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The Half-Life of Bacterial Messenger RNA

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THE HALF-LIFE OF BACTERIAL MESSENGER RNA

by

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INTRODUCTION

Prokaryotic organisms are broadly grouped into three general classes based on their optimum growth temperatures. Psychrophiles are organisms which grow best between 0^{o} and 20^{o}, mesophiles are organisms which grow best between 20^{o} and 45^{o}, and thermophiles are organisms which grow best between 45^{o} and 80^{o}. Thermophilic organisms are interesting organisms for study because they grow and flourish at temperatures at which molecules necessary for survival are ordinarily denatured or destroyed.

A variety of thermophilic organisms have been isolated from several prokaryotic genera. Work done in this laboratory has used two species of bacteria from the genus Bacillus for comparison. The mesophilic organism is B. licheniformis and the thermophilic organism is B. stearothermophilus. The present work was undertaken to shed some light on the phenomenon of thermophily, particularly into the stability of the messenger ribonucleic acid (mRNA) of the two organisms.

Three theories have been put forward to explain the phenomenon of thermophily. These three theories postulate that thermophily may be due to: 1) the protective action of lipids, 2) a high metabolic state, and 3) increased macromolecular stability. The first theory attempts to explain thermophily on the basis of the fact that cell membranes of thermophiles have more saturated lipid material than the cell membranes of mesophiles or psychrophiles (1). Psychrophiles have been shown to have membranes rich in unsaturated fatty acids while thermophiles had membranes rich in saturated fatty acids. Since

saturated fatty acids have higher melting points than unsaturated fatty acids, one would expect that the thermophilic cell membrane should contain higher melting lipid material in order to maintain the membrane fluidity at approximately the same level as that in mesophile or psychrophile. Additionally, the hydrophobic interactions between saturated fatty acids are stronger than those between unsaturated fatty acids making the thermophilic membrane more stable at elevated temperatures. One of the problems in interpreting the evidence supporting this theory is that not only will a change in growth temperature change the fatty acid make-up of the cell membrane but a small change in the composition of the growth medium will have the same effect. Therefore, there is no unique fatty acid composition for psychrophilic, mesophilic, and thermophilic cell membranes.

The second theory, or the kinetic theory, postulates a high metabolic state where cell components are broken down and resynthesized more rapidly in thermophiles than in mesophiles and psychrophiles (2). The growth rates of thermophilic organisms at their optimum growth temperatures are faster than the growth rates of mesophilic and psychrophilic organisms. This is due to the fact that enzymatic and chemical reations occur more rapidly at higher temperatures. Some evidence for the kinetic theory has come out of this laboratory. It was noted that B. licheniformis has fewer active ribosomes than B. stearothermophilus at the common growth temperature of 46^{o} (3). Presumably B. stearothermophilus also has more ribosomes in general than B. licheniformis and, therefore, B. stearothermophilus is able to synthesize protein at a faster rate than B. licheniformis.

The third theory postulates that macromolecules of thermophilic organisms are more heat stable because they differ in physical - chemical properties from corresponding macromolecules of mesophiles (4). This theory has received the most experimental support so far. Evidence for physical - chemical differences between macromolecules (mainly proteins) of thermophilic and mesophilic organisms has been known for some time. The proteins of bacterial flagella (5,6) and purified enzymes (7) of thermophilic bacteria, including amino acid activating enzymes (8), have been shown to be unusually heat stable. Past work in this laboratory also supports the existence of physical - chemical differences between the macromolecules of mesophilic and thermophilic bacteria. The ribosomal RNA of the thermophiles was shown to have a higher guanine + cytosine content than that of the mesophiles (9). The thermophilic ribosomal RNA was more stable to denaturation by heat and urea than the mesophilic ribosomal RNA. The DNA of the thermophiles was likewise shown to have a higher guanine + cytosine ration than that of the mesophiles (10). Thermal denaturation profiles showed that the thermophilic DNA was more heat stable than the mesophilic DNA. The DNA's had T_m values of about 92^o and 88^o, respectively. A cell free amino acid incorporating system from B. stearothermophilus was shown to be more heat stable and also more active than the cell free system from a mesophile (11).

The present work was undertaken to investigate the stability of the mRNA of the two organisms commonly used in this laboratory. Knowledge of the stability of the mRNA would provide valuable information about the phenomenon of thermophily because mRNA is the macromolecular

link between the genetic material and the protein synthesizing machinery, both of which have been shown to be more heat stable in the thermophile. Furthermore, such information would indicate the applicability of the second and third theories of thermophily discussed above. This investigation involves a study of the overall half-life of the mRNA of a mesophilic and a thermophilic organism. Previous work in this laboratory dealt with the acitivities and numbers of ribosomes from mesophilic and thermophilic preparations (3). For that work, a procedure was developed involving the isolation and resolution of various lengths of polyribosomes (polysomes) from the two organisms, B. licheniformis and B. stearothermophilus. By making use of this procedure for the present work, the breakdown of different polysome fractions (i.e. mRNA strands with varying numbers of ribosomes attached) could be studied.

MATERIALS AND METHODS

Growth and Harvest of Organisms

The two bacterial organisms used for these experiments were Bacillus licheniformis (NRS 243) and Bacillus stearothermophilus 10.

The bacteria were cultured on slants containing 0.2% yeast extract (Difco), 1.0% Trypticase (BBL), and 2.0% Bactoagar (Difco). The slants were incubated approximately 12 hours at 37^{o} for the mesophile and about 10 hours at 55^{o} for the thermophile. Broth cultures were started by inoculating 500 ml of sterile broth (1% Trypticase and 0.2% yeast extract) with the bacteria from two slants. The bacteria on the slants were washed into the broth with 5.0 ml of sterile water.

The cells were grown in one liter shake flasks in an incubator shaker (New Brunswick Scientific Co. model G-25) in 500 ml batches. B. licheniformis was grown at 37^{o} and 46^{o}, and B. stearothermophilus was grown at 46^{o} and 55^{o}. Growth curves were obtained by following cell concentration turbidimetrically at 540 nm.

Cells to be used for polysome preparations were harvested at an absorbance of 0.5 at 540 nm. This represents a concentration of cells in approximately the middle of the logarithmic phase of growth for both organisms at all four temperatures. Growth was terminated by pouring each 500 ml of cells together with 200 ml of liquid nitrogen over ice. The cells were sedimented using either a continuous flow system (Sorvall model KSB-R) in a refrigerated centrifuge (Sorvall

RC-2) at 0^{o} and 30,000 x g or by batch centrifugation using a Sorvall GSA rotor at 0^{o} and 10,400 x g depending on the volume of cells collected. All the cells were then combined in one centrifuge tube, frozen, and stored in liquid nitrogen until use.

Cells to be used for labeling experiments were grown to an absorbance of 0.45 at which time 100 ml of cells were transferred from the one liter shake flask to a sterile 250 ml erlenmeyer flask which had been previously equilibrated at the appropriate temperature in a water bath shaker (Research Specialties Co.). The cells were then allowed to shake for 10 minutes before the label was added (i.e. an absorbance of approximately 0.5 at the time of label addition).

Measurement of Overall Half-Life of mRNA

The procedure used in these experiments was similar to that used by Grinsted (12). To a shake flask containing 100 ml of cell culture (5,6-^{3}H) uridine (sp. act. 49 Ci/M mole) was added at time zero to a final concentration of 2.0 μCi/ml. Sampling was begun immediately. After approximately 60 seconds of incorporation of the labeled uridine, rifampicin was added to a final concentration of 30 μg/ml along with a 1,000 fold excess (i.e. to a final conc. of 0.041 mM) of unlabeled uridine. All the samples were 0.5 ml in volume and were taken as rapidly as possible using a Pipetman repeating pipet. The samples were squirted into 6.0 ml of 5.5% (w/v) trichloroacetic acid (TCA) which had been previously equilibrated at 0^{o} in an ice bath. The solution, containing TCA and sample, was mixed on a vortex stirrer and replaced in the ice bath.

