AN ABSTRACT OF THE THESIS OF

Bruce A. Ramundo for the Master of Science

in Biology presented on 15 May 1981

Title: The Effect of Temperature on the mRNA of Aeromonas Species

Abstract approved: [redacted]

Fifty-five Aeromonas species previously isolated from various environmental sources were grown at two temperatures (25 and 35 C). Eight of these isolates, when grown and subjected to 48 major biochemical and antimicrobial susceptibility tests at 25 C, had reactions characteristic of an Aeromonas sp. However, when these same eight isolates were grown and subjected to the same tests at 35 C, they showed reactions characteristic of species in the family Enterobacteriacea. One of these isolates, from a squirrel monkey, was used in RNA competition hybridization experiments and it was determined that the RNAs of this isolate grown at 25 and 35 C were not homologous indicating that growth temperature perturbs RNA transcription.

THE EFFECT OF TEMPERATURE ON THE mRNA OF *AEROMONAS* SPECIES

A Thesis
Submitted to
the Division of Biological Sciences
Emporia State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Bruce A. Ramundo
May, 1981

Approved for Major Department

Approved for Graduate Council

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to Dr. Rodney J. Sobieski for his guidance and continuous help during this research. I also would like to thank my wife, Rosemary, for her patience and love throughout this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The bacterial genus Aeromonas, a member of the family Vibrionaceae, was characterized by Kluyver and Van Neil (1936). Since then eight species have been described at one time or another. At this time the eighth edition of Bergy's Manual of Determinative Bacteriology recognizes only three, Aeromonas hydrophila, A. salmonicida and A. punctata. These bacteria are gram negative rods which ferment carbohydrates with the production of acid or acid and gas and are oxidase positive, occurring singularly, in pairs or chains. They range in size from one to 3.5 microns long and from 0.4 to one micron wide. Motile species possess a single polar flagellum (Buchanan and Gibbons, 1971).

Rouf and Rigney (1971) studied the growth temperature ranges of 13 A. hydrophila strains. Of these 13, six were psychrophiles having optimal growth between 15 and 20 C with a maximal growth temperature of between 40 to 45 C. The other seven were classified as mesophiles, three of which had a maximal growth temperature of 55 C.

Aeromonads grow well on nutrient agar, blood agar and on enteric media such as MacConkey and S.S. agars. On enteric media colonies may resemble either lactose fermentors or non-fermentors. Most strains of A. hydrophila show beta hemolysis on blood agar (Von Graevenitz and Mensch, 1968).

Frequently aeromonads are isolated from stagnant and streaming water, wells, soil and occasionally tap water. At this time the primary habitat of Aeromonas species is thought to be nonfecal sewage high in organic substances which may act to inoculate the water (Ewing and Hugh, 1974).

Aeromonads have been recognized for many years to be pathogenic for

many cold blooded animals. They are known to cause such diseases as red leg in frogs, septicemia in snakes and salamanders, and furunculosis in fish (Kulp, W.L., 1942; McCarthy, 1975).

From studies done in the 1960's and early 1970's the incidence of Aeromonas species in the human population was found to be extremely low. Catsarras and Buttiaux (1965) found A. hydrophila in only 0.7% of 4,426 children under the age of two. Von Graevenitz and Zinterhofe (1970) recovered six Aeromonas isolates from 188 random stool specimen samples in patients with intestinal infections. Thus, with such an apparent low incidence in the human population, aeromonads have rarely been implicated as a cause of disease in man. However, this may be partly due to our failure to recognize their human pathogenic potential and more importantly the use of improper diagnostic tests (Bulger, Sherris, 1966; Conn, 1964). It is thought that more cases of intestinal infections would be attributed to A. hydrophila if they were properly identified. This misidentification is due to the similarity of reactions between Escherichia coli and aeromonads to such common tests and reactions as urease, IMViC and the fact that the growth of Aeromonas species on Endo agar produces metallic colonies which are virtually indistinguishable from E. coli. Therefore A. hydrophila infections are often misdiagnosed as E. coli (Bulger and Sherris, 1966; Neilson, 1978). With the utilization of better diagnostic methods Aeromonas species have been isolated from skin lesions, throat, blood, gallbladder, normal feces and patients with gastroenteritis and abscesses (Ewing et al., 1961; Gilardi et al., 1970). Most often Aeromonas infections occur in patients undergoing immunosuppressive chemotherapy for malignancy, organ transplant, or some other chronic or debilitating disease. However, in 1973 an Aeromonas

infection was reported as the probable cause of fatal septicemia in a thirty year old male with no past history of debilitating disease (Kovarik and Sides, 1973).

Titcomb (1978) sampled a zoo environment for the presence of Aeromonas species. Subsequently, she found that when 55 Aeromonas isolates were subjected to 48 biochemical and antimicrobial tests[1] at 25 C, their reactions were characteristic of an Aeromonas sp. However, when these same 55 isolates were subjected to the same 48 biochemical and antimicrobial susceptibility tests at 35 C, seven of the isolates were identified as being other than an Aeromonas sp. Most often they were identified as not belonging to the Vibrionceae but as members of another enteric group, the family Enterobacteriacea. The genus within the latter family was Enterobacter.

Shown in Table I, are those biochemical and antimicrobial susceptibility tests which differed when the bacteria were grown and tested at 25 C and 35 C. Due to these phenotypic changes the squirrel monkey isolate was identified as an A. hydrophila (Titcomb #Z3-5b-2) at 25 C and an E. cloace at 35 C. Tests such as oxidase, Simmon's citrate and sorbitol were lost at 25 C while other tests such as maltose and growth on S.S. agar were positive at 35 C. Thus reactions were both gained and lost at the two temperatures.

---

1. Dextrose - phenol red, dextrose - OF, MacConkey's agar, oxidase, Simmon's citrate, indol production, H_2S production, urea, ornithine decarboxylase, lysine decarboxylase, arginine dehydrolase, acetate, DNAase, esculin, lactose, sucrose, mannitol, dulcitol, adonitol, inositol, sorbitol - phenol red, raffinose - phenol red, rhamnose - phenol red, xylose - OF, maltose - OF, growth at 42 C, Shigella-Salmonella agar, nutrient agar, lipase, lecithin, hemolysis, starch, gelatin, motility, arabinose, ampicillin, carbencillin, cephalothin, tricarcillin, ceflamonadol, gentamycin, amikacin, tobramycin, kanamycin, chloramphenicol, furadantin, tetracycline and colistin (Titcomb, 1978).

Table I. Biochemical and antimicrobial assays of Z3-5b-2 at two growth temperatures

REACTION OR TEST	GROWTH AND TEST TEMPERATURE	
	25C	35C
Oxidase	+	-
Simmon's citrate	+	-
Sorbitol, phenol red	+	-
Xylose, phenol red	-	+
Maltose, phenol red	-	+
Lactose	+	-
Urease	+	-
Growth on SS agar	-	+
Motility	+	-
Ampicillin	+	-
Carbenicillin	+	-
Tricarcillin	+	-

The purpose of my research was to determine a mechanism by which these phenotypic changes occurred which had caused the seven isolates to be correctly identified as an Aeromonas species at 25 C and at 35 C identified as some other organism. At least 45 other aeromonads remained true to speciation at 35 C. I considered three possible explanations for this phenomenon: 1.) The DNAs of those Aeromonas species which gain and lose biochemical and antimicrobial characteristics when grown at the two different temperatures in some way were non-homologous, 2.) The DNAs were the same but different sequences of the DNA were coded for at the two temperatures and therefore the RNAs were not the same, and, 3.) The DNAs and RNAs were the same at both temperatures, however at some time during or after translation there was some type of modification of cellular proteins.

