Ribosomes and the S-100 fraction from the psychrophile *M. sphaerosporus* were preincubated for 10 min from 35 to 45 C, before assay, whereas the corresponding components from the thermophile *M. pusillus* were preincubated from 65 to 75 C. The complete polyU-directed ^{14}C -phenylalanine incorporation system contained the following in a final vol of 1.0 ml: 60 μmol Tris-hydrochloride at pH 7.8, 10 μmol MgCl_2 , 25 μmol KCl, 0.5 μmol β -mercaptoethanol, 0.05 μmol guanosine triphosphate (GTP), 1 μmol adenosine triphosphate (ATP), 2.5 μmol phosphoenolpyruvate, 100 μg pyruvate kinase (Calbiochem, A grade), 200 μg polyU (Schwarz Bioresearch), 200 μg tRNA (from *M. sphaerosporus* or *M.*

pusillus), 1 μ curie ^{14}C -phenylalanine (Schwarz BioResearch), washed ribosomes (250 μg protein), and 200 μg of dialyzed high-speed supernatant (S-100) protein (activating enzyme). The reaction mixture was incubated 60 min at 20 C in the case of the psychrophile, and 60 min at 45 C in the case of the thermophile. The reaction was stopped by adding 2 ml of 10% trichloroacetic acid (TCA) containing 3 mg/ml ^{14}C -phenylalanine. Samples were prepared for counting by a filter disc method as described by Mans and Novelli (1960).

Ribosomal binding of ^{14}C -phenylalanyl-tRNA was also determined by the method of Nirenberg and Leder (1964). Psychrophile ribosomes were preincubated for 10 min at temperatures from 35 to 45 C, whereas thermophile ribosomes were preincubated at 65, 70, and 75 C. Each incubation mixture contained the following in a final volume of 0.2 ml: 250 μg ribosomal protein, 200 μg poly U, 50 μg ^{14}C -phenylalanyl-tRNA (prepared from *M. sphaerosporus* or *M. pusillus*), 10 μmol Tris-hydrochloride (pH 7.2), 2.5 μmol MgCl_2 , and 5 μmol KCl. After incubation for 30 min at 20 C for the psychrophile and 45 C for the thermophile, reaction mixtures were collected by filtration through Millipore membrane filters (type HA, 0.45 μm). The material on the filter was washed with 5 ml of TMK buffer at 4 C, and counted.

Radioactive samples were suspended in 5 ml of PPO-POPOP [2,5-diphenyloxazole/1,4-bis-2-(5-phenyloxazolyl)-benzene], and counted in a Nuclear-Chicago Mark I scintillation counter.

General assay procedures. The DNA was measured by the diphenylamine assay described by Schneider (1957), and RNA was determined by the method of Ceriotti (1955). Protein was assayed by the Lowry procedure (Lowry et al. 1951). Base compositions of rRNA and tRNA were determined following hydrolysis in 0.3 N KOH and the separation of 2'-3'-ribonucleotides, according to the method of Katz and Comb (1963).

RESULTS AND DISCUSSION

Maximum Growth Temperatures of Fungi

Potato dextrose agar slants were inoculated with spore suspensions of the phycomycetes and development of mycelia at different incubation temperatures were evaluated after 7 d. The maximum growth temperatures for the thermophiles *M. pusillus* and *M. miehei* were 58 C and 55 C, respectively. The following maximum growth temperatures were determined for the psychrophiles: *M. strictus*, 25 C; *M. sphaerosporus*, 22 C; and *Z. psychrophilus*, 17 C. These temperature maxima were similar to those reported by others for the same species of thermophilic (Crisan 1973) and psychrophilic (M. A. A. Schipper, pers. comm.) phycomycetes.

Thermal Denaturation of DNA

The T_m values of DNA isolated from thermophiles and psychrophiles were determined from thermal denaturation curves. The data in Table 1 show that the DNA of the thermophiles was more heat-stable than the DNA of the psychrophiles. The T_m values also correlated with the maximum growth temperatures of the phycomycetes. Likewise, the DNA of thermophiles had a higher %G + C value than the DNA of psychrophiles. Overall, the range

TABLE 1. T_m values for fungal DNA preparations

Source	T_m (C)	G + C Mole % ^a
<u>Thermophiles</u>		
<i>M. pusillus</i>	90.4	51.4
<i>M. miehei</i>	89.0	48.0
<u>Psychrophiles</u>		
<i>M. strictus</i>	86.5	41.9
<i>M. sphaerosporus</i>	86.2	41.2
<i>Z. psychrophilus</i>	85.3	39.0

$$^a T_m = 69.3 + 0.41(\% G + C).$$

of %G + C values (39-51 mole %) was nearly identical to the value reported for phycomycetes (40-48 mole %) by Storck (1966).