The samples were kept in the cold for at least one hour and the acid precipitated material was then collected on membrane filters (Millipore 0.45μ type HA). The filters were then washed with two 3.0 ml portions of 5.5% TCA and with two 3.0 ml portions of 1.0% (v/v) acetic acid. The efficiency of filtration was checked by preparing identical samples and centrifuging one and filtering the other. Both precipitates were then counted as described below.

The filter papers were placed in scintillation vials and were allowed to dry overnight. To the vials were then added 10.0 ml of scintillation cocktail (4.00 g PPO and 0.05 g POPOP per liter of toluene). The samples were then counted in a liquid scintillation

counter (Searle Isocap 300). The counting efficiency was about 40% as measured by the channels ratio method.

The half-lives were determined for each organism at two temperatures. The temperatures were 37^{o} and 46^{o} for B. licheniformis and 46^{o} and 55^{o} for B. stearothermophilus.

Lysis of Cells for Polysome Preparation

The procedure described here is essentially the same as that developed by Shen (3) with several modifications. All operations were performed at 4^{o}.

Frozen cells from two liters of broth culture were allowed to thaw for 1.5 to 2 hours in the cold. The thawed cells were suspended in 2.0 ml of 0.02 M tris(hydroxymethyl)aminomethane (tris;Sigma) at pH 8.6 which contained 25% (w/v) sucrose (Mann Research; ribonuclease free) and 2.0 ml of lysozyme solution (16 mg/ml; Sigma) were then added. This mixture was allowed to stand for one hour with occasional stirring. After one hour the following solutions were added in the order indicated: 2.0 ml of 52.0 mM magnesium acetate, 26.0 mM potassium chloride, and 6.00 mM spermidine trihydrochloride (ICN Pharmaceuticals); 2.0 ml of 10 mg/ml potassium polyvinylsulfate (General Biochemicals); 1.33 ml of 0.02 M tris at pH 7.4 containing 25% (w/v) sucrose; 2.0 ml of 0.1 M tris at pH 7.4 and 1.33 ml of 5% (w/v) Brij (Emulsion Engineering Inc.). The cells were allowed to lyse in the cold for two hours with occasional stirring. At the end of two hours, 0.66 ml of a 0.15 mg/ml solution of deoxyribonuclease I (Worthington Biochemical Corp.; RNase free) was added. The mixture was allowed to stand for 15 minutes at 4^{o}C.

When polysomes were prepared from cells treated with rifampicin only 500 ml of cell culture were collected. The lysis and fractionation procedures were as above except that the volumes of the solutions used for lysis were cut in half.

Preparation and Fractionation of Polysomes

The lysis mixture was centrifuged at 10,800 x g for five minutes. The supernatant was collected and centrifuged at 81,000 x g for 35 minutes. The supernatant was discarded. The pellet, consisting of polysomes, was suspended in 0.15 ml of standard buffer (22 mM tris at pH 7.4, 8.66 mM magnesium acetate, and 4.33 mM spermidine trihydrochloride).

A 15 - 30% (15% w/v and 30% w/v sucrose in standard buffer) linear sucrose density gradient was prepared using a Buchler linear density gradient maker. Into the left chamber of the density gradient maker were pipetted 2.3 ml of the 15% sucrose solution and 2.2 ml of the 30% sucrose solution were pipetted into the right chamber. A vibrator stirrer was placed in the right chamber and the mixture was pumped into a 5.0 ml Beckman polyallomer centrifuge tube. The linearity of the gradients prepared in this fashion was checked using a refractometer.

About 0.05 - 0.10 ml of the polysome suspension was layered carefully onto the density gradient using a large bore pipet. The gradients were then centrifuged in a Spinco SW-39 swinging bucket rotor at 35,000 rpm for 90 minutes. The distribution of the polysomes throughout the gradient was determined using a density gradient fractionator (ISCO Model D) which pushed the gradient through a flow cell having quartz windows at a constant rate of 0.2 ml per minute. The absorbance at 254 nm was monitored with an ultraviolet analyzer (ISCO Model UA-2).

RESULTS AND DISCUSSION

Growth of Organisms

The temperatures chosen for the growth of the organisms were 37° and 46° for B. licheniformis and 46° and 55° for B. stearothermophilus. These are the temperatures at which the two organisms are commonly grown in this laboratory. At the common growth temperature of 46° the temperature variable is eliminated so that data for the two organisms are readily compared. Additionally, measurement at two temperatures allows an examination of the effect of temperature on the half-life of mRNA.

The growth curves for the organisms in the shake flask are presented in Figure 1. The doubling times for the cells at the point of growth where either label was added or where the cells are harvested for polysome preparation (absorbance of 0.5) were calculated from the following equation: $d = \frac{(t)(\log 2)}{\log (x/a_o)}$

where t is time, a_o is the initial concentration, and x is the concentration at time t. The doubling times are listed in Table 1.

Table 1

Doubling Times of the Organisms

Organism	Temperature	Doubling Time (minutes)
B. licheniformis	37°	46.7
B. licheniformis	46°	40.6
B. stearothermophilus	46°	82.8
B. stearothermophilus	55°	51.9