MATERIALS AND METHODS

All bacterial cultures were obtained from Dr. R. J. Sobieski, Division of Biological Sciences, Emporia State University.

Chemicals and Reagents

All chemicals and reagents utilized in this study were reagent grade in quality. C^{14} adenine (55.6 mCi/mmol) was obtained from New England Nuclear, Boston, Massachusetts. Protease was obtained from Sigma Chemical Co., deoxyribonuclease from United States Biochemical, and ribonuclease from Worthington Biochemical and Sigma Chemical Co.

Buffers

Listed below are buffers used in nucleic acid isolation and hybridization procedures.

Saline Citrate (SSC)

0.15 M NaCl
0.02 M Na-Citrate

TES

0.05 M TRIS
0.005 M EDTA
0.05 M NaCl
pH 8.0

TKM

0.001 M TRIS
0.001 M KCl
0.001 M $MgCl_2$
pH 7.4

Media

Bacteria were grown in tryptone (0.5%), glucose (0.1%), enriched yeast (0.25%) broth (TGEB) for isolation of DNA and RNA. Bacteria were maintained on tryptone, glucose, enriched yeast agar (1.5%) plates (TGEA). All isolation procedures utilized inoculum cells obtained from

one day old slants.

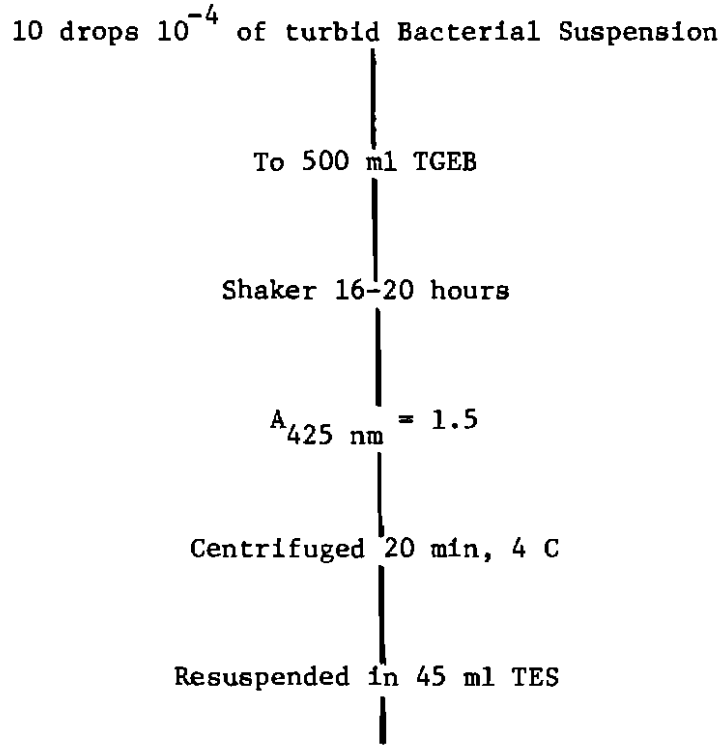
Simmon's citrate was used regularly to insure that cellular phenotypic differences existed at 25 and 37 C.

