Characterization of Bacterial Isolates from an Acidic Soil Environment Using 16S rRNA Phylogeny Techniques

Jerrie Caroline Haney

University Honors 458
Senior Thesis
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UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - PROSPECTUS

Name: Jerrie Caroline Haney

College: Agriculture Department: Microbiology

Faculty Mentor: Dr. Gary Stacey ; Dr. Ray Stapleton

PROJECT TITLE: "The Characterization of Microbial Diversity in an Acidic Soil Sample Using 16s rRNA Phylogeny"

PROJECT DESCRIPTION (Attach not more than one additional page, if necessary):

Please see attached sheet.

Projected completion date: May 1998

Signed: Jerrie Caroline Haney

I have discussed this research proposal with this student and agree to serve in an advisory role, as faculty mentor, and to certify the acceptability of the completed project.

Signed: Raymond P. Stapleton 2-24-98, Faculty Mentor

Date: 2-25-98

Return this completed form to The University Honors Program, F101 Melrose Hall, 974-7875, not later than the beginning of your last year in residence.

Prospectus

There are several steps that one must follow to analyze microorganisms from a molecular standpoint. The purpose of this thesis will be to analyze an acidic soil sample for two sets of data. The first goal is to analyze the portion of the sample that is made up of culturable organisms. These are the microbes that can be grown on laboratory media. The second goal is to examine the portion of the soil sample that cannot be cultured on laboratory media.

To characterize the organisms that can be grown, one must first enrich for them on solid media. The isolated colonies are restreaked several times to make sure that the colony is pure. Once there are well isolated colonies visible, they can be plated again to produce higher numbers of organisms. Next, the DNA is extracted from these isolates. Using polymerase chain reaction (PCR), the gene encoding the 16s rRNA is amplified. These PCR products are run on an agarose gel to confirm that the appropriate DNA product was amplified. After this is verified, the PCR product is ligated into a cloning vector, a plasmid, and then this plasmid is used to transform competent *Escherichia coli* cells. These transformed cells are then plated on selective media. They are allowed to grow overnight and then colonies are selected based on a color screening assay for further culturing and analysis. If a colony is blue after incubation, it has not been transformed. If a colony is white, then it has been transformed. The plasmid is then extracted from the white colonies using a mini-plasmid-preparation technique. The extracted plasmids are restriction digested and then run on a gel. The gel is used to make sure the desired 16s rRNA gene insert fragment is present. After this is verified, the fragment is sequenced using an automated technique. The sequence is then analyzed on the computer for its phylogenic relation to other known and/or sequenced microorganisms.

To characterize organisms that cannot be cultured, DNA is extracted directly from the soil sample. This DNA is then amplified, verified, and analyzed using the same techniques already described. This portion of the work will allow examination and identification of microbes that cannot be grown or studied in the laboratory, but that may be contributing to the ecology of the soil sampling site.

UPDATED PROSPECTUS

There are several steps that one must follow to analyze microorganisms from a molecular standpoint. The purpose of this research will be to analyze acidic soil sample bacterial isolates for their phylogenetic relationships. These microorganisms are readily grown on laboratory media.

To characterize these bacteria, one must first enrich for them on solid media. The isolated colonies are restreaked several times to make sure that the colony is pure. Once there are well isolated visible colonies, they can be plated again to produce higher numbers of organisms. Next the DNA is extracted from the isolates. The gene encoding the 16S ribosomal RNA (rRNA) portion of the ribosome is amplified using polymerase chain reaction (PCR) following the DNA extraction. These PCR products are electrophoresed on an agarose gel to confirm that the appropriate size DNA product was amplified. After this is verified, the PCR product is ligated into a plasmid cloning vector and this plasmid is used to transform competent *Escherichia coli* cells which are then plated on selective media. After growth overnight, colonies are selected based on a color screening assay for further culturing and analysis. If a colony is blue after incubation, it has not been transformed. If a colony is white, then it has been successfully transformed. The plasmid is extracted from the white colonies using a mini-plasmid-preparation technique. The extracted plasmids are restriction digested and the resulting DNA fragments are separated by gel electrophoresis. The gel is used to make sure the desired 16S rRNA gene insert fragment is present. After this is verified, the fragment is sequenced using an automated technique. The sequence is then analyzed on the computer for its phylogenetic relation to other known and/or sequenced microorganisms.