Figure 1

Growth Curves of the Organisms

Ordinate: Absorbance at 540 nm

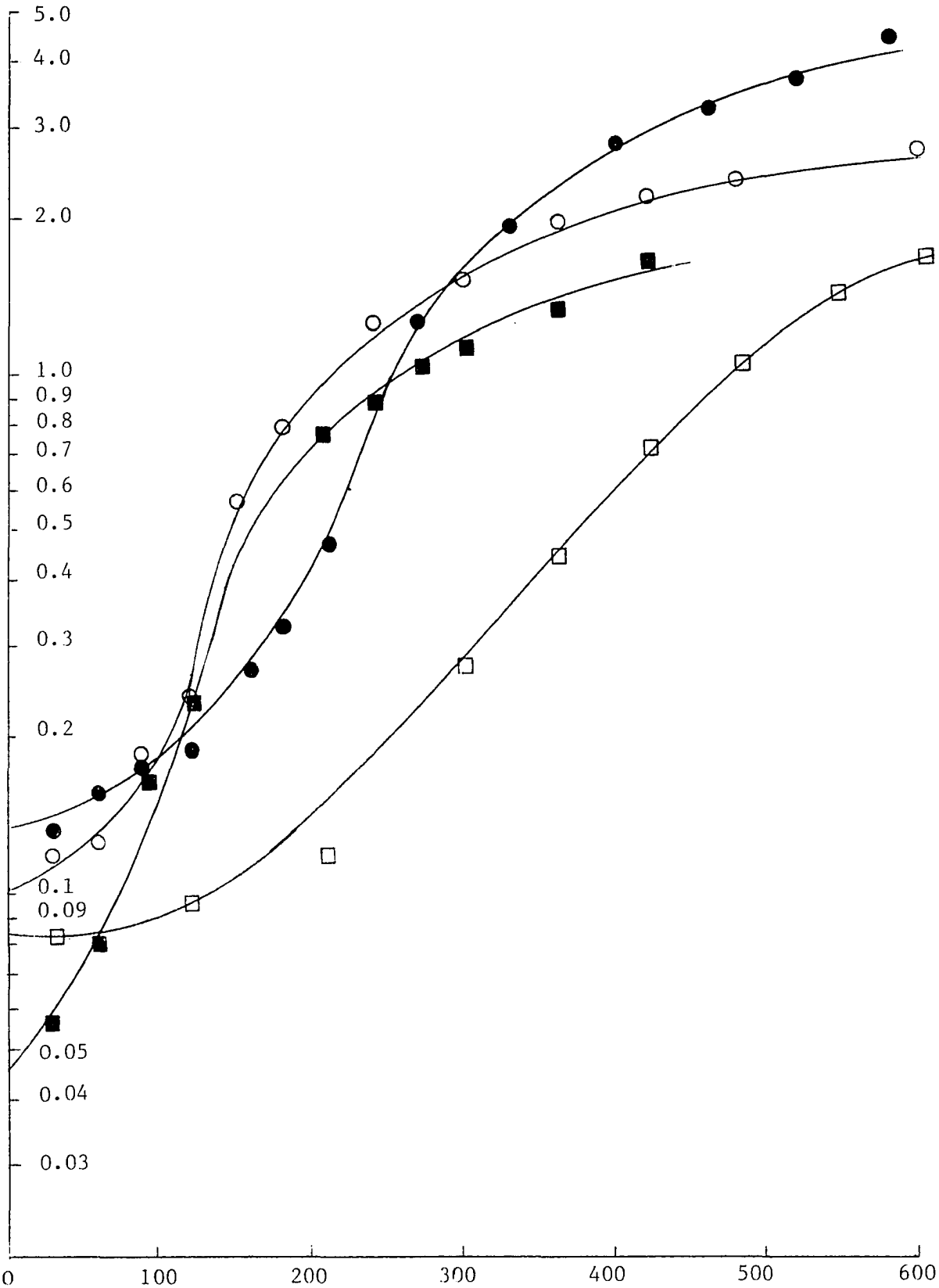
Abscissa: Time in minutes

(●) B. licheniformis grown at 37°

(○) B. licheniformis grown at 46°

(■) B. stearothermophilus grown at 46°

(□) B. stearothermophilus grown at 55°



Overall Half-life of mRNA

The half-life of a compound in a biological system is the time that it takes for the concentration of that compound to reach one half of its initial value. The overall half-life of mRNA can be measured by adding a drug which blocks the synthesis of mRNA and then following the decay in mRNA concentration. The drug used in these experiments was rifampicin (3-{4-methylpiperazinyliminomethyl}rifamycin SV). This drug is known to inhibit mRNA synthesis. It binds tightly to E. coli RNA polymerase with a $K_d = 3 \times 10^{-9}$M (14). Its mode of action is believed to be the blocking of the initiation of transcription. Evidence indicates, however, that the first phosphodiester bond is formed even in the presence of rifampicin (15). A new mode of action has recently been postulated (16). According to the latter author the rifampicin binds to DNA dependent RNA polymerase but the binding is decreased in the presence of DNA. This decrease in binding is pronounced at low rifampicin concentrations, but is not apparent at high rifampicin concentrations. Once the RNA polymerase forms its tight complex with DNA, rifampicin will not bind to the enzyme and transcription will continue. The net effect of rifampicin appears to be the blocking of initiation but actually initiation appears to take place but the drug prevents the synthesis of oligonucleotides after the formation of the first phosphodiester bond.

There has been some disagreement as to the concentration of rifampcin that should be used in experiments such as the ones being reported here. Coote et. al. reported that rifampicin has a great cytotoxic effect even at concentrations as low as 1.0 µg/ml (17).

The organisms grew well at all temperatures except for B. stearothermophilus at 46° where growth was sluggish. B. licheniformis at 46° grows faster than B. licheniformis at 37° as indicated by the doubling times. This is as one would expect, however, B. stearothermophilus at 55° grows more slowly than B. licheniformis at either temperature. Generally the growth rate of an organism increases as temperature increases as long as the cells are being grown within their optimum temperature range. Two possible reasons for the slower growth of B. stearothermophilus at 55° are as follows. First, the doubling times were calculated at an absorbance of 0.5 which is slightly later in the log phase for B. stearothermophilus than it is for B. licheniformis. Second, it has been stated that thermophilic growth has an extraordinarily high requirement for oxygen (13). This requirement, coupled with the fact that oxygen solubility decreases as temperature increases, may explain why the thermophile grows somewhat more slowly than might be expected.

The absolute amount of growth obtained was higher for B. licheniformis than it was for B. stearothermophilus.

It was noted that, during lysis for polysome preparations, that B. licheniformis cells grown at 46° were consistently more resistant to lysis than B. licheniformis cells grown at 37°. This indicates that a change occurs in the cell wall of B. licheniformis when it is grown at the higher temperature so that the cell wall becomes more resistant to hydrolysis by lysozyme. This may prove to be an interesting area for further study because of the temperature dependence of this phenomenon.

Based on the data obtained the authors reported that in their estimation no reliable values for mRNA half-life can be obtained using a drug that is so cytotoxic. In a more recent paper it has been shown that the half-lives of bacterial mRNA determined by DNA:RNA hybridization in a rifampicin inhibited system and in an uninhibited system are the same (18). The concentration of rifampicin used in these experiments was 10 µg/ml. The authors suggest that a rifampicin concentration of less than or equal to 30 µg/ml will not affect the breakdown of mRNA.

Preliminary experiments in the present study were carried out with a rifampicin concentration of 10 µg/ml. The half-life plots showed a considerable amount of scatter. The scatter was essentially the same when only rifampicin or only cold uridine were used. The scatter was, however, reduced significantly when a rifampicin concentration of 30 µg/ml together with a uridine concentration 1.0×10^{-4} mg/ml were used. These latter conditions were, therefore, chosen for all of the subsequent experiments.

Decay curves of the mRNA are presented in Figures 2 through 5. (^{3}H)-uridine is rapidly incorporated into the mRNA until the addition of rifampicin and unlabeled uridine at which time the mRNA begins to breakdown almost immediately (within the time necessary to take the first sample). The mRNA concentration decreases exponentially and levels off at a particular concentration. The concentration at which the decay curve levels off represents the concentration of stable RNA (ribosomal RNA and transfer RNA). The exponential portion of the curve depicts the breakdown of the labile RNA (mRNA).

Figure 2

mRNA Decay Curve for B. licheniformis Grown at 37°

Ordinate: Percent of Maximum Counts Per Minute

Maximum Number of Counts Obtained: 129, 418 cpm

Abscissa: Time (seconds)