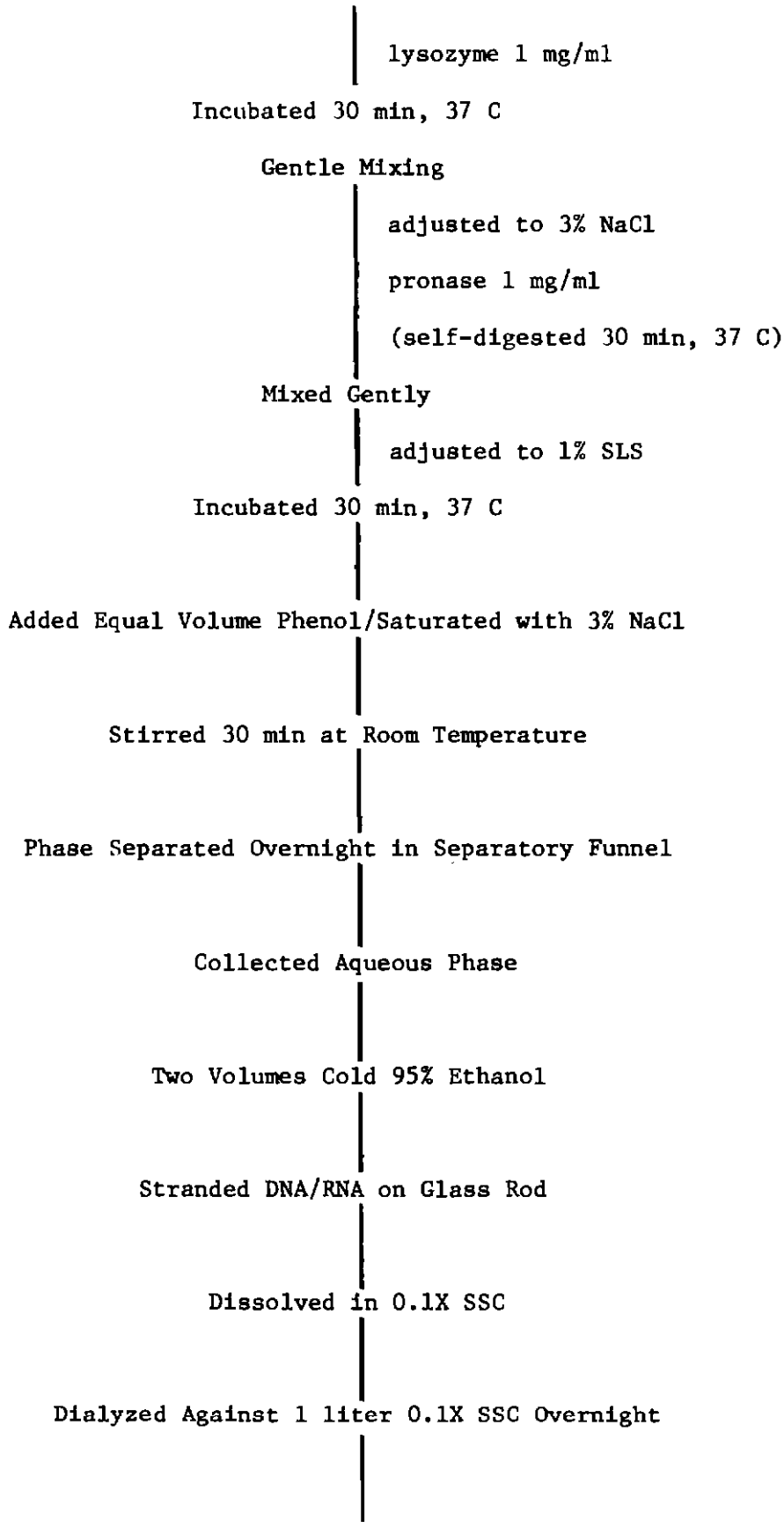
Cell Maintenance

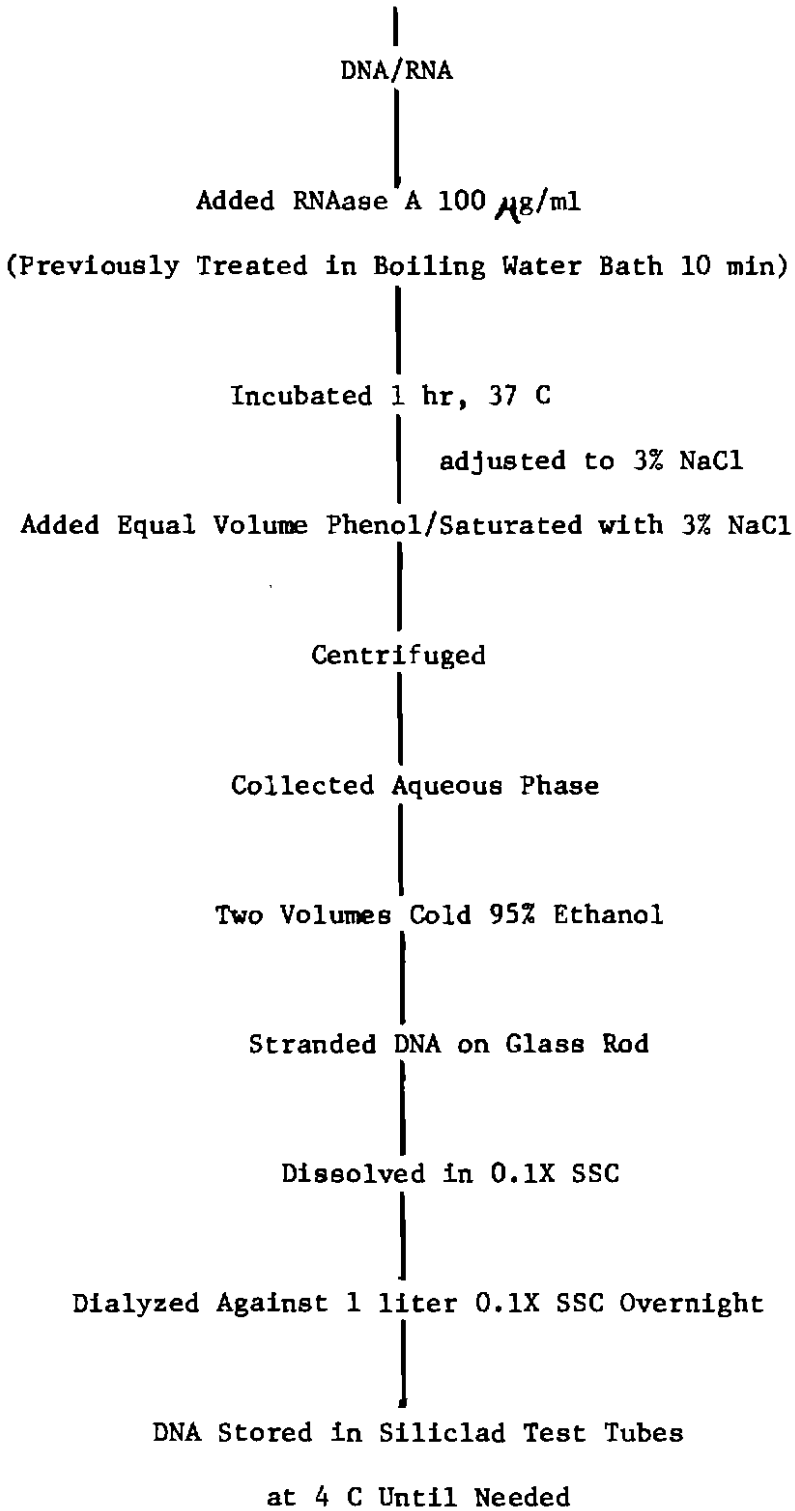
To insure the integrity of the bacteria, the culture was suspended in one milliliter of TGEB until turbid, mixed, lawn streaked on a TGEA plate and incubated at 30 C for one day. Under the isolation hood cells were harvested, suspended in 30 ml TGEB to $A_{425\ nm} = 0.4$ and three ml of sterile glycerol were added. Using a Pasteur pipette 0.3 ml of the broth-glycerol-cell suspension mixture was dispensed into small sterile vials and frozen. As fresh cultures were needed a vial was thawed and dilution streaked for isolated colonies on TGEA.

DNA Isolation

Isolation was by a modification of a technique by Currier and Nester (1976) and is given below.



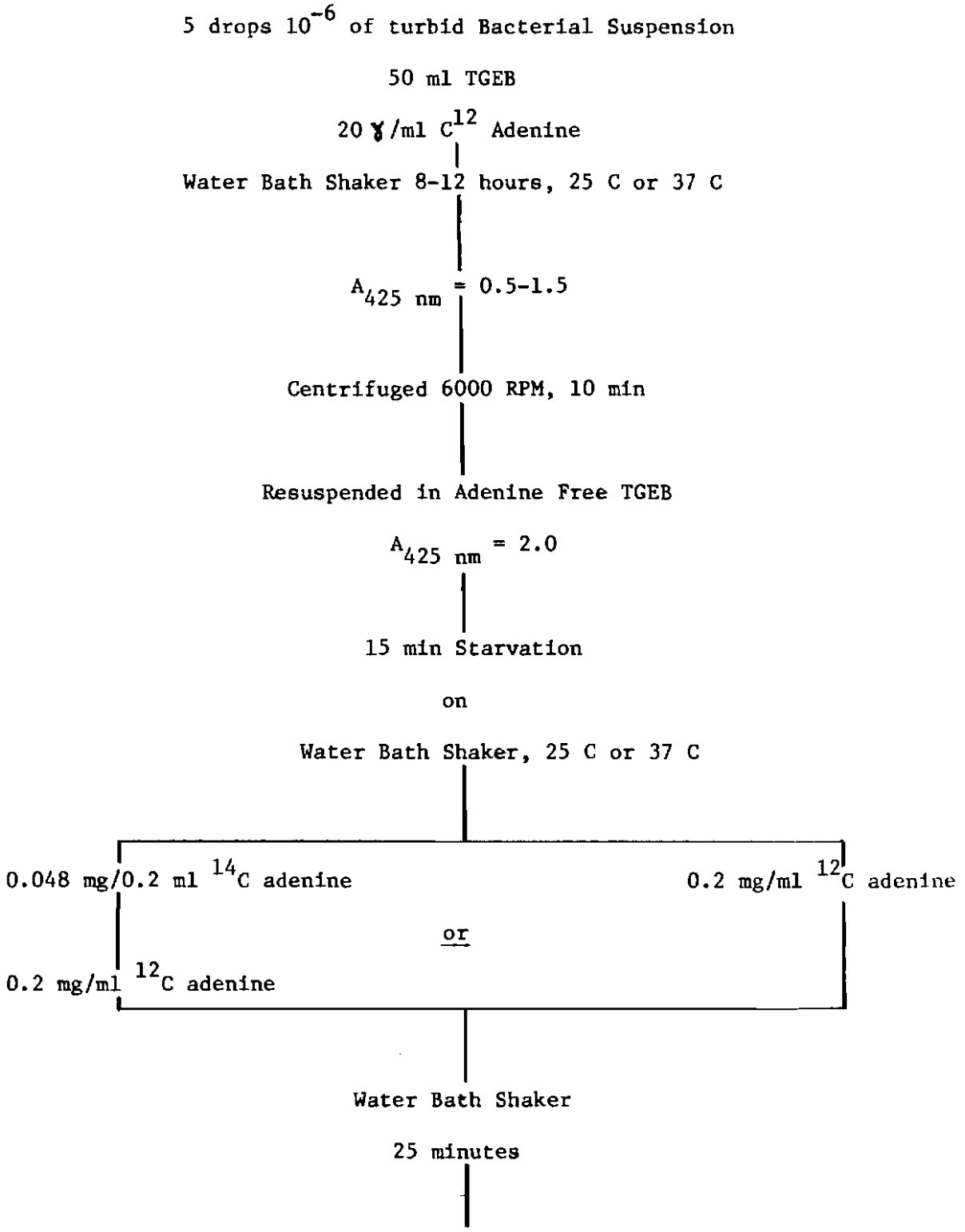

lysozyme 1 mg/ml
Incubated 30 min, 37 C
Gentle Mixing
adjusted to 3% NaCl
pronase 1 mg/ml
(self-digested 30 min, 37 C)
Mixed Gently
adjusted to 1% SLS
Incubated 30 min, 37 C
Added Equal Volume Phenol/Saturated with 3% NaCl
Stirred 30 min at Room Temperature
Phase Separated Overnight in Separatory Funnel
Collected Aqueous Phase
Two Volumes Cold 95% Ethanol
Stranded DNA/RNA on Glass Rod
Dissolved in 0.1X SSC
Dialyzed Against 1 liter 0.1X SSC Overnight