Typical thermal denaturation profiles are given in Fig. 1 which show that the DNA of the thermophile *M. pusillus* was heat-resistant up to about 65 C, whereas the DNA of *M. strictus*, a psychrophile, began showing a hyperchromic effect around 50 C. Other investigators have shown that DNA from thermophilic bacteria was more heat-resistant and had higher T_m values than DNA from mesophilic and psychrophilic species of the same genus (Stenes et al. 1968; Irwin et al. 1973).

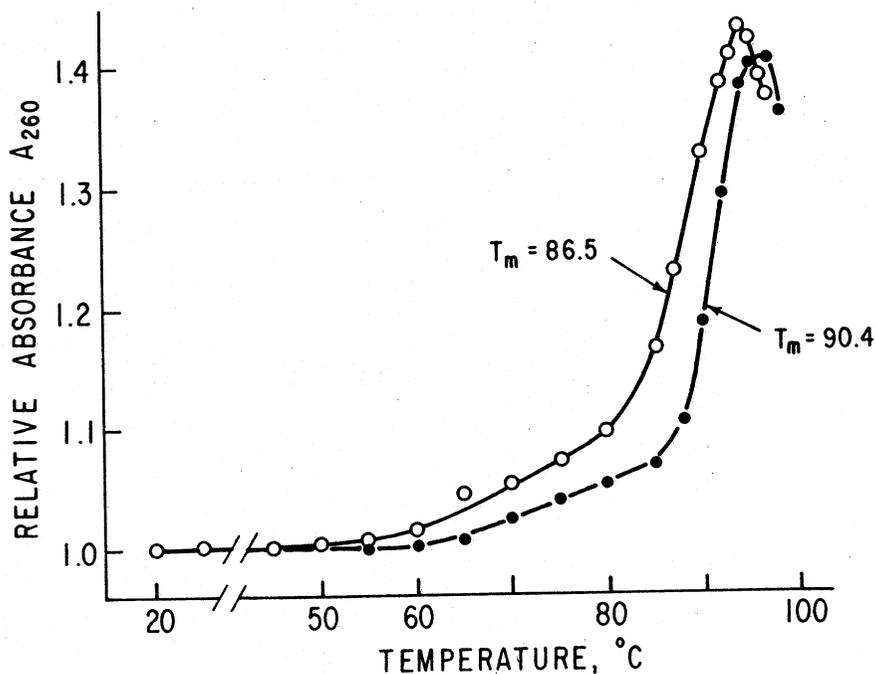


FIG. 1. Thermal denaturation profiles of DNA from *M. pusillus* and *M. strictus*, in 0.015 M sodium citrate - 0.15 M NaCl, pH 7.0; O--O, *M. strictus*; ●--●, *M. pusillus*.

Thermal Stability of Ribosomes

The chemical composition of purified ribosomes is shown in Table 2. The

TABLE 2. Chemical composition of fungal ribosomes

Source	Percent	
	Protein	RNA
<u>Thermophiles</u>		
<i>M. pusillus</i>	45.0	55.0
<i>M. miehei</i>	44.2	55.8
<u>Psychrophiles</u>		
<i>M. strictus</i>	44.0	56.0
<i>M. sphaerosporus</i>	45.4	54.6
<i>Z. psychrophilus</i>	45.0	55.0

data given are averages of duplicate determinations performed on two different ribosome preparations. Values obtained for the RNA and protein contents of individual ribosome preparations were comparable. The average values for the two thermophiles were 44.6% protein and 55.4% RNA. In the case of psychrophilic phycomycetes, the average values for protein and RNA contents were 44.8%, and 55.2%, respectively. In contrast, ribosomes of the obligately psychrophilic yeast *Candida gelida* were reported to contain 35% protein and 60% RNA (Nash and Grant 1969).

The thermal stability of ribosomes (80S) and ribosomal subunits (60S and 40S) of the two thermophiles was nearly identical (Table 3).