 - Addition of Rifampicin

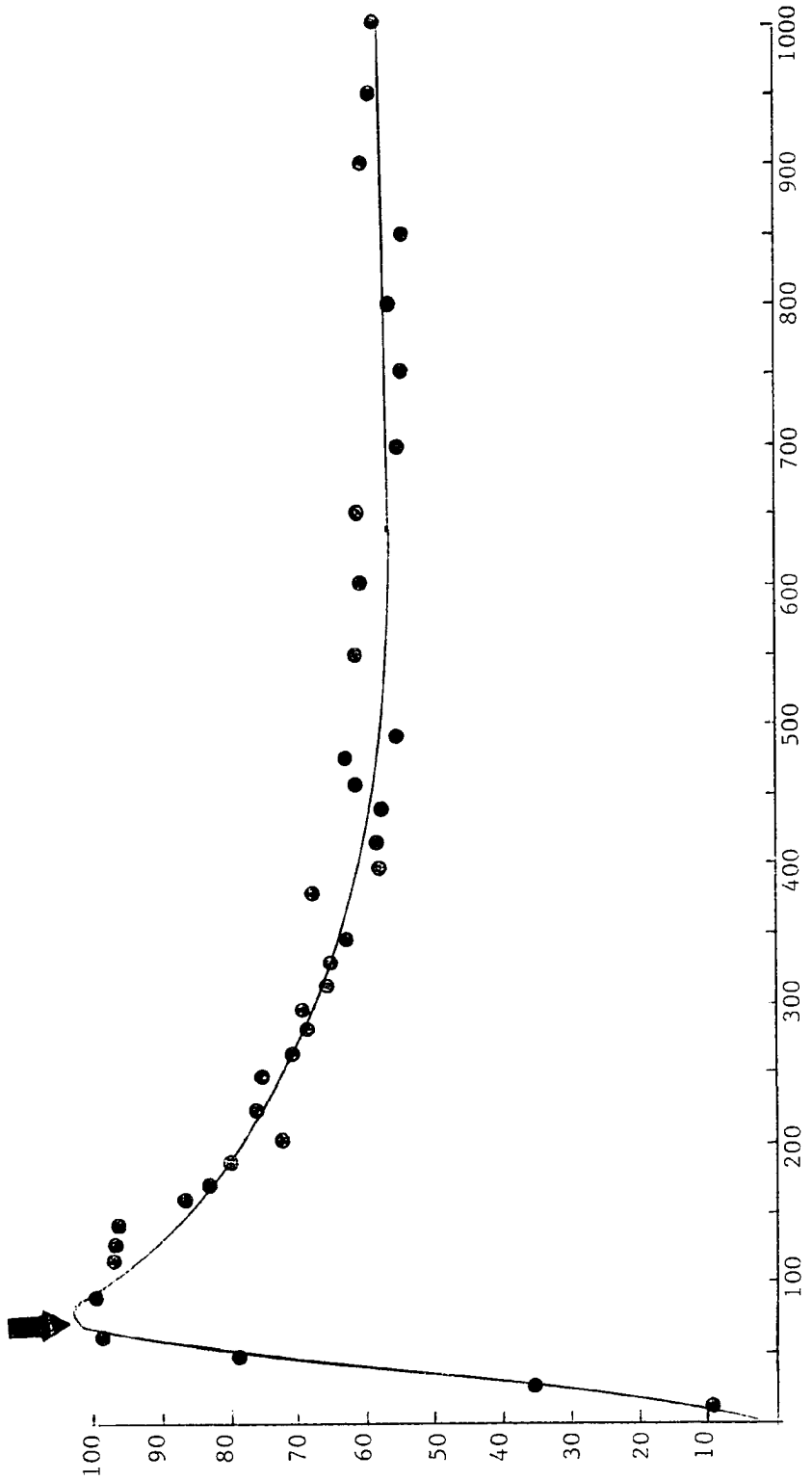


Figure 3

mRNA Decay Curve for B. licheniformis Grown at 46°

Ordinate: Percent of Maximum Counts Per Minute

Maximum Number of Counts Obtained: 88, 132 cpm

Abscissa: Time (seconds)

 - Addition of Rifampicin

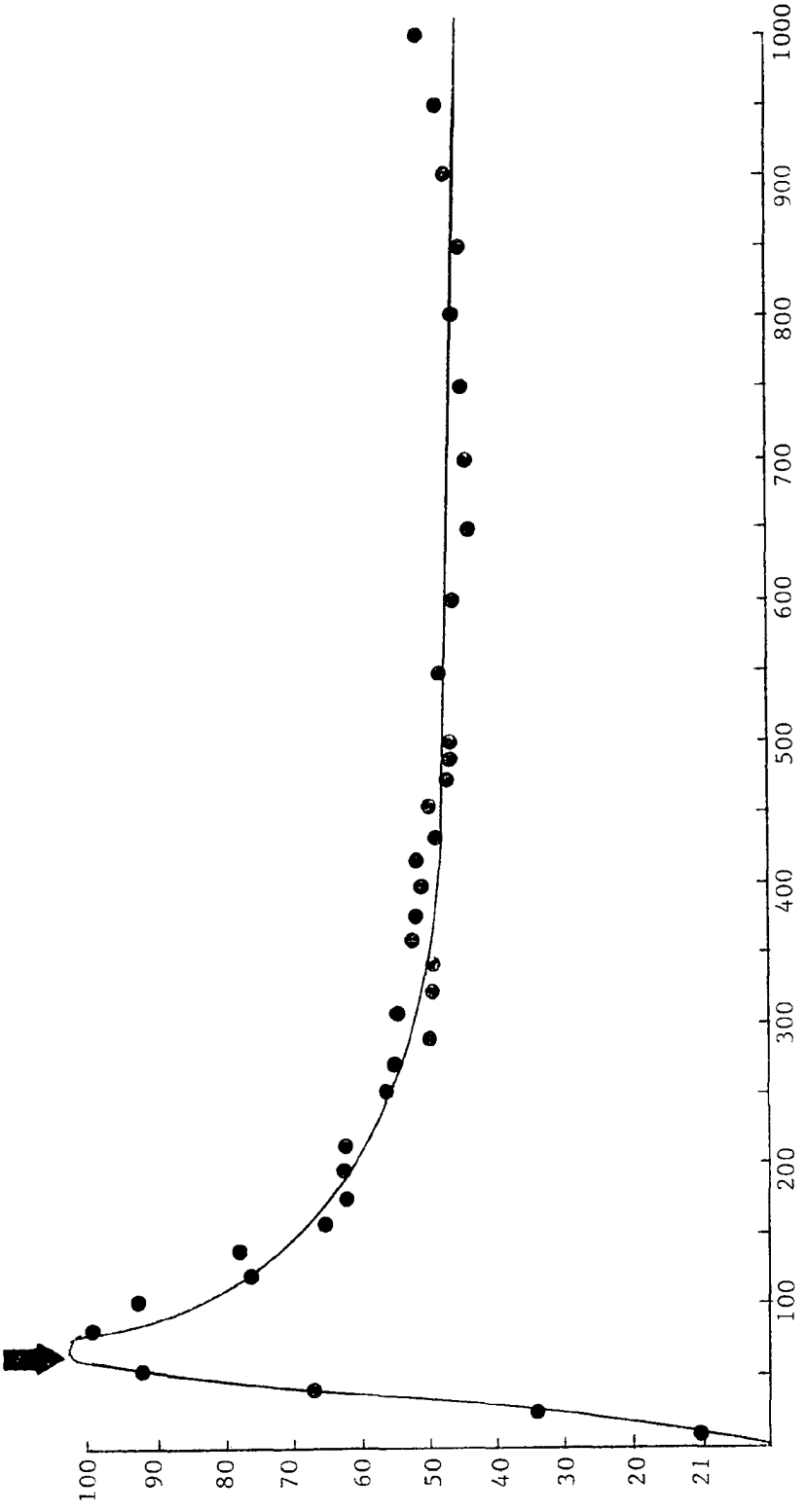


Figure 4

mRNA Decay Curve for B. stearothermophilus Grown at 46^{o}

Ordinate: Percent of Maximum Counts Per Minute

Maximum Number of Counts Obtained: 177, 354 cpm

Abscissa: Time (seconds)