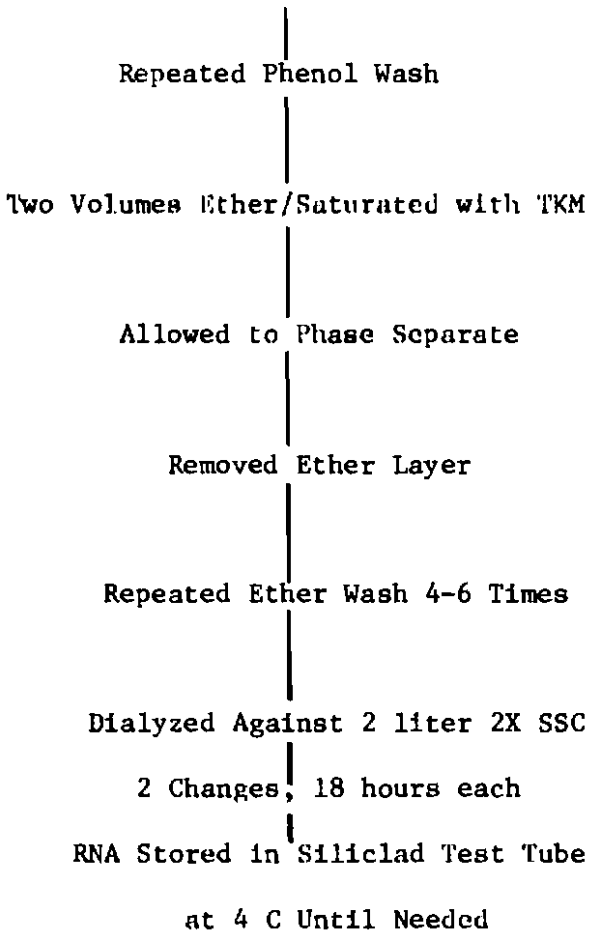


DNA isolated in this manner had a 260/280 nm ratio of 1.6 - 1.9. The typical yield was three mg from four liters of cells.

RNA Isolation

Isolation was by a modification of the technique of Taylor et al. (1967) and is given below.





RNA isolated in this manner had a 260/280 nm ratio of 2.1 to 2.7. The typical yield during RNA isolation was 1.5 mg from 200 ml of cells.

Hybridization

DNA template and hybridization procedures were modified from Gillespie and Spielgemann (1965).

Filter Preparation. Membrane filters (24mm diameter, 0.45 μm pore size) were obtained from Schleicher and Schuell. Two days before use, both sides of the filters were washed with 50 ml of 6X SSC and soaked in 6X SSC at 4 C until used. Filters were handled with toothless forceps to prevent damage.

DNA Template. The desired amount of isolated DNA was pipetted into

siliclad 16 x 125 mm Kimax screw cap test tubes. Enough 0.1X SSC was added for a total volume of one milliliter. Tubes were then placed in a boiling water bath for 20 minutes and quickly placed in an ice water bath in the cold room (4 C). Nine milliliters of cold 2X SSC were added to the tubes and gently mixed. Using a Millipore Microanalysis Filterholder the contents of each tube were filtered onto presoaked membrane filters and test tubes and filter assembly were washed with two milliliters of 6X SSC. Following air drying, filters were placed under vacuum overnight at room temperature. The next day filters were placed in a vacuum oven at 80 C for 2-1/2 hours at 26-28 mm Hg.

Hybridization. For competition hybridization, DNA template filters were placed in siliclad scintillation vials which contained increasing ratios of non-radioactive (cold) RNA to a constant amount of radioactive (hot) RNA. Enough 2X SSC was added to the filter - RNA mixtures for a final volume of one milliliter. Vials were incubated in a 60 C water bath for 22-24 hours, the filters were removed from vials and washed in three changes of 10 ml of 2X SSC for 10 minutes. Each side of the filter was then washed with 10 ml 2X SSC in the filter apparatus. Filtration rates were such that it took eight minutes to filter 10 ml. Filters were placed in siliclad scintillation vials containing two ml 2X SSC and 0.04 mg RNAase and incubated at 25 C for one hour. Following incubation, both sides of the filter were washed with 10 ml of 1X SSC, air dried, and placed in separate vials containing scintillation fluid. Radioactivity of each filter was counted for 10 minutes.

Controls. RNA, DNA and filter membrane blanks were run in each of the hybridization experiments. At no time were there radioactive counts above background.

RESULTS AND DISCUSSION

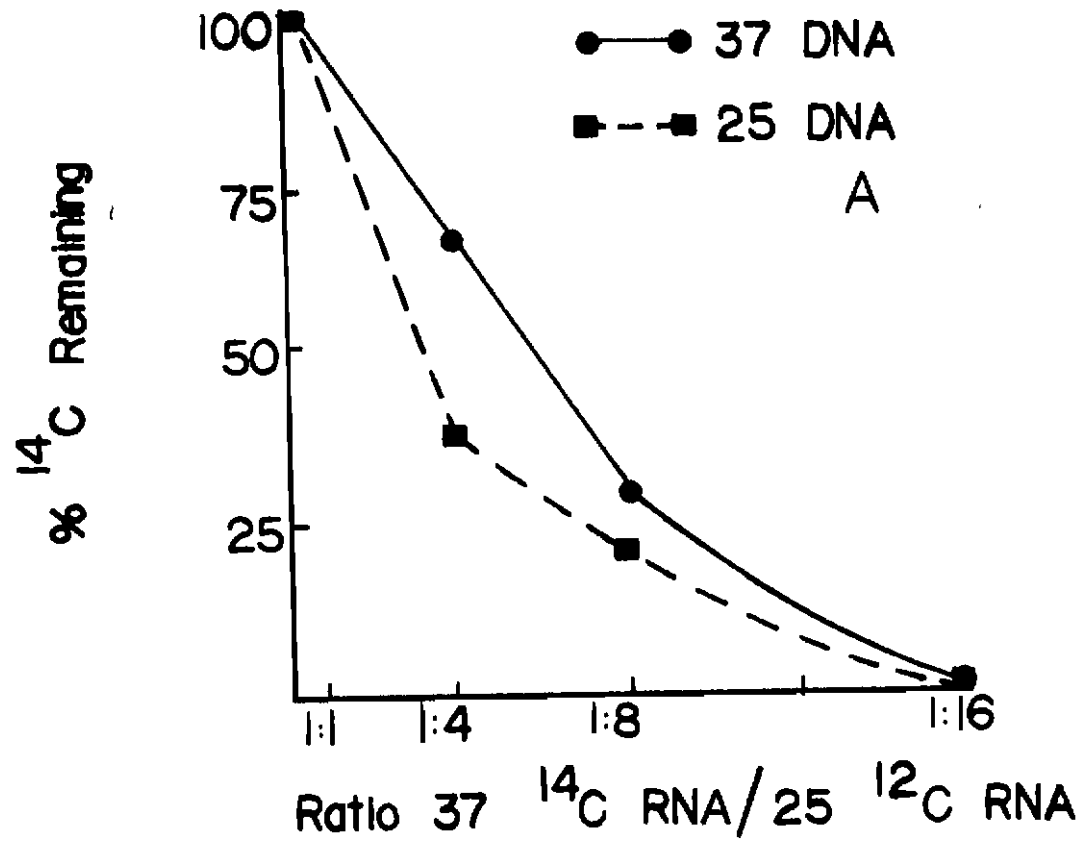
RNA hybridization competition was based on the following logic. The DNA, radioactive (hot) RNA and non-radioactive (cold) RNA isolated from cells grown at 25 C and 37 C were used in competition hybridization experiments. If the increasing amounts of cold RNA are able to compete against a constant amount of hot RNA for DNA sites, the radioactive counts would decrease as the ratios increased indicating there were no differences in the RNAs from the two incubation temperatures.