TABLE 3. T_m values for ribosomal particles

Source	T_m (C)		
	80S	60S	40S
<u>Thermophiles</u>			
<i>M. pusillus</i>	61.5	58.2	54.6
<i>M. miehei</i>	61.0	58.0	54.2
<u>Psychrophiles</u>			
<i>M. strictus</i>	48.5	46.8	45.0
<i>M. sphaerosporus</i>	47.8	46.2	44.4
<i>Z. psychrophilus</i>	46.2	45.0	42.8

The T_m values obtained for 80S, 60S, and 40S ribosomal particles were 2-3 C higher than the corresponding values, 58.4 C, 55.9 C, and 52.3 C, respectively, determined by others for the ribosomal particles of the thermophilic fungus *Penicillium duponti* (Miller and Shepherd 1973). The ribosomes and ribosomal subunits of psychrophilic phycomycetes were substantially less heat-stable and showed average T_m values of 47.5 C (80S), 46 C (60S), and 44 C (40S). The T_m values for the psychrophiles were about 3 C lower than that reported for 80S ($T_m = 50.5$ C), 60S ($T_m = 49.9$ C), and 40S ($T_m = 47.2$ C) ribosomal particles from the mesophilic fungus *P. notatum* (Miller and Shepherd 1973). In another study, a T_m value of 49 C was found for ribosomes of *C. gelida*, an obligate psychrophilic yeast (Nash and Grant 1969).

Figure 2 illustrates the effect of increasing temperature on the stability of purified ribosomes in TMK buffer. The ribosomes of the thermophile (*M. pusillus*) did not show a rise in absorbance until 55 C was reached and had a T_m of 61.5 C. On the other hand, ribosomes of the psychrophilic *M. strictus* were only stable up to 40 C and they demonstrated a T_m of 48.5 C.

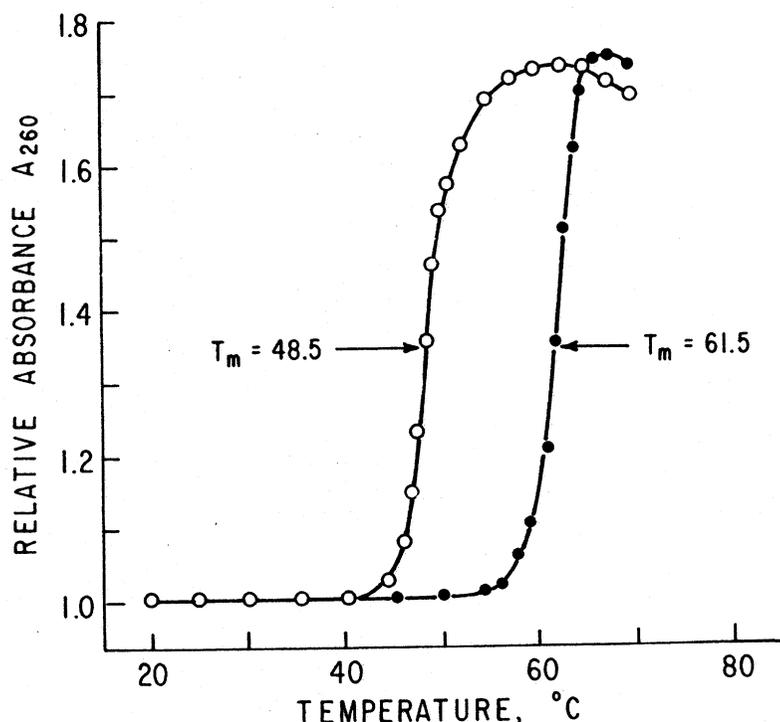


FIG. 2. Thermal denaturation profiles of purified ribosomes in TMK buffer; O--O, *M. strictus*; ●—●, *M. pusillus*.

The thermal stability of ribosomes (T_m values ranged from 46.2 to 61.5 C for 80S particles) correlated with the maximal growth temperature of the test organisms (17 to 58 C). Similar observations were made in studies of other fungi (Miller and Shepherd 1973), yeasts (Nash and Grant 1969), and bacteria, including thermophiles, mesophiles, and psychrophiles. However, ribosomes of thermophilic bacteria (maximal growth temperature of 50-60 C) and psychrophilic bacteria (maximal growth temperature of 18-20 C) differed in T_m values by about 5 C only (Pace and Campbell 1967; Irwin et al. 1973), whereas in this study the difference in T_m values of thermophilic and psychrophilic ribosomes varied from 12-13 C (40S and 60S subunits) to almost 15 C (80S particles). In addition, there was an interesting correlation between the temperature at which denaturation of ribosomes of the thermophile *M. pusillus* initiates (56-58 C) and the maximum growth temperature of the organism. Similar observations were made in other studies on the denaturation of ribosomes of the thermophilic bacterium *Thermus aquaticus* (Zeikus et al. 1970). On the other hand, no correlation existed between the initiation of ribosome denaturation and the maximum growth temperature for the psychrophilic phycomycete *M. strictus*, which were 44-45 C and 25 C, respectively.