 - Addition of Rifampicin

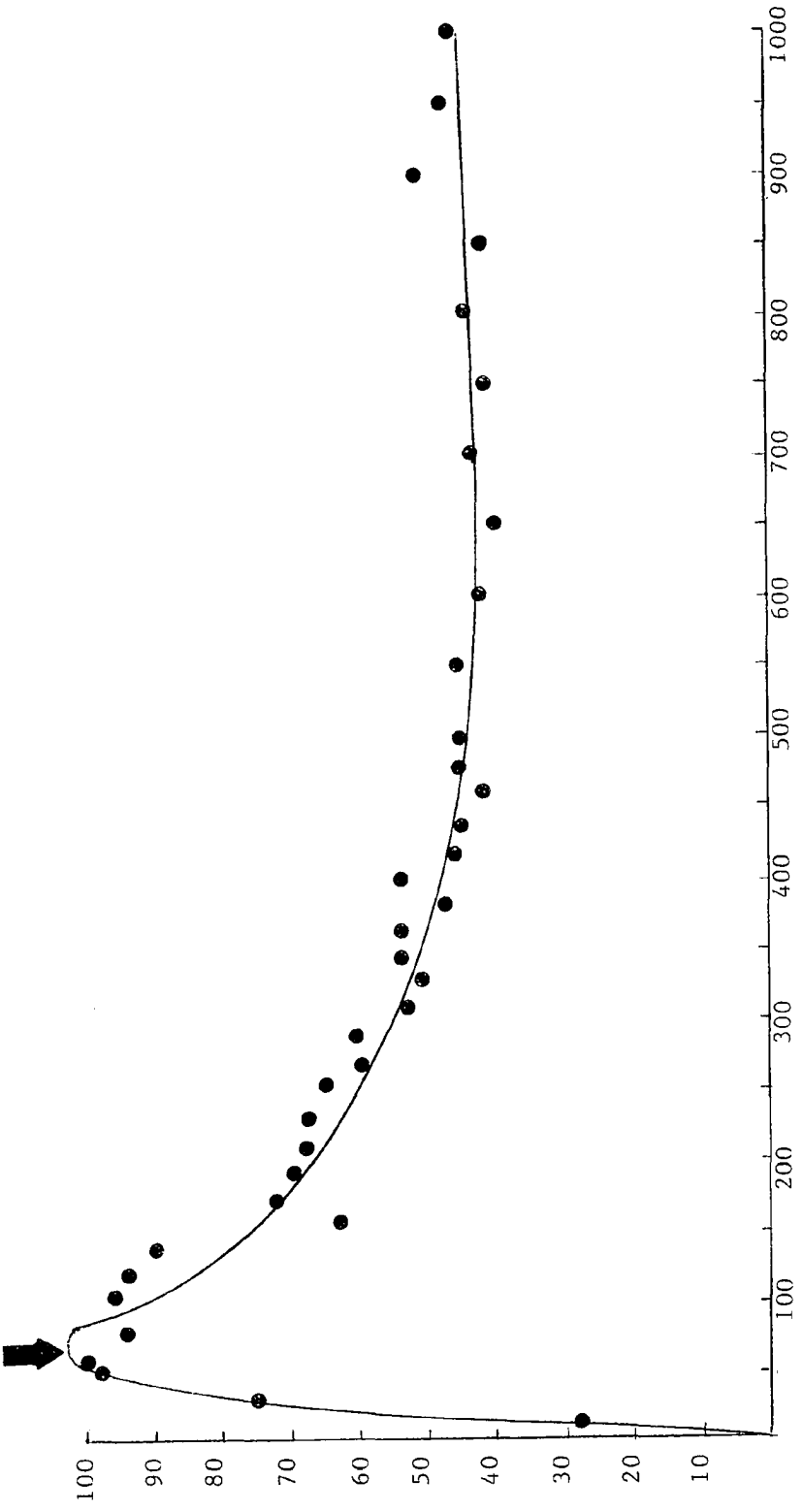


Figure 5

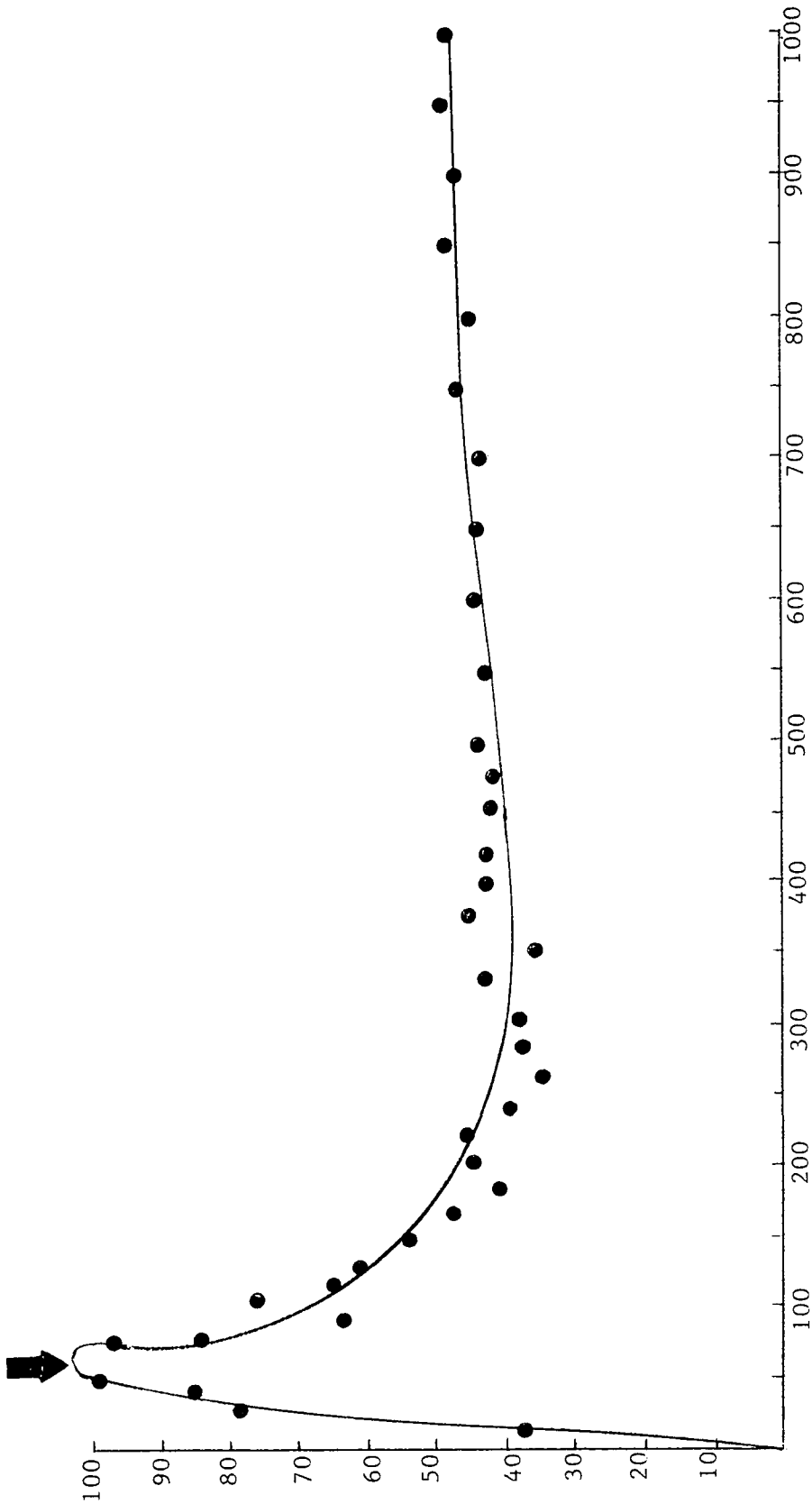
mRNA Decay Curve for B. stearothermophilus Grown at 55^{o}

Ordinate: Percent of Maximum Counts Per Minute

Maximum Number of Counts Obtained: 109, 631 cpm

Abscissa: Time (seconds)

 - Addition of Rifampicin



The data of Figures 2 - 5 were converted to linear plots by assuming that the decay curve was exponential. Semi-log plots were prepared (see Figure 6) by subtracting the concentration of stable RNA from the concentration of labile RNA and plotting the log of this difference vs. time (concentrations are expressed in terms of the percentage of the maximum cpm). If it is assumed that the decay is a first order process then the slope of the line is equal to -k, where k is the rate constant. The derivation is as follows. For a first order reaction the integrated rate equation is:

$$k = (1/t)\ln(a_o/a_o-x)$$

where k is the rate constant, t is the time, a_o is the initial concentration, and a_o-x is the concentration at time t. The equation may be rearranged to give:

$$\ln(a_o-x) = -kt-\ln(a_o)$$

which is of the form y = mx + b. When $\ln(a_o-x)$ is plotted vs. time the slope is equal to -k. The half-life is the time it takes for one half of the initial concentration to disappear. The rate equation becomes:

$$k = 1/t_{\frac{1}{2}}\ \ln\{a_o/(a_o-a_o/2\}} = 1/t_{\frac{1}{2}}\ \ln(2)$$

Rearranging this equation the following equation is obtained:

$$t_{\frac{1}{2}} = \ln(2)/k$$

The slopes of the lines of the semi-log plots were determined by least squares analysis and the standard deviation of the slope was also obtained.