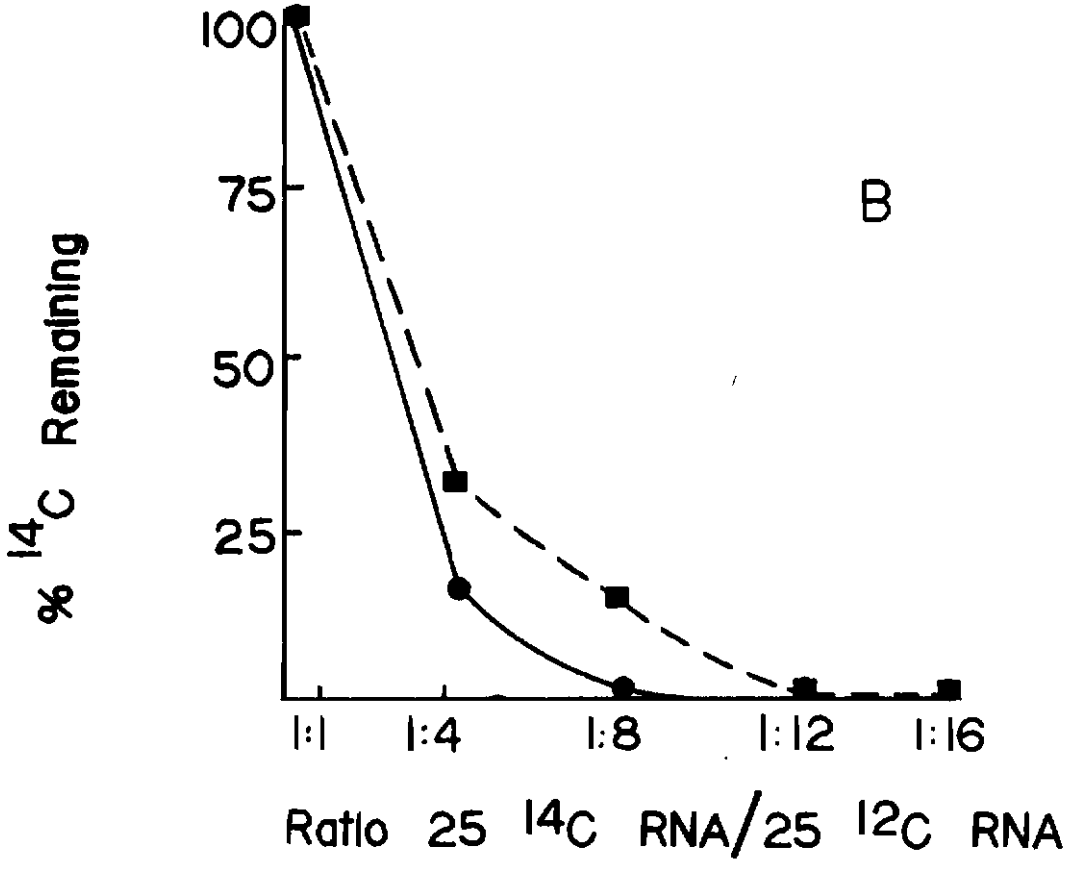
This would occur because the hot and cold RNAs were competing for the same complementary nucleotide sequences on the DNA. However, if the increasing amounts of cold RNA were unable to compete against the radioactive RNA for DNA sites it would indicate that the RNAs made at the two temperatures differed in some of their sequences. This would be illustrated by a leveling of radioactive counts present somewhere above background radioactivity. This lack of competition between RNA made at one temperature and RNA made at the other temperature would establish a major starting point at the molecular level for the phenotypic changes between E. haffnia (identified at 37 C) and this aeromonad (identified at 25 C). The competition between RNAs made at the two temperatures would indicate one of two possible explanations. Either post transcriptional modification of RNA was eliminating some molecular species preventing their translation into protein or there was an activation or inactivation of different proteins. Thus these phenotypic changes would be ribosomal in origin.

Figure 1A illustrates the results when 25 cold RNA competed with 37 hot RNA for complementary sites on 25 and 37 DNA. At a 1:4 ratio the 25 RNA was competing for 30% more of the radioactive counts of the 37

Figure 1A. Competition hybridization between a constant amount of 37 C radioactive RNA vs. increasing amounts of 25 C non-radioactive RNA with 25 and 37 C DNA.

Figure 1B: Competition hybridization between a constant amount of 25 C radioactive RNA vs. increasing amounts of 25 C non-radioactive RNA for sites on 25 and 37 C DNA.


100
75
50
25
% 14C Remaining
37 DNA
25 DNA
A
1:1
1:4
1:8
1:16
Ratio 37 14C RNA/25 12C RNA


100
75
50
25
% 14C Remaining
B
1:1
1:4
1:8
1:12
1:16
Ratio 25 14C RNA/25 12C RNA

hot RNA when using 25 DNA template filters as opposed to 37 DNA. This difference decreased as the ratio increased to less than 5% at 1:8 and by 1:16 the 25 RNA was competing against all the 37 hot RNA regardless of which DNA was being used.