Nucleotide Composition and T_m of rRNA

The base composition and T_m value of ribosomal RNA from thermophilic and psychrophilic phycomycetes are shown in Table 4. The %G + C values

TABLE 4. Nucleotide composition and T_m of ribosomal RNA

Source	Base Composition, ^a				Mole % G + C	T_m (C)
	GMP	CMP	AMP	UMP		
Thermophiles						
<i>M. pusillus</i>	28.3	16.5	26.4	28.6	44.8	76.0
<i>M. miehei</i>	27.8	17.2	27.1	27.9	45.0	76.4
Psychrophiles						
<i>M. strictus</i>	29.6	13.7	24.8	31.9	43.3	73.0
<i>M. sphaerosporus</i>	31.4	11.5	21.8	35.5	42.9	72.6
<i>Z. psychrophilus</i>	30.7	14.6	28.1	26.6	45.3	72.0

^aAbbreviations: GMP, guanosine monophosphate; CMP, cytosine monophosphate; AMP, adenosine monophosphate; UMP, uridine monophosphate.

of rRNA from the thermophiles were slightly higher than from the psychrophiles, except in the case of *Z. psychrophilus*. Correspondingly, T_m values of rRNA from thermophiles (76 C) were higher than T_m values of psychrophilic rRNA, which ranged between 72 and 73 C. Pace and Campbell (1967) reported similar differences in procaryotic microorganisms and found that rRNA from psychrophilic bacteria had lower G + C (mole %) contents and lower T_m values than rRNA from thermophilic bacteria.

Figure 3 illustrates the thermal denaturation of rRNA from *M.*

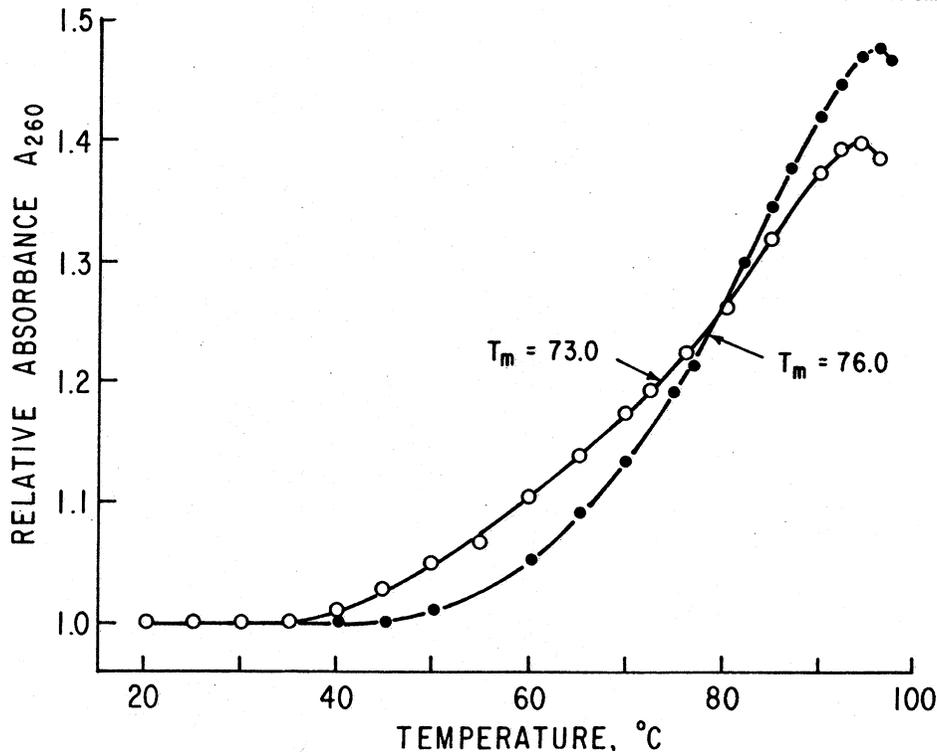


FIG. 3. Thermal denaturation profiles of rRNA in 0.15 M NaCl; O--O, *M. strictus*; ●--●, *M. pusillus*.

pusillus and *M. strictus*. The rRNA of the psychrophile began showing signs of denaturation (e.g., increase in absorbance) at about 40 C, whereas the rRNA of the thermophile was stable up to 48 C.