Figure 6

Semi-log Plots

Ordinate: ln (concentration of labile RNA - concentration of stable RNA)

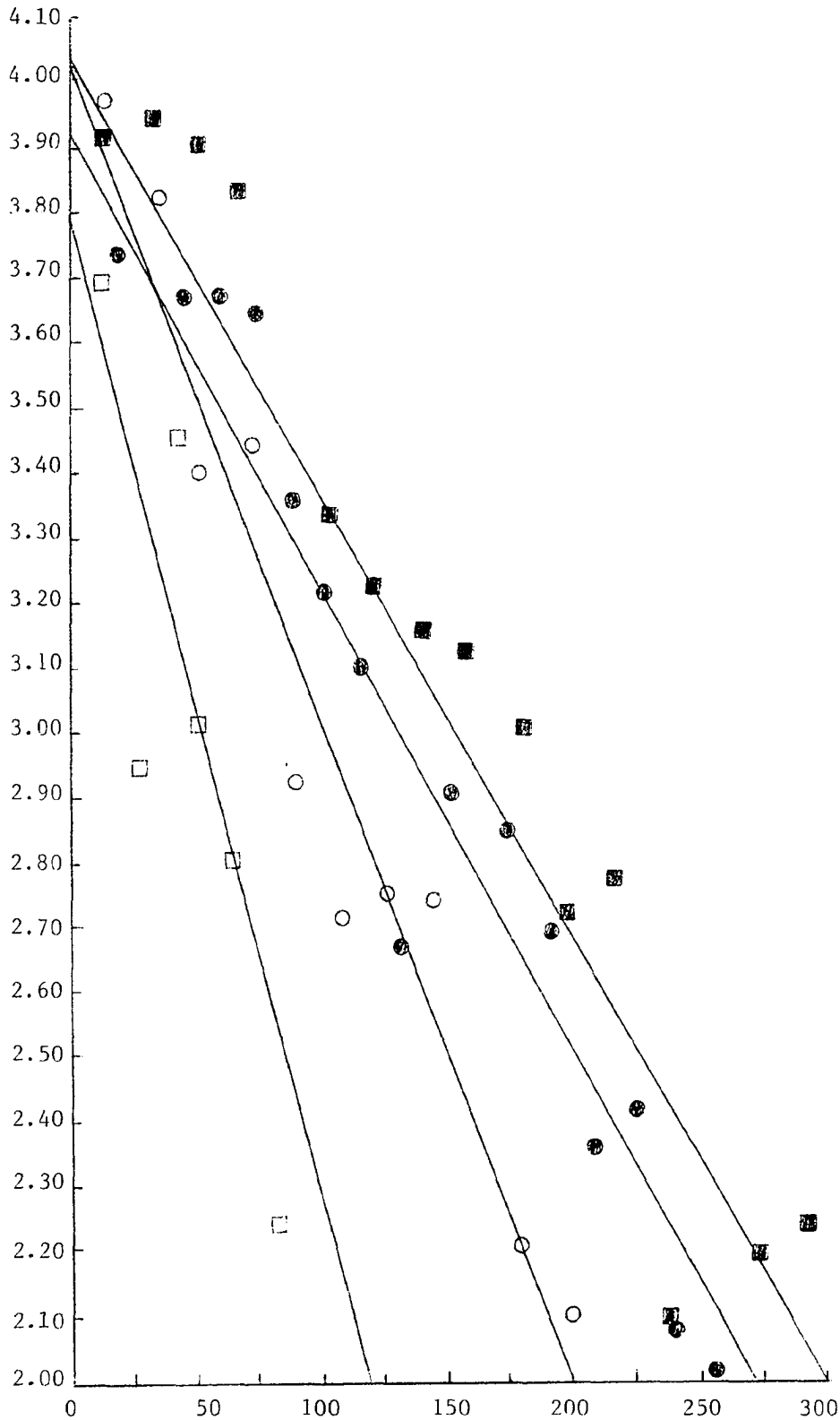
Abscissa: Time (seconds)

(●) *B. licheniformis* grown at 37^0

(○) *B. licheniformis* grown at 46^0

(■) *B. stearothermophilus* grown at 46^0

(□) *B. stearothermophilus* grown at 55^o



The half-life data are summarized in Table 2. At the common growth temperature of 46° the half-life of the mRNA from the mesophile is shorter than the half-life of the thermophilic mRNA, therefore, the thermophilic mRNA is degraded less rapidly than the mesophilic mRNA. In both organisms an increase in growth temperature caused an increase in the rate of degradation of mRNA. Additionally, the half-lives of the thermophilic mRNA at 46° and the mesophilic RNA at 37° are essentially the same.

The rate of breakdown of any molecule in an organism is going to be dependent on the organism's metabolic state. The more rapidly an organism is growing, the more rapidly metabolites are likely to be broken down. Hence, in order to take account of the rate of growth of the cells, the half-life was divided by the doubling time. The larger this number is the less mRNA degradation occurs per doubling time. It can be seen from the stability index that the mRNA of the mesophile is more stable than the mRNA of the thermophile, even at the common growth temperature of 46°. The stability index decreases for both organisms as the growth temperature is increased. Furthermore, it appears that the stability of the mRNA decreases as the growth temperatures are increased from 37° to 55°.

How then do these data relate to the kinetic and macromolecular theories of thermophily? Considering first the macromolecular theory, there are two possible reasons for the lower stability of the thermophilic RNA. Firstly, there may be structural differences in the thermophilic mRNA which makes it more labile and secondly, the ribo-

Table 2

Half-life and Stability Index of mRNA

Organism	Temperature (^{o}C)	Half-life (seconds)	Doubling Time (seconds)	Stability Index (half-life/doubling time)
B. licheniformis	37	92.5 ± 6.1	2.80×10^{3}	3.30×10^{-2}
B. licheniformis	46	69.0 ± 5.8	2.44×10^{3}	2.83×10^{-2}
B. stearothermophilus	46	97.4 ± 9.5	4.97×10^{3}	1.96×10^{-2}
B. stearothermophilus	55	40.0 ± 11.5	3.11×10^{3}	1.29×10^{-2}

nucleases for the thermophile may also be more active than those of the mesophile.

The kinetic theory states that the macromolecules of the thermophilic organisms are broken down and resynthesized more rapidly. These data provide evidence that mRNA of the thermophilic organism is indeed less stable and is degraded more rapidly. The rate of mRNA systhesis, however, appears to be essentially the same for both organisms at all four temperatures judging by the rate of incorporation of uridine into the mRNA. It may be, however, that the rate of transport of uridine into the cell and not the rate of mRNA synthesis is the rate limiting step in this process.

The level at which the decay curves level off gives a measure of the relative concentration of stable RNA. The level for B. licheniformis grown at 46^{o} and B. stearothermophilus grown at both 46^{o} and 55^{o} is essentially the same (about 45 - 50%). The level for B. licheniformis grown at 37^{o} is considerably higher (about 60%).

Polysome Profiles in the Absence of Rifampicin

The polysome profiles obtained in the absence of rifampicin (see Figure 7) are like those obtained by Shen (3) except for the following differences. The peak resolution has been increased by usually layering a smaller amount of polysome suspension on the gradient and by using the 0 - 0.5 absorbance range rather than the 0 - 2.5 range. Furthermore, Shen reported that peak 11 was absent in B. licheniformis and B. stearothermophilus grown at 46°, however, peak 11 is seen in both of the profiles shown here. The increase in peak resolution probably accounts for the appearance of this peak.

Peak 1 represents low molecular weight UV absorbing compounds at the top of the gradient. Peaks 2 and 3 represent 30 S and 50 S ribosomal subunits, respectively, and are sometimes obscured by peak 1. Peaks 4 and 5 are 70 S ribosomes and 100 S ribosome dimers, respectively. Peaks 6 through 11 are messenger RNA strands with increasing numbers of ribosomes attached and peak 12 represents membrane fragments which are stirred up by the chase solution and always signifies the bottom of the density gradient.