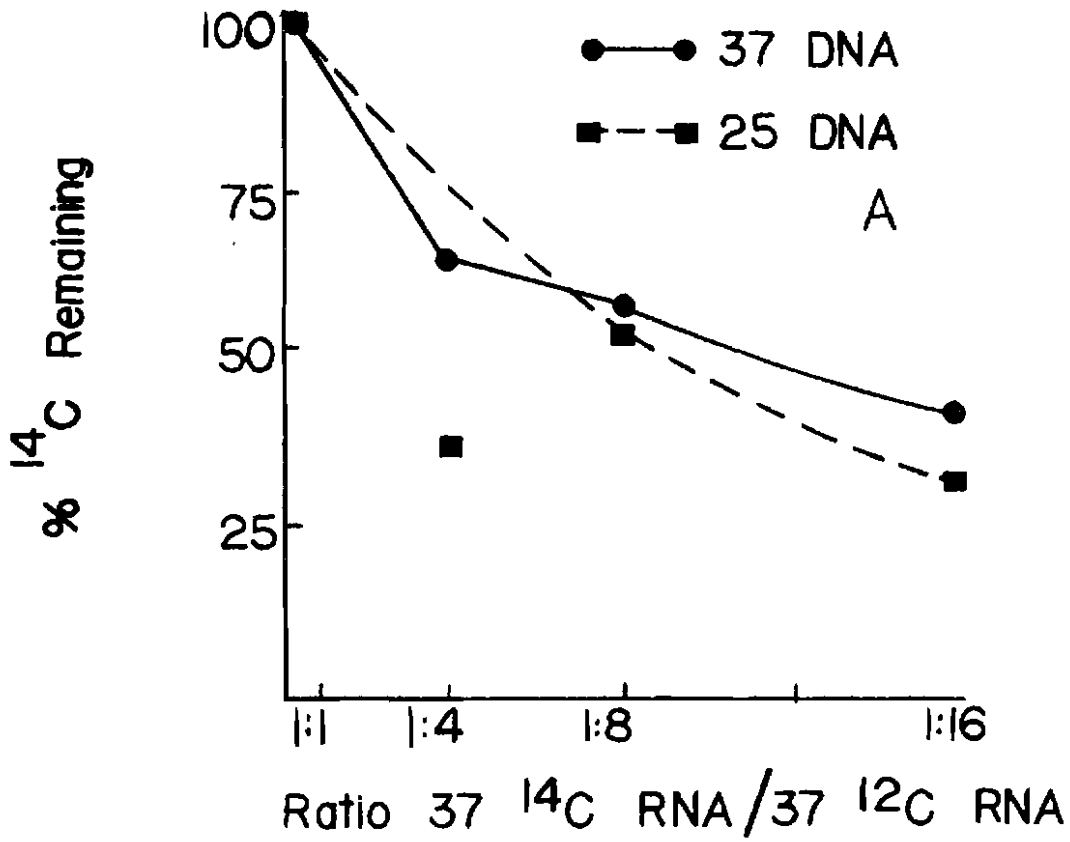
Figure 1B illustrates results of competition hybridization between 25 cold and hot RNA for sites on 25 and 37 DNA. As this figure shows the 25 cold RNA at ratios of 1:12 and greater competed against all the radioactive counts of the 25 hot RNA regardless of whether the DNA was isolated from 25 or 37 grown cells. At this point the results of Figure 1 would lead to the conclusion that the phenotypic differences in this aeromonad were due to either translational or post transcriptional mechanisms.

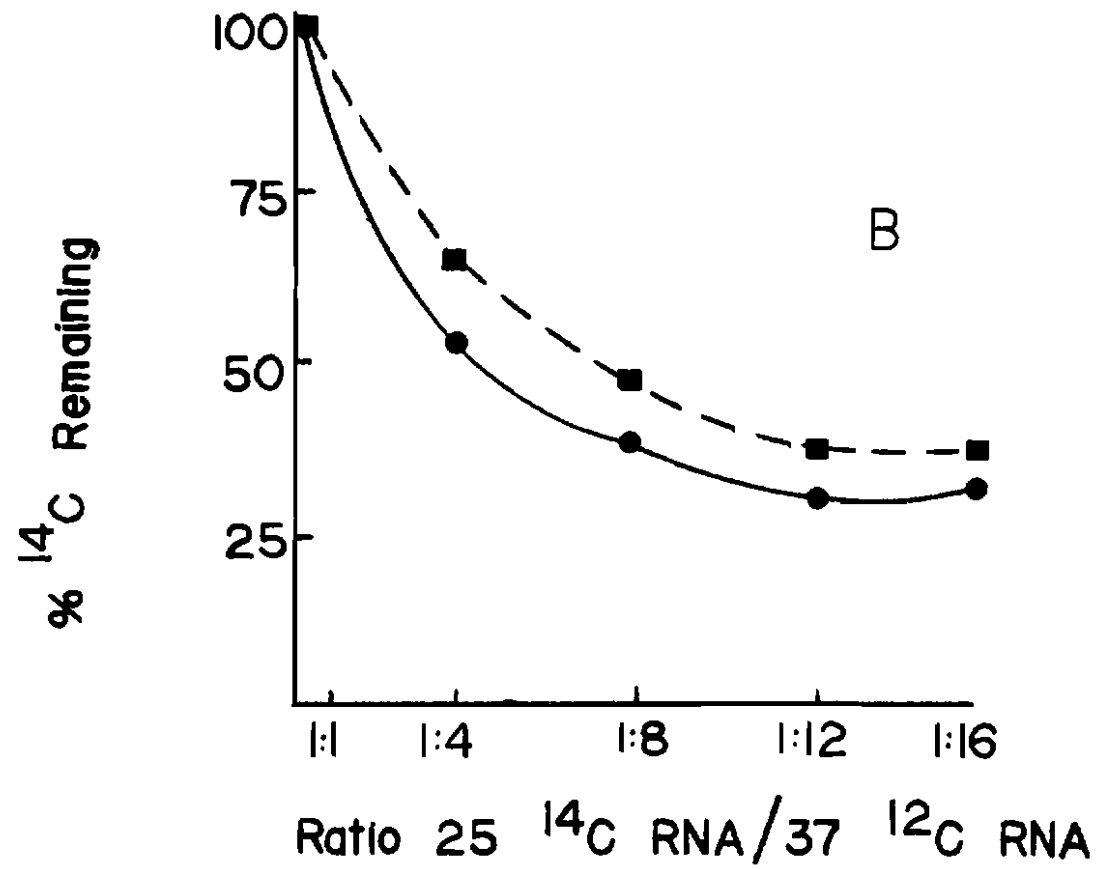
Figure 2 represents the results of 37 cold RNA competing against 37 hot RNA in the "A" part of the figure and against 25 hot RNA in the "B" part. In both experiments 25 and 37 C DNA were used. The inability of 37 cold RNA to compete for 100% of the 37 hot RNA was shown by a stabilization of radioactivity at 33%. From the previous discussion, the fact that the 37 cold RNA was only able to compete for 67% of the radioactive counts in 37 hot RNA indicates that these two RNAs must differ in some manner. It is important to remember however that the two RNAs in this particular experiment were isolated from cells grown at the same temperature and differed only in that one was radioactive and one was not. This apparent discrepancy will be considered in the following pages.

The lower figure illustrates competition between cold 37 RNA and hot 25 RNA. Again the cold 37 RNA was not able to compete against all of the counts in the 25 hot RNA. The leveling of counts was at 30% of

Figure 2A. Competition hybridization between a constant amount of 37 C radioactive RNA vs. increasing amounts of 37 C non-radioactive RNA for sites on 25 and 37 C DNA.

Figure 2B. Competition hybridization between a constant amount of 25 C radioactive RNA vs. increasing amounts of 37 C non-radioactive RNA for sites on 25 and 37 C DNA.


100
75
50
25
37 DNA
25 DNA
A
% 14C Remaining
1:1
1:4
1:8
1:16
Ratio 37 14C RNA/37 12C RNA


100
75
50
25
B
% 14C Remaining
1:1
1:4
1:8
1:12
1:16
Ratio 25 14C RNA/37 12C RNA

input, similar to the results when 37 hot and 37 cold were competed.

Whether 25 or 37 C DNA was used as a template did not affect the experimental outcome in any of the tested combinations. Therefore the phenotypic differences seen were not due to differences in the cellular DNA at the two temperatures (25 and 37 C). Good competition was observed when 25 cold RNA was competed against homologous and heterologous hot RNA (Fig. 1A, B). These results also indicate that there were no differences in the RNAs at the two temperatures. However, when one compares the outcome of 37 hot RNA competed by 25 (Fig. 1A) and 37 cold RNA (Fig. 2A) competition was not identical suggesting that some differences exist between 25 and 37 C RNA. Analysis of these apparently contradictory RNA results has led to the following model which is depicted in Figure 3. At 25 C the mRNA is coded from one strand on the DNA referred to as the "A" strand and its resulting reactions were characteristic of an Aeromonas sp. However at 37 C, mRNA was not only coding from this "A" strand, but temperature in some way affected transcription so that small segments of the complementary DNA strand, the "B" strand, were also read. The reading of different segments at 37 C produced transcripts which when translated, changed the phenotypic expression so that at 37 C its reactions were typical of an Enterobacter species.