The %G + C contents of rRNA preparations from either thermophilic or psychrophilic phycomycetes fell within the range reported by Storck (1965) for total ribonucleic acid (tRNA plus rRNA) of the mucorales group of fungi. However, the T_m value of either thermophilic or psychrophilic rRNA was disproportionately higher than the 57 C melting temperature reported by Miller and Shepherd (1973) for both thermophilic and mesophilic rRNA species from fungi of the genus *Penicillium*. This suggested that rRNA from some fungi may have unique physicochemical properties. The data also indicated that rRNA preparations from either psychrophilic or thermophilic sources had substantially higher T_m values than their corresponding ribosomal species. This suggested that the nucleic acid component probably contributed to the overall thermal stability of ribosomes. However, since the T_m values of rRNA's from thermophiles and psychrophiles differed by only 3-4 C, it would appear that the rRNA component alone cannot account for the significantly greater differences that exist in the thermal stability of ribosomes from the two groups of fungi.

Nucleotide Composition and T_m of tRNA

The data in Table 5 show that tRNA from thermophilic phycomycetes had a

TABLE 5. Nucleotide composition and T_m of transfer RNA

Source	Base Composition, ^a			Mole % UMP	G + C	T_m (C)
	GMP	CMP	AMP			
<u>Thermophiles</u>						
<i>M. pusillus</i>	26.6	30.4	28.7	14.3	57.0	77.0
<i>M. miehei</i>	27.6	28.8	27.4	16.2	56.4	76.4
<u>Psychrophiles</u>						
<i>M. strictus</i>	28.7	26.8	22.6	21.9	55.5	70.0
<i>M. sphaerosporus</i>	28.2	23.0	24.5	24.3	51.2	68.2
<i>Z. psychrophilus</i>	27.8	24.8	22.8	24.7	52.6	68.4

^aAbbreviations: see Table 4.

higher average %G + C value (56.7%) than tRNA from psychrophilic fungi (53.1%). The average T_m value for thermophilic tRNA (76.7 C) was correspondingly higher than the average T_m calculated (68.9 C) for the tRNA from psychrophilic phycomycetes.

The thermal stability of *M. pusillus* tRNA was compared with *M. strictus* tRNA in TMK buffer and is shown in Fig. 4. The psychrophilic tRNA began to show hyperchromicity at about 45 C, whereas tRNA from the thermophilic *M. pusillus* began melting at 62 C.

Functional Stability of Ribosomes and Activating Enzymes

Heat treatment of the various components of the protein-synthesizing system impaired the polyU-directed incorporation of ¹⁴C-phenylalanine by cell-free extracts of *M. sphaerosporus*, an obligately psychrophilic phycomycete (Table 6). Ribosomes preheated at 35 and 40 C already lost a substantial amount of their initial activity. The finding that ribosomes of the psychrophile were inactivated at a temperature lower than