Figure 7

Polysome Profile of the Organisms

Ordinate: Absorbance at 254 nm, Range 0 - 0.5 A

Abscissa: Milliters From the Tope of the Gradient

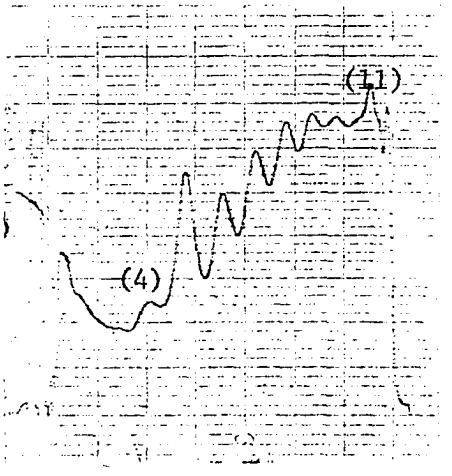
(1 cm corresponds to 1.2 ml)

(a) B. licheniformis grown at 37°

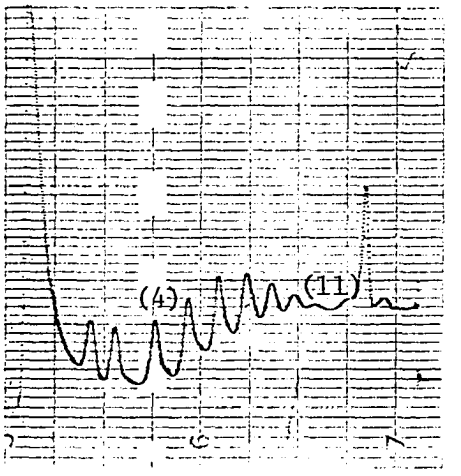
(b) B. licheniformis grown at 46°

(c) B. stearothermophilus grown at 46°

(d) B. stearothermophilus grown at 55°



(a)



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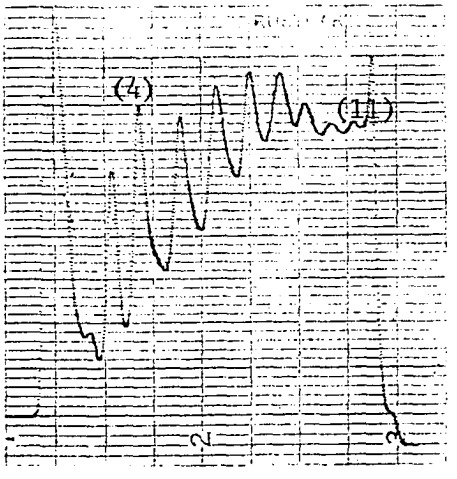

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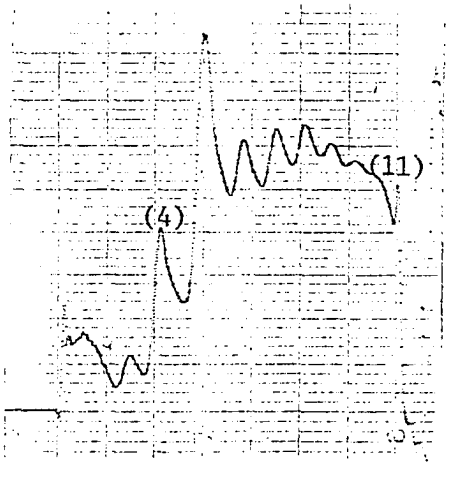


(d)

Polysome Profiles in the Presence of Rifampicin

In these experiments the bacterial cells were grown as usual and the cells were then exposed to the drug rifampicin for varying lengths of time. The cells were harvested and the polysomes were isolated and fractionated. It was hoped that a decrease in one or more peaks in the polysome profile, and a concommitant increase in another (or several other) peaks, could be observed as a function of exposure time time to rifampicin. This would then indicate the relative stability of the different polysomes.

The profiles obtained are shown in Figures 8 and 9. The apparent rate of degradation of the mRNA observed in the polysome profiles agrees well with the overall half-lives of the mRNA. B. stearothermophilus polysomes break down much more rapidly than the B. licheniformis polysomes. There is some observable decay in the B. stearothermophilus polysomes at the time of the first sample (30 seconds), whereas, there is no observable decay in the B. licheniformis polysomes even after 60 seconds exposure to rifampicin.

In both organisms, the large polysomes were degraded rapidly, thereby leading to an increase in the 70 S peak. As time went on, however, the 70 S peak decreased and the 100 S peak increased. At the end of 10 minutes there were essentially only 100 S ribosome dimers present in both organisms.

The differences in peak heights were not sufficient to obtain information concerning the relative stability of the different polysomes. The larger polysomes were degraded to smaller ones and even-

Figure 8

Polysome Profiles of B. licheniformis Grown at 37°

and Exposed to Rifampicin

Ordinate: Absorbance at 254 nm

Abscissa: Milliters From the Top of the Gradient

(1 cm corresponds to 1.2 ml)

Length of Rifampicin Exposure (seconds)

(a) 30

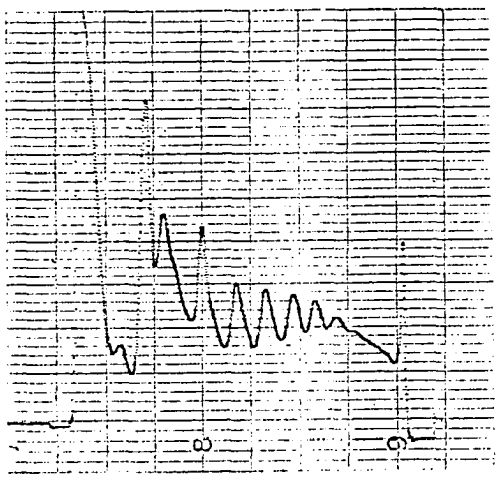
(b) 60

(c) 150

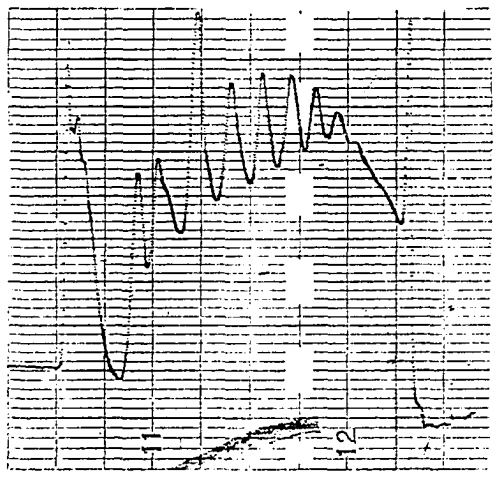
(d) 180

(e) 240

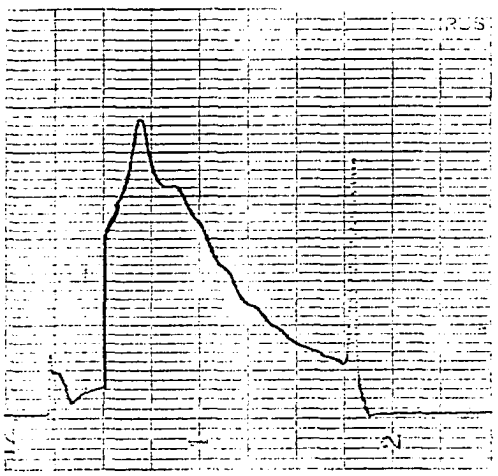
(f) 600



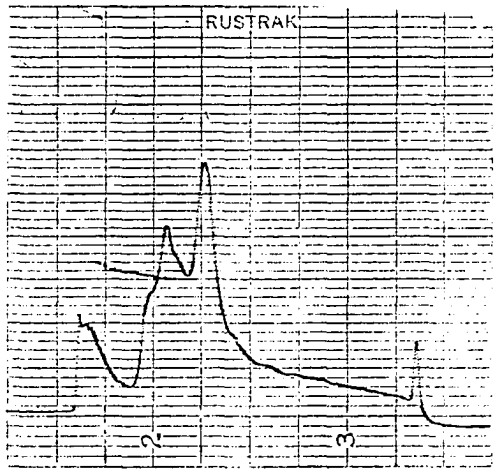
(a)



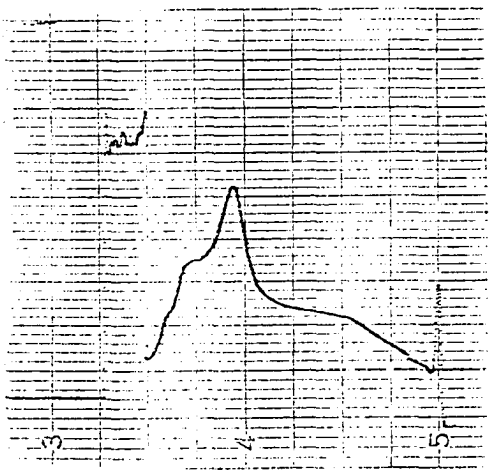
(b)