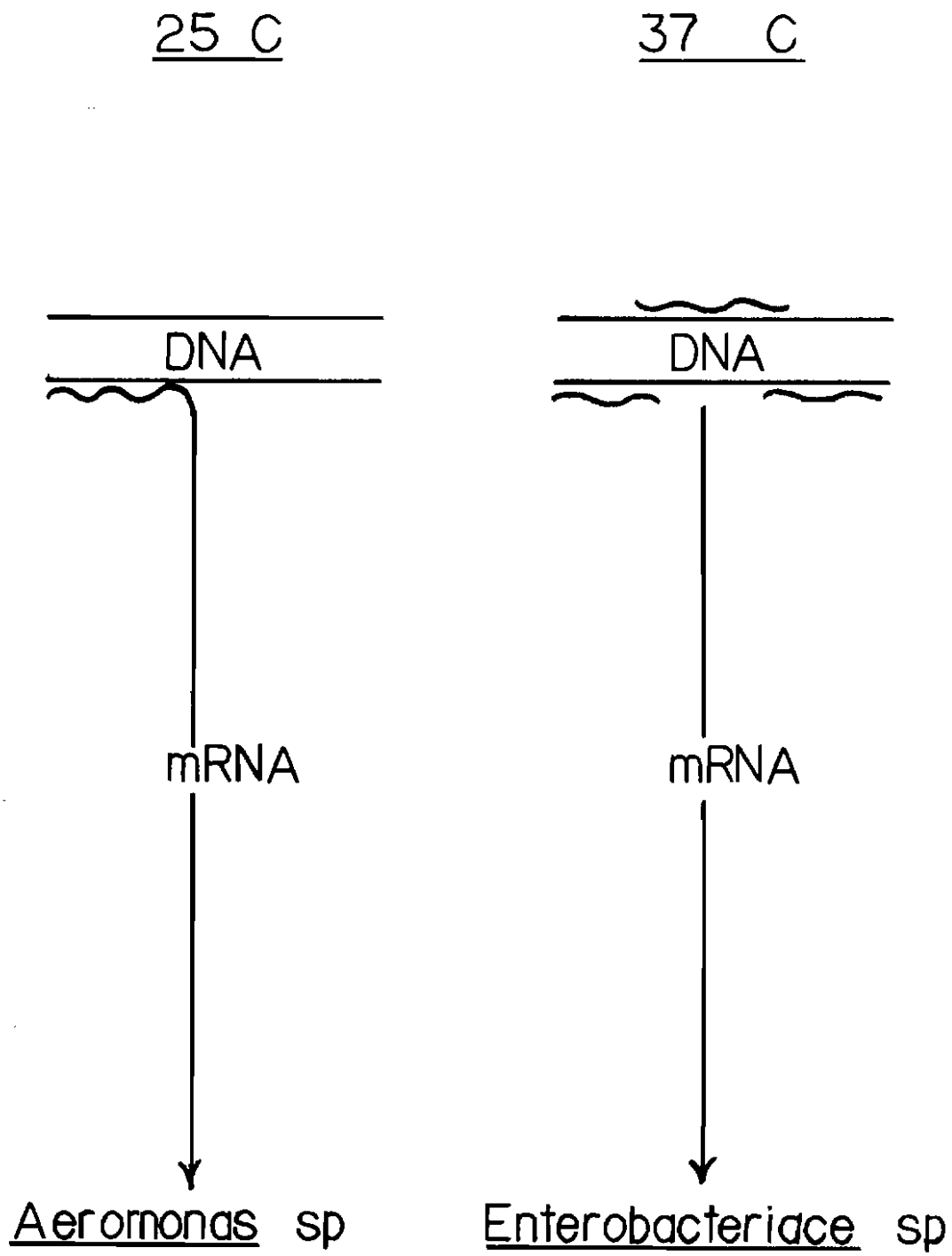
This model accounts for the observed experimental results in the following manner:

1) When 25 C hot and 25 C cold RNA competed (Fig. 1B) there was good competition because only RNA read from the "A" strand of DNA was present to react with the DNA.

2) When 37 C hot and 25 C cold RNA competed (Fig. 1A) the "B"

Figure 3. Proposed model of differences in certain Aeromonas sp. during transcription when grown at two temperatures 25 and 37 C.

MODEL



strand transcripts present only in the 37 hot RNA, were in such low concentration that the excess of 25 cold RNA effectively prevented self annealing of the "A" and "B" transcripts in the hot RNA preparation. Since Figure 1A does indicate complete competition it points out the limits of sensitivity in hybridization systems which are based on total cellular RNA.

3) It was only during those experiments when cold 37 RNA was present in excess concentrations (Fig. 2A, B) that RNA coded from the "B" strand of the DNA was present in sufficient numbers to influence the experimental results. In those instances "B" strand RNA complements were able to anneal with "B" strand DNA as well as to "A" strand RNA transcripts, effectively preventing cold "A" strand transcripts from competing completely against hot RNA "A" strands for sites on the "A" strand of DNA.

Thus the model accounts for the case when 37 hot RNA was tested against 25 cold (Fig. 1A) and against 37 cold (Fig. 2A) RNA. In the first case the low amounts of "B" strand DNA in the hot preparation were not detected, but in the second case self annealing of the large excesses of cold RNA allowed some hot RNA to anneal to template DNA. Likewise in Figure 2B it is seen that 37 cold RNA does not compete completely against 25 hot RNA because self annealing within the cold RNA also eliminates competition for sites on DNA by 25 hot RNA. Hence, only when 37 cold RNA is the competitor can these hybridization differences be detected.

This model was presented on the basis of experimental results in previously published research with both bacteria and viruses.

In the early 1970's molecular biologists believed the genetic

systems of bacteria to be simple and well understood. However, Barrel et al. (1976) published a paper on the genetics of ØX174, a virus which infects E. coli. They had found overlapping genes, which was to say that genes did not always exist as separate entities, as previously believed, but sometimes shared nucleotide base sequences. In ØX174 they reported two different types of overlap, one where only a single nucleotide was shared and another in which the sequence of one gene was completely contained within another gene. Since their work a third gene has been discovered in ØX174 completely encoded within another sequence (Fiddes and Godson, 1979; Smith et al., 1977; Weissbock et al., 1977). Thus the apparent simplicity of one gene coding for one useful RNA was not the hard, fast rule in procaryotic systems that it was once thought to be.

As a result of research on the genome of Simian Virus 40 (SV 40) it was discovered that not only does some overlapping of genes occur but that one strand of DNA was read for early RNA, that initiated viral genome replication and the other strand of the DNA was read to direct the synthesis of structural proteins, that is, late RNA (Reddy et al., 1978). In addition, overlapping here results in shared base sequences between the 5' OH end of one strand of RNA with the 3' OH end of the RNA from the other DNA template. Therefore both strands of the DNA were being read for complete SV 40 replication.

Platt and Yanofsky (1975) reported that gene overlapping already known to occur in viruses, also occurred in the bacterium E. coli. They found that the termination signal of one gene was overlapping the initiation signal of the adjacent gene.

Besides overlapping genes, bacterial genetic systems carry transposons which are structurally and genetically discrete segments of DNA which have the ability to move around the cell's chromosomes and among any extrachromosomal DNA plasmids. Structurally, transposons consist of nucleotide sequences ranging in size from a few to 1,400 nucleotides. Starlinger and Saedler (1965) working with Gal$^-$ mutants of E. coli suggested that this unusual pheontypic characteristic was due to the insertion of additional nucloetides into the normal Gal gene. Since that time E. coli DNA has been found to include three classes of insertion sequences. One of these classes, insertion sequence II, was found by Pilacinski et al. (1977) to exert either a positive or negative effect on nearby genes depending on its orientation. As reported by Cohen and Shapiro (1980) investigators have found that the expression of one Salmonella flagellum gene or its counterpart was dependent on the inversion of a segment of the bacterial chromosome. This inversion occurred only in the absence of certain proteins needed for homologous recombination. Thus bacteria are known to possess elements which can change their location in the chromosome and as a consequence activate new genes and inactivate the transcription of others. In this Aeromonas study this idea supports a temperature dependent transposon being the cause of the changes detected by RNA-DNA hybridization.