This thesis will utilize major headings consistent with a graduate student thesis in the area of Microbiology at the University of Tennessee, Knoxville.

ABSTRACT

Many soil environments have been altered by industrial processes such as mining and chemical waste dumping. There are many changes in the soils at these sites such as a depressed pH and increased contamination. Many times, the main contaminant present will be polyaromatic hydrocarbons or PAHs. These PAHs can be the harmful byproducts of coal refining or petroleum dumping. Because this problem is becoming more and more widespread, bioremediation methods are becoming very important. For bioremediation to be an option in the acidic soil environment, the bacterial microorganisms present must be better understood. I studied a site at the Westinghouse Savannah River Laboratory in Aiken, South Carolina. There are seven coal-fired plants, the soil pH is 3.0, and there are many different PAHs present. A 16S rRNA gene library was constructed by polymerase chain reaction (PCR) amplification and cloning of isolated cultures obtained from this site. Sequence analysis of this library was used to construct a series of phylogenetic trees. These trees graphically show the relationship of the isolates to known organisms and potentially to each other.

KEY WORDS

16S rRNA; acidic pH; acidophile; coal byproducts; contamination; PAHs; phylogenetic tree

INTRODUCTION

Many natural environments have become contaminated by pollution from human alteration of the Earth's surface. It has been observed that these sites have an altered pH and sometimes hydrocarbon pollutants are present. It is important for researchers to examine these sites and to understand the natural processes occurring which may lead to eventual return of the site to its former neutral pH and non-contaminated state. The site studied for this thesis was in Aiken, South Carolina. The low pH and contamination with PAHs was a result of coal byproduct run-off into the nearby land areas.

Review of Literature

There have been several studies examining the effects of pollution on the natural state of land. For example, in studies conducted at sites impacted by long-term coal storage, it was determined that the major microorganisms populating these sites were iron- and sulfur-oxidizing bacteria (Radway 1988). Another study from the 1990's looked at the bacterial diversity present at these sites and found many unique attributes of the microorganisms existing under these extreme growth conditions (Pizzaro 1996).

After it was established that life can exist at different polluted sites, scientists wanted to examine the possible biochemical potentials of the microbial communities. Because many of these sites are contaminated with hydrocarbons as well as having extreme growth conditions such as low pH, it is important to note findings from other sites such as the Savannah River Laboratory Site. One study of pyrene degradation, proposed that two microorganisms, a bacterium and a fungus, were symbiotically working to degrade the hydrocarbon present at the site examined (Wiesche 1996). This is important because current research from the Savannah River Laboratory Site suggests that

no single microbial isolate is capable of degrading the PAH contaminant present (Stapleton, April 1998).

Into the early 1980's, there was a consensus among scientists that the organisms present on the Earth could be divided into five kingdoms, Monera, Protista, Fungi, Plantae, and Animalia (Whittaker 1969). The early classification scheme is shown in Figure 1. This view began to change as the power of molecular techniques became more widely recognized. Scientists began to use the 16S rRNA gene to analyze microbial classification taxonomy and classification. A two-dimensional representation of the structure of 16S rRNA can be seen in Figure 2 (NCBI). This new research led scientists to change their ideas about the kingdoms of life.

Figure 3 shows how the molecular techniques separated the living organisms on Earth. It became evident that there were three domains of life -- Eukarya, Bacteria, Archaea -- into which all organisms fit (Pace 1996). These three domains are further subdivided into kingdoms and then further into phyla. There have already been several phyla studied and characterized. Now, there seems to be a new proposed phylum that describes many of the microorganisms found in extreme acidic environments. These organisms are grouped into a phylum called *Holophaga/ Acidobacterium* (Ludwig 1997).