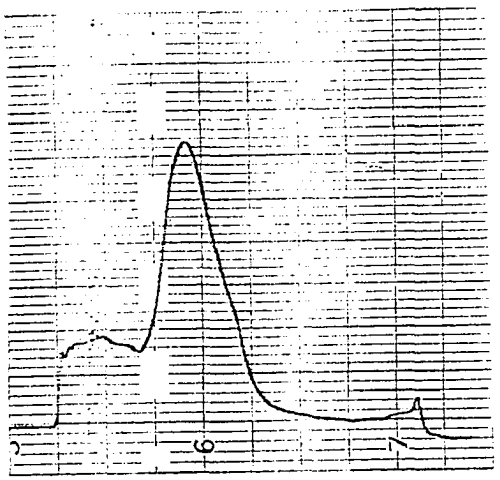
(c)



(d)



(e)



(f)

Figure 9

Polysome Profiles of B. stearothermophilus Grown at 55°

and Exposed to Rifampicin

Ordinate: Absorbance at 254 nm

Abscissa: Milliters From the Top of the Gradient

(1 cm corresponds to 1.2 ml)

Length of Rifampicin Exposure (seconds)

(a) 30

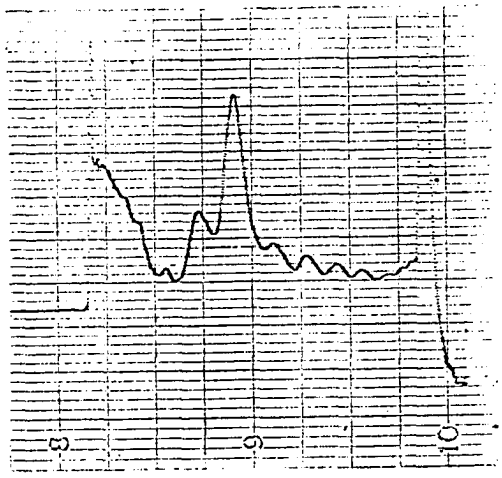
(b) 60

(c) 90

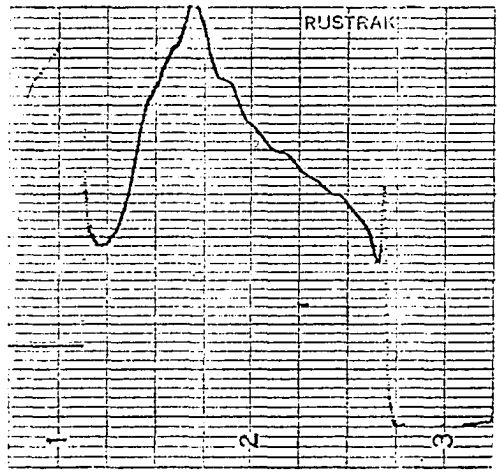
(d) 120

(e) 180

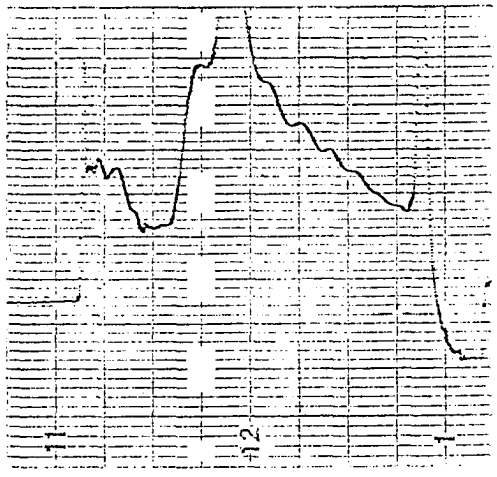
(f) 600



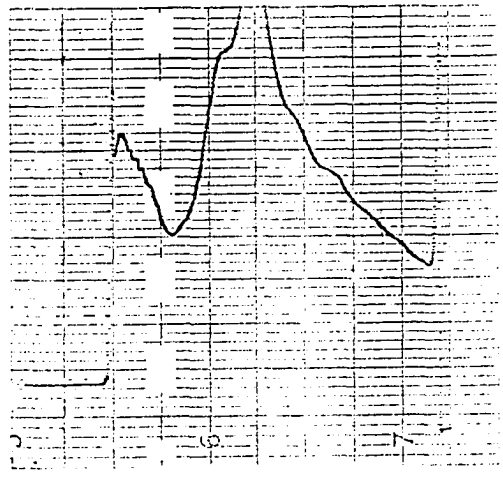
(a)



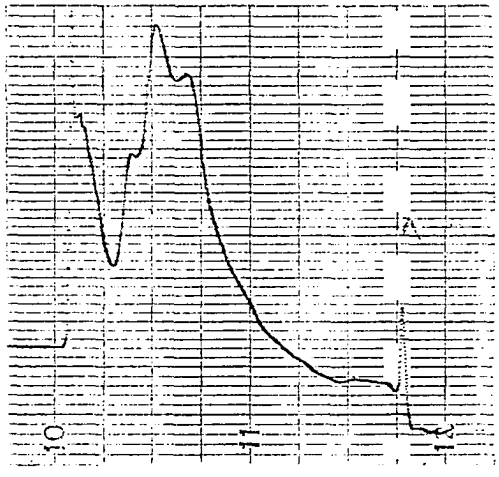
(b)



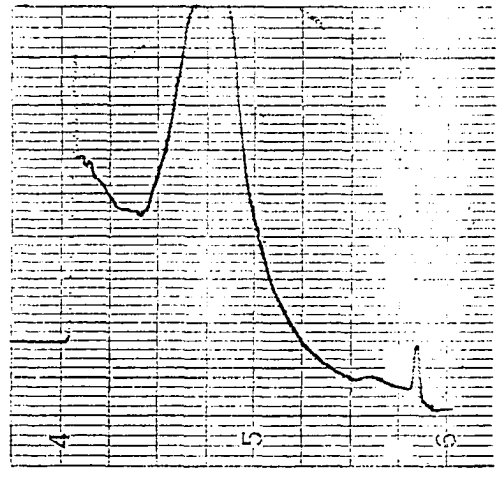
(c)



(d)



(e)



(f)

tually to 70 S ribosomes which associated under the conditions used in these experiments to yield 100 S ribosome dimers.

BIBLIOGRAPHY

1. Gaughran, E. R. L., J. Bacteriol. 53, 506 (1947).

2. Allen, M. B., Bacteriol. Rev. 17, 125 (1953).

3. Shen, P. Y., Ph. D. Dissertation, Western Michigan University (1972).

4. Campbell, L. L. and Pace, B. J., Appl. Bacteriol. 31, 24 (1971).

5. Stenesh, J. and Koffler, H., Federation Proceedings 21, 406 (1962).

6. Manning, G. B. and Campbell, L. L., J. Biol. Chem. 236, 2952 (1961).

7. Arca, M., Calvori, C., Frontali, L. and Tecce, G., Biochim. Biophys. Acta 87, 440 (1964).

8. Shen, P. Y., Coles, E., Foote, J. L., and Stenesh, J., J. Bacteriol. 103, 479 (1970).

9. Stenesh, J. and Holazo, A. A., Biochim. Biophys. Acta 138, 286 (1967).

10. Stenesh, J., Roe, B. A., and Snyder, T. L., Biochim. Biophys. Acta 161, (1968).

11. Stenesh, J., Schechter, N., Shen, P. Y., and Yang, C., Biochim. Biophys. Acta 228, 259 (1971).

12. Grinsted, J., Biochim. Biophys. Acta 179, 268 (1969).

13. Allen, M. B., Bacteriol. Rev. 17, 125 (1953).

14. Yarbrough, L. R., et. al., Biochem. 15, 2669 (1976).

15. So, A. G. and Downy, K. M., Biochem. 9, 4788 (1970).

16. Kessler, C. and Guido, H. R., Biochem. and Biophys. Res. Comm. 74, 50 (1977).

17. Coote, J. G., Wood, D. A., and Mandelstam, J., Biochemical Journal 134, 263 (1973).

18. Coleman, G. and Brown, S., J. of Gen. Microbiol. 92, 200 (1976).

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