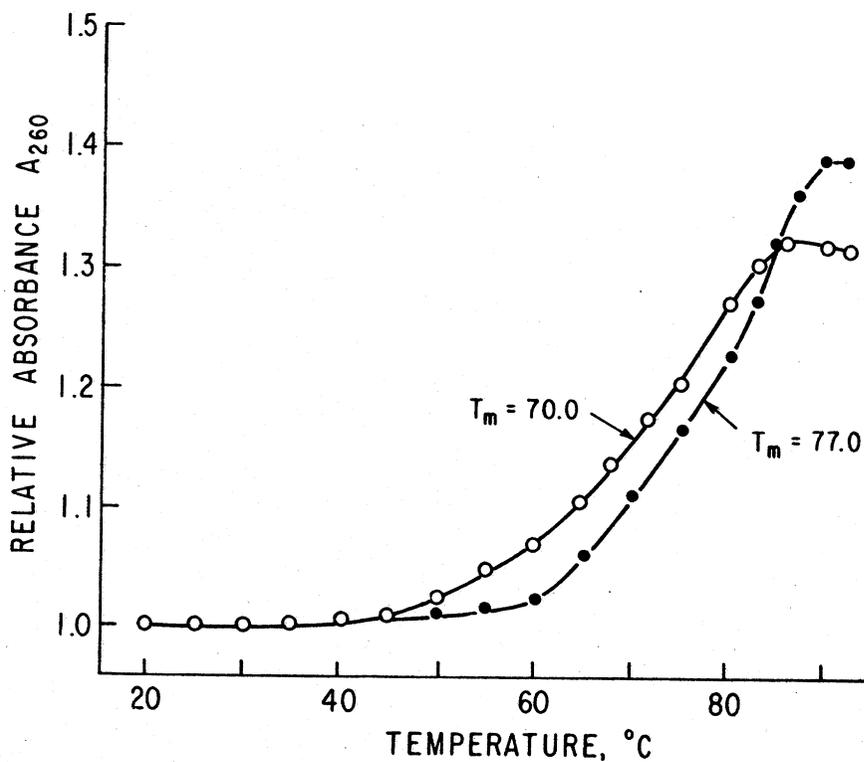


FIG. 4. Thermal denaturation profiles of tRNA in TMK buffer; 0--0, *M. strictus*; ●—●, *M. pusillus*.

TABLE 6. PolyU-directed ^{14}C -phenylalanine incorporation into protein with cell-free extracts of *M. sphaerosporus*

Treatment	Specific Activity ^a	% Loss
Unheated control	3,600	0
Ribosomes preheated at		
35 C	2,160	40
40 C	432	88
45 C	60	98
S-100 preheated at		
35 C	1,878	48
40 C	620	83
45 C	20	99

^aValues expressed as counts/min per mg of ribosomal protein.

its T_m value (47.8 C) implied that melting of the ribosomes was probably not required for inactivation.

The effect of heat on the activity of phenylalanyl-tRNA synthetase also was tested with heated and unheated S-100 fractions of the psychrophile. Phenylalanyl-tRNA synthetase activity was reduced by nearly 50% after the S-100 fraction containing the activating enzymes was

preheated for 10 min at 35 C. Preincubation at 45 C for 10 min resulted in the total loss of phenylalanyl-tRNA synthetase activity (Table 6).

In order to characterize more fully the temperature-sensitive nature of protein synthesis, the capacity of *M. sphaerosporus* ribosomes to bind charged tRNA was studied following a 10-min preincubation at different temperatures. The data in Table 7 show that the heat treat-

TABLE 7. Phenylalanyl-tRNA binding by *M. sphaerosporus* ribosomes

Treatment	Specific Activity ^a	% Loss
Unheated control	5,780	0
Ribosomes preheated at		
35 C	4,046	30
40 C	2,774	52
45 C	1,270	78

^aCounts/min of aminoacyl-tRNA bound per mg of ribosomal protein. Values are based on the amount of ribosomes and radioactivity retained on the Millipore filters.

ment adversely affected the capacity of psychrophile ribosomes to specifically bind phenylalanine-tRNA. After the ribosomes were preincubated at 35, 40, and 45 C, binding capacity of ribosomes was reduced by 30, 52, and 78%, respectively.

Experiments similar to those described above also were carried out to test the functional stability of ribosomes from *M. pusillus*, a thermophilic member of the same genus. Ribosomes of the thermophile were incubated for 10 min at 65, 70, and 75 C, before assay. As a result, polyU-directed incorporation of ¹⁴C-phenylalanine was reduced by 34, 65, and 92%, respectively. Preincubation of ribosomes at the temperatures indicated resulted in a 40, 56, and 82% loss of the phenylalanyl-tRNA binding capacity, respectively.

The molecular organization of intracellular membrane-bound organelles has been suggested as the probable cause for the inability of eucaryotic microorganisms, including fungi, to grow at temperatures above 60-62 C (Tansey and Brock 1972). Presumably, the intracellular organellar membranes surrounding nuclei and mitochondria in eucaryotes become leaky at elevated temperatures, which is not compatible with thermostability.

On the basis of the information obtained in this study it may be concluded that the heat-induced lesions in the protein-synthesizing machinery of both thermophilic and psychrophilic phycomycetes definitely involve aminoacyl-tRNA synthetases and the aminoacyl-tRNA binding capacity of ribosomal particles. Thus, thermolabile soluble and ribosomal components apparently are among the molecular determinants that contribute to the inability of *M. pusillus* and *M. sphaerosporus*, and probably other thermophilic and psychrophilic fungi, to grow above their maximum growth temperatures.

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