The new phylum can be seen in Figure 4. It is characterized by the varied habitats that are occupied by its members. The ability of these microorganisms to exist at low pH and the greatly uncharacterized biochemical potential of representatives are other characteristics. The samples that fall into this new phylum were obtained from many different habitats such as snow drifts, marine sediment, and also soils (Ludwig 1997).

Kingdom	Characteristics	Number of Phyla	Representative Organisms
I. Monera	Prokaryotic; no nuclear membrane; no mitochondria; single circular chromosome; binary fission; photoheterotrophic or photoautotrophic; unicellular; filamentous; mycelial	14	Bacteria; myxobacteria; actinomycetes; cyanobacteria (blue-green algae)
II. Protista	Eukaryotic; nuclear membrane; more than one chromosome; hetero-trophic or photoautotrophic; mitotic division; uni- or multi-cellular; cilia; plastids; mitochondria	3	Protozoans; mycetozoans (slime molds); brown algae; red algae; green algae; hypochytrids; oomycetes; chytrids
III. Fungi	Absorptive nutrition; unicellular or mycelial; haploid or dikaryotic; cell walls contain chitin; have lysine pathway	2	Zygomycota; Dikaryomycota (Ascomycota); Basidiomycota; lichens
IV. Plantae	Photoautotrophic; highly differentiated; diploid phase; DAP lysine pathway; develop from nonblastular embryo	9	Liverworts; mosses; ferns; conifers; seed plants; etc.
V. Animalia	Heterotrophic; multicellular; diploid blastula	32	Coelenterates; flatworms; mollusks; insects; reptiles; birds; mammals

Figure 1: Adapted Five-Kingdom Classification of Life

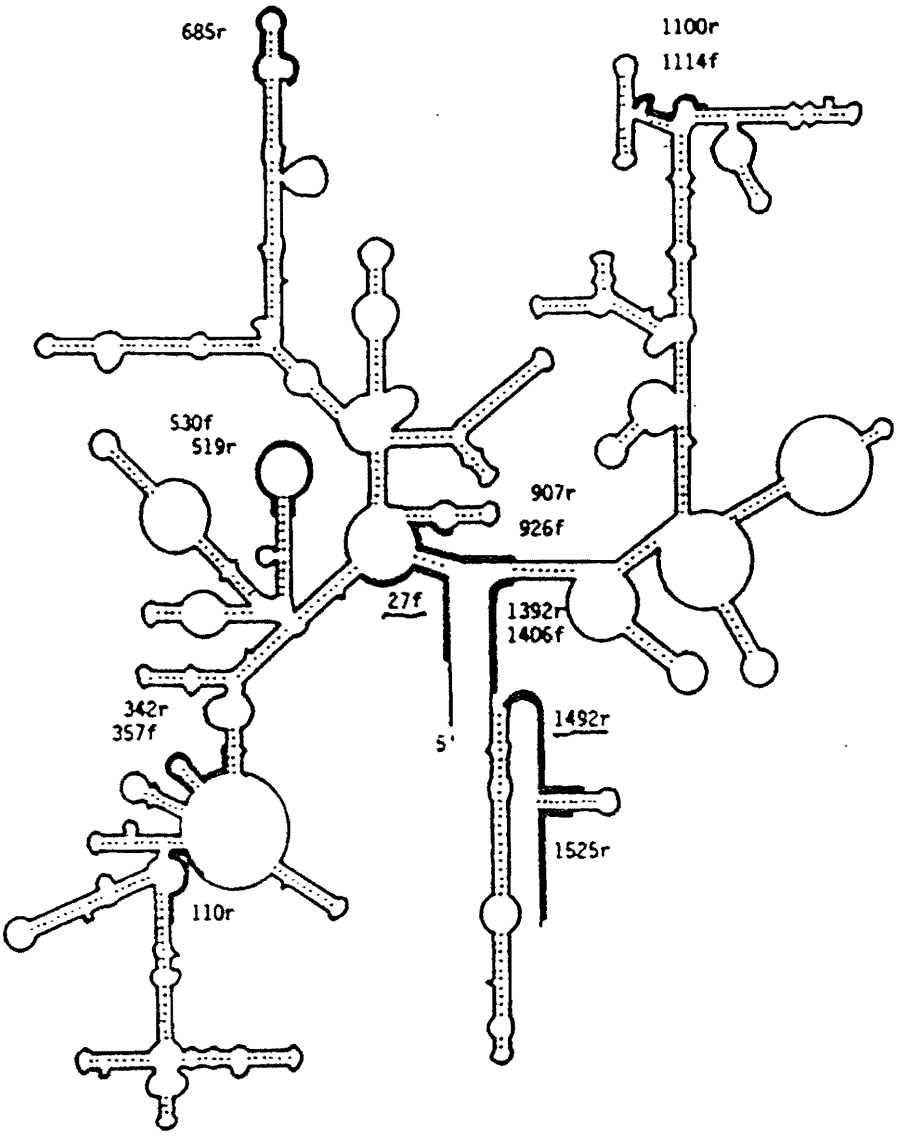


Figure 2: Adapted Two-Dimensional Structure of 16S rRNA

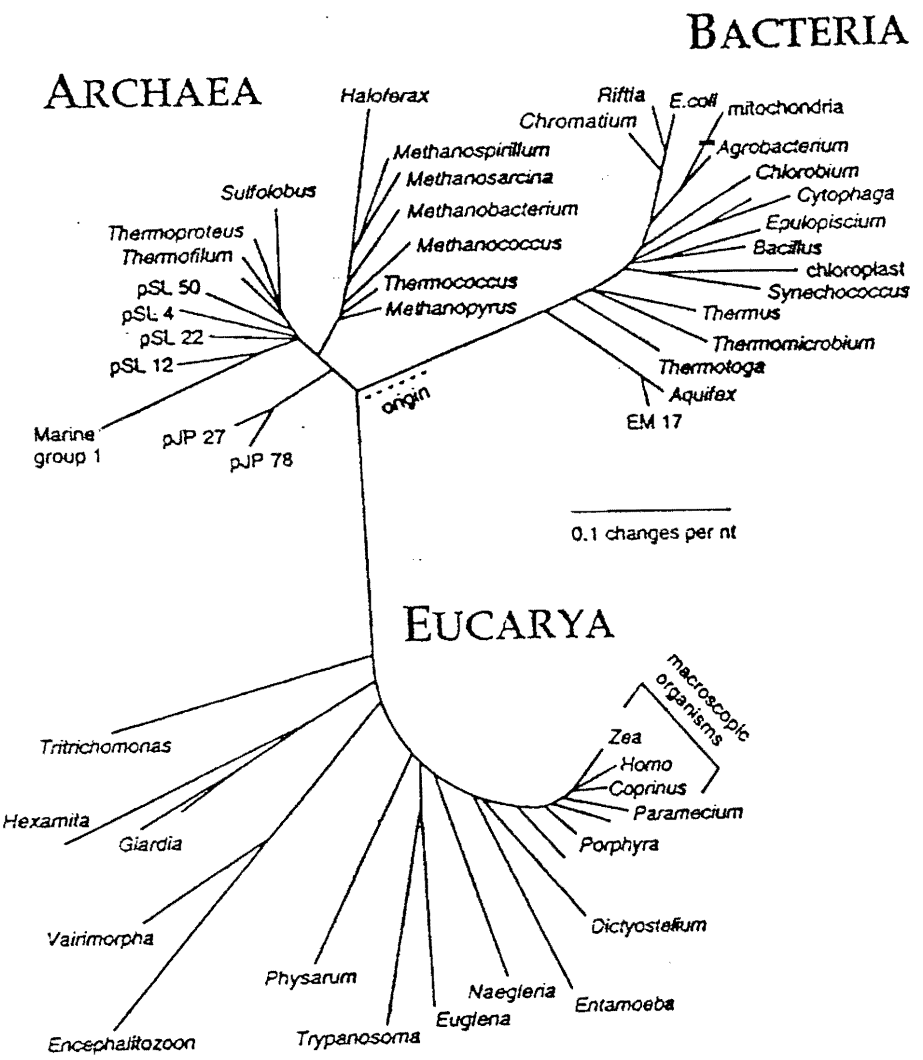


Figure 3: Adapted Three-Domain Tree of Life

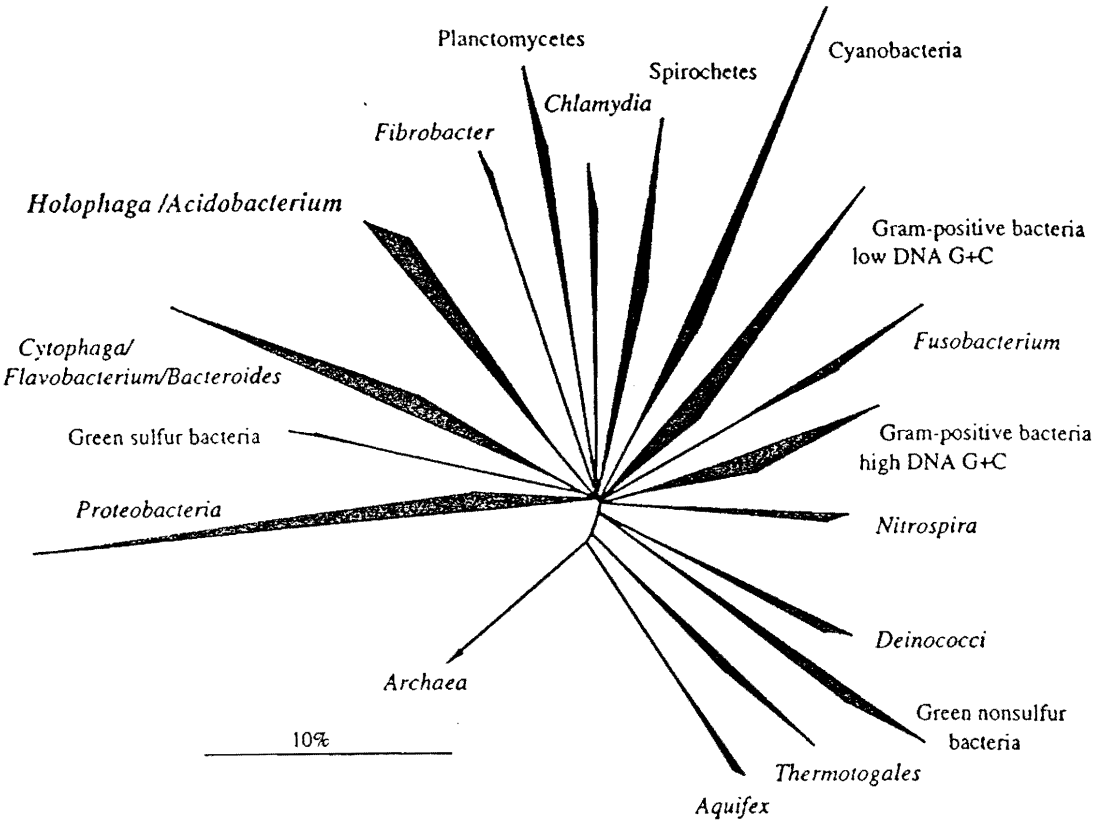


Figure 4: Adapted Bacterial Phyla Showing New *Holophaga/ Acidobacterium* Branch

Other characteristics of this new phylum are likely to be discovered through ongoing research efforts.

Purpose Statement

The work completed for this thesis is one part of a larger study. To understand how the results of this work are useful, it is necessary to provide a greater context for evaluation. Dr. Raymond Stapleton conducted a study of the Savannah River Laboratory Site that examined PAH degradation in extremely acidic environments while working for the Center for Environmental Biotechnology at the University of Tennessee, Knoxville. His research determined the following things: 1) PAH degradation does occur in extremely acidic environments, 2) no microbial isolate alone could not degrade any PAH, and 3) these findings suggest that a consortia of microorganisms are responsible for the PAH degradation. This work is reported in an unpublished paper that has been submitted (Stapleton, April 1998).

To further examine and characterize the diversity of the microorganisms at the Savannah River Laboratory Site, the researcher completed a 16S rRNA phylogenetic study. This thesis focuses on characterization of the bacterial isolates recovered in the previous study completed by Dr. Stapleton. This study will hopefully provide insight into the microorganisms in the PAH degrading consortia and will also show which phyla are capable of existence at other sites like the one examined in this thesis.

The purpose of this thesis shall be threefold. First, the researcher will acquire a suite of isolated microorganisms from a natural sample obtained from the Aiken, South Carolina Savannah River Laboratory Site that are acidophillic and, when in a consortia,

may be capable of degrading contaminant hydrocarbons, PAHs. Secondly, the researcher will phylogenetically describe the isolates using computer analysis of partial sequence data obtained from the 16S rRNA gene recovered from DNA extraction of pure cultures, PCR amplification, vector cloning, and automated sequencing. Thirdly, a determination of the phylogenetic diversity of the microorganisms found at the site will be made.

Hypothesis

This thesis will attempt to support the following three part hypothesis:

1. Isolates from the sample site will be acidophiles and will grow best on acidic media in the laboratory;
2. DNA (i.e. 16S rRNA gene) recovery will describe different microorganisms having similar physiological characteristics and capabilities;
3. There will be some diversity in the isolates, but a majority of them will group into the *Holophaga/ Acidobacterium* phylum.

MATERIALS AND METHODS

Organisms were isolated from the Savannah River Site in Aiken, South Carolina soil samples by enrichment plate methods. This work was part of a larger study and was completed by Dr. Raymond Stapleton. DNA was extracted from pure cultures and the 16S rRNA gene was PCR amplified (Lane 1991). The gene fragment was ligated into a pCR II vector (Invitrogen, San Diego, CA) and transformed into *Escherichia coli.* Colonies were selected for further analysis by a color screen assay on selective media. The cloned vector was removed, digested, and electrophoresed on a one percent agarose gel to verify the 16S rRNA gene insert was present (Holmes, et al 1981). Positive microorganisms were subcultured. Large scale plasmid preparation was performed and the plasmid digested to verify the insert was still present after subculture (Promega 1992). Positive preparations were sequenced in the University of Tennessee Biological Resource Facility using an ABI sequencer. All sequence data was computer analyzed. It was searched against the National Center for Biotechnology Information (NCBI) database using a basic local alignment search tool (BLAST) program to determine and identify relatedness to other sequenced microorganisms (Altschul 1990). Partial sequences were then used to construct phylogenetic tress in the ribosomal database project (RDP). Figure 5 graphically represents this process.

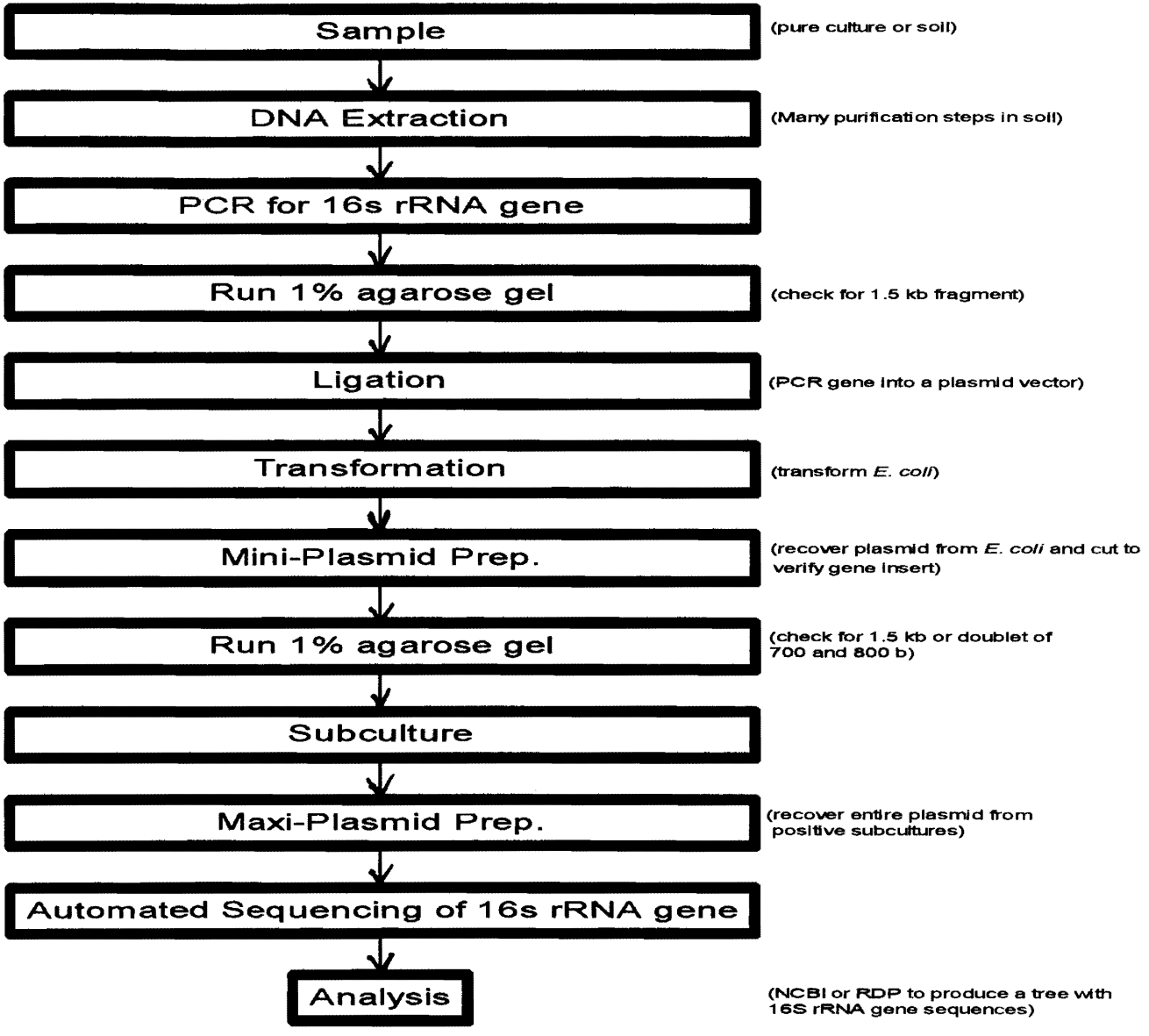


Figure 5: Materials and Methods Flow Chart

RESULTS

The laboratory work conducted for this thesis was successful in most regards and resulted in the accumulation of useful data. Microorganisms were isolated from the acidic Savannah River Laboratory Site in Aiken, South Carolina. DNA was extracted from the pure cultures and the 16S rRNA gene was PCR amplified. The gene was cloned into *Escherichia coli* using a pCR II vector (Invitrogen, San Diego, CA). The vector was recovered and sequenced in an ABI sequencer. Partial sequence data for three isolates was obtained. A computer analysis of these sequences was conducted using the NCBIs BLAST program. Identity to known organisms was determined. Table 1 and Table 2 show the data obtained. The partial sequence data for the three organisms was also used to construct phylogenetic trees using the suggest tree program available through the RDP computer database. Figures 6, 7, and 8 show the results of this program for each partial sequence.

ISOLATE	PHYSIOLOGICAL CHARACTERISTIC
SRS1	Acidophile
SRS2	Acidophile
SRS4	Neutrophile

Table 1: Isolates Used in the Study

ISOLATE	SEQUENCING PRIMER	CLOSEST DATABASE MATCH	% SIMILARITY
SRS1	27f	*Acidiphilium facilis*	96
		Acidiphilium aminolytica	95
SRS2	27f	*Acidiphilium aminolytica*	97
		Acidiphilium facilis	95
SRS4	27f	*Xylella fastidiosa*	89
		Xylella albilineans	88

** 27f Primer sequence is the following: 5′ AGAGTTTGATCMTGGCTCAG... **

Table 2: 16S rRNA Partial Gene Sequence Analysis Using a Computer Assisted Methodology and Database

Figure 6: Phylogenetic Comparison of SRS1 with Closely Related Microorganisms

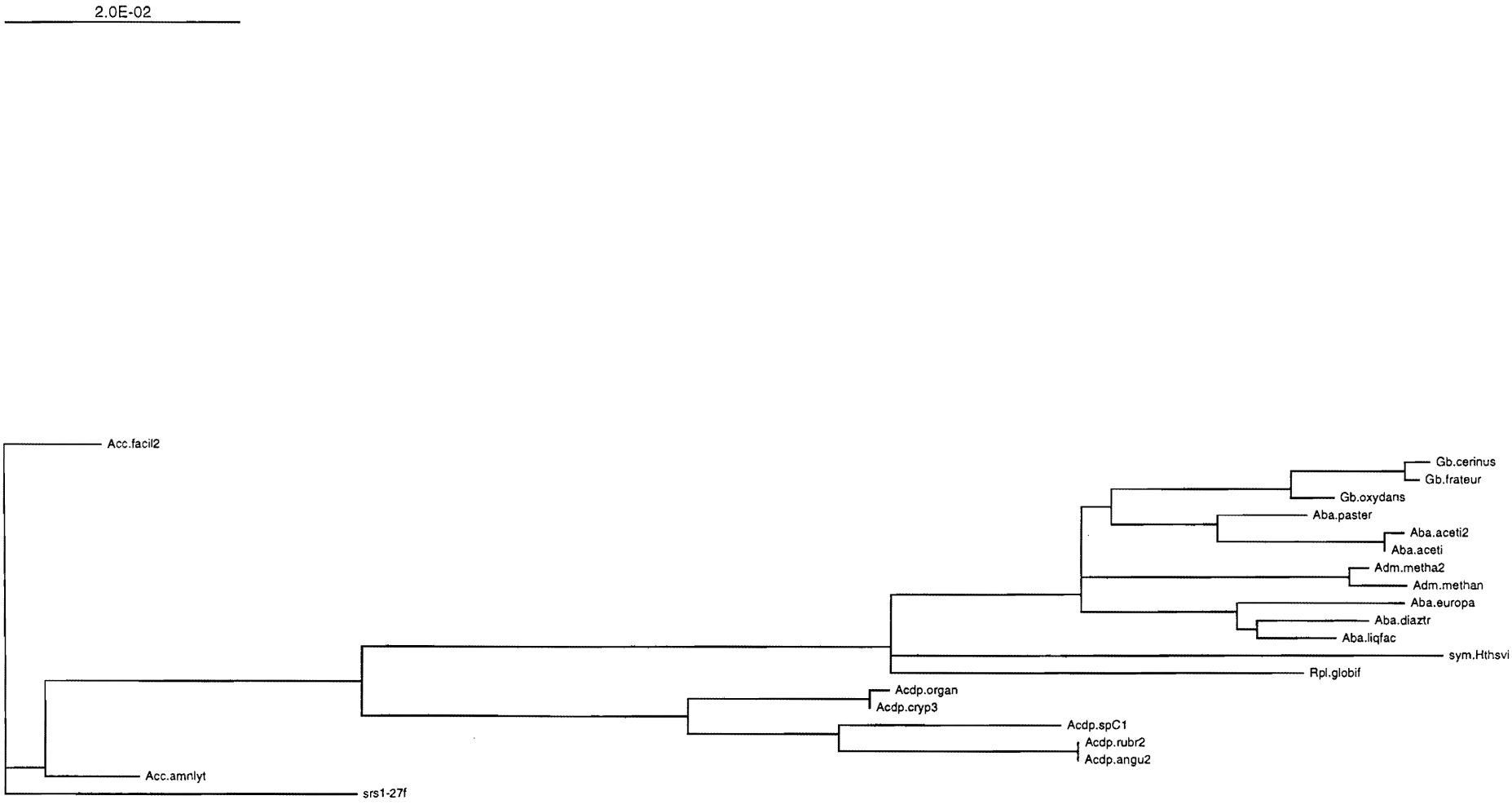


Figure 7: Phylogenetic Comparison of SRS2 with Closely Related Microorganisms