The intent of this survey of previous research on the genetic systems of bacterial cells is intended to explore the potential for diversity in the bacterial gene. What once was considered a simple, well understood system is actually a complex, multi-optional system which is still not completely understood.

In order to further examine this phenotypic phenomenon and the suggested model, additional research is needed and two possible experiments are suggested here. The first would be to isolate and radioactively label mRNA from 37 C cells, allow this mRNA to self anneal in hybridization fluid, in the absence of DNA. The mRNA would be treated with the nuclease RNAase which digests only single strands and evaluate for the presence of radioactivity. If the model is correct and mRNA transcripts are self annealing, radioactivity significantly above background, will be present. An approach to determine the specific differences between 25 C and 37 C RNA could utilize the diffusion of different RNA species on polyacrylamide gel electrophoresis. In this procedure the analysis and comparison of the different bands at 25 and 37 C RNAs would show any differences in RNA species between the two different temperatures.

SUMMARY

1.) RNA competition hybridization was not affected by the temperature at which the DNA for the template was isolated (25 or 37 C). Thus the DNAs at the two temperatures are homologous.

2.) The experimental results do not implicate post transcriptional modification as the primary cause of phenotypic differences at the two temperatures (25 and 37 C).

3.) RNA isolated at 25 and 37 C was found to be non-homologous. These differences may be responsible for the initiation of the phenotypic changes previously noted.

LITERATURE CITED

LITERATURE CITED

Barrell, B.G., G.M. Air and C.A. Hutchinson. 1976. Overlapping genes in Bacteriophage ØX174. Nature (London) 264:34-41.

Buchanan, R.E. and N.E. Gibbons. 1971. Bergy's Manual of Determinative Bacteriology. 8th ed. Baltimore, MD: Williams and Wilkins Co. 341-349.

Bulger, R.J. and J.C. Sherris. 1966. The Clinical Significance of Aeromonas hydrophila. Arch. Intern. Med. 118:562-564.

Catsaras, M. and R. Buttiaux. 1965. Les Aeromonas dans les Matieres fecalse humaines. Ann Inst. Pasteur Lille. 16:85-88.

Cohen, S.N. and J.A. Shapiro. 1980. Transposable Genetic Elements. Sci. Am. 242(2):36-45.

Conn, J.R. 1964. Spontaneous Peritonitis and Bacteremia in Laennec's Cirrhosis Caused by Enteric Organisms. Ann. Int. Med. 60:568-580.

Currier, T.C. and E.W. Nester. 1976. Isolation of Covalently Closed Circular DNA of High Molecular Weight from Bacteria. Analy. Biochem. 76:431-441.

Dean, J.M. and R.M. Post. 1967. Fatal Infection with Aeromonas hydrophila in a Patient with Acute Myelogenous Leukemia. Ann. Int. Med. 66:1177-1179.

Ewing, W.H., R. Hugh and J.G. Johnson. 1961. Studies on the Aeromonas group. Atlanta: Public Health Service Communicable Disease Center. 1-8.

Ewing, W.H. and R. Hugh. 1974. Aeromonas. In, Manual of Clinical Microbiology. E.H. Lennete, E.H. Spaulding and J.P. Truant, Eds. American Society of Microbiology, Washington, D.C. 230-237.

Fiddes, J.C. and G.N. Godson. 1979. Evolution of the Three Overlapping Gene Systems in G4 and ØX174. J. Mol. Biol. 133:19-43.

Gilardi, G.L., E. Tonnone and M. Bernbaum. 1970. Unusual Fermentative, Gram-Negative Bacilli isolated from Clinical Specimens. App. Microbiol. 20:156-159.

Gillespie, D. and S. Spiegelman. 1965. A Quantitative Assay for DNA-RNA Hybrids with DNA Immobilized on a Membrane. J. Mol. Biol. 12:829-842.

Kovarik, J.L., L.J. Sides and J.R. Becky. 1973. Fatal Septicemia with Aeromonas hydrophila. Rocky Mountain Med. J. 70:36-38.

Kulp, W.L. and D.G. Borden. 1942. Further Studies on Proteus hydrophilis the Etiological Agent in "Red Leg" Disease of Frogs. J. Bacteriol 44:673-685.

McCarthy, D.H. 1975. Detection of Aeromonas salmonicida Antigen in Diseased Fish Tissue. J. Gen. Microbiol. 88:384-386.

Neilson, A.H. 1978. Occurrence of Aeromonads in Activated Sludge: Isolation of Aeromonas sobia and its possible Confusion with Escherichia coli. J. App. Bacteriol. 44:259-264.

Pilacinski, W., E. Mosharrafa, R. Edmondson, J. Zissler, M. Fiandt, and W. Szybalski. 1977. Insertion Sequences IS2 Associated with int-Constitutive Mutants of Bacteriophage Lambda. Gene 2:61-74.

Platt, T. and C. Yanofsky. 1975. An Intercistronic Region and Ribosome Binding Site in Bacterial messenger RNA. Proc. Natl. Acad. Sci. USA 72(6):2399-2403.

Reddy, V.B., R. Dhar and S.M. Weissman. 1978. Nucleotide Sequence of the Genes for the Simian Virus 40 Proteins VP2 and VP3. J. Biol. Chem. 253:621-630.

Rouf, M.E. and M.M. Rigney. 1971. Growth Temperatures and Temperature Characteristics of Aeromonas. App. Microbiol. 22(4):503-506.

Schubert, R.H.W. 1967. In, The Taxonomy and Nomenclature of the Genus Aeromonas. Kluyver and Van Neil 1936: Part I. Suggestion on the Taxonomy and Nomenclature of the Aerogenic Aeromonas species. Int. J. Syst. Bacteriol. 17:23-27.

Smith, M., N.L. Brown, G.M. Air, B.G. Barrell, A.R. Coulson, C.A. Hutchinson, III, and F. Sanger. 1977. DNA Sequence at the C Termini of the Overlapping Genes A and B in Bacteriophage ØX174. Nature (London) 265:702-705.

Starlinger, P. and H. Saedler. 1976. IS-Elements in Microorganisms. Current Top. Microbiol. Immunol. 75:111.

Taylor, K., Z. Hradecna and W. Szybalski. 1967. Asymmetric Distribution of the Transcribing Regions on the Complimentary Strands of Coliphage Lambda DNA. Proc. Natl. Acad. Sci. 57:1618-1625.

Titcomb, D. 1978. A Local Environmental Survey of Aeromonas Species. Wichita, KS. Wichita State Univ. Thesis 1-57.

VonGraevenitz, A. and A.H. Mensch. 1968. The Genus Aeromonas in Human Bacteriology. New Eng. J. Med. 278:246-249.

VonGraevenitz, A. and L. Zinterhofe. 1970. The Detection of Aeromonas hydrophila in Stool Specimens. Health Laboratory Science 7(3):124-127.

Weissback, P.J., W.E. Borrias, S.A. Langeveld, P.D. Baas, and G.A. VanArkel. 1977. Bacteriophage ØX174: Gene A Overlaps Gene B. Proc. Natl. Acad. Sci. USA 74(6):2504-2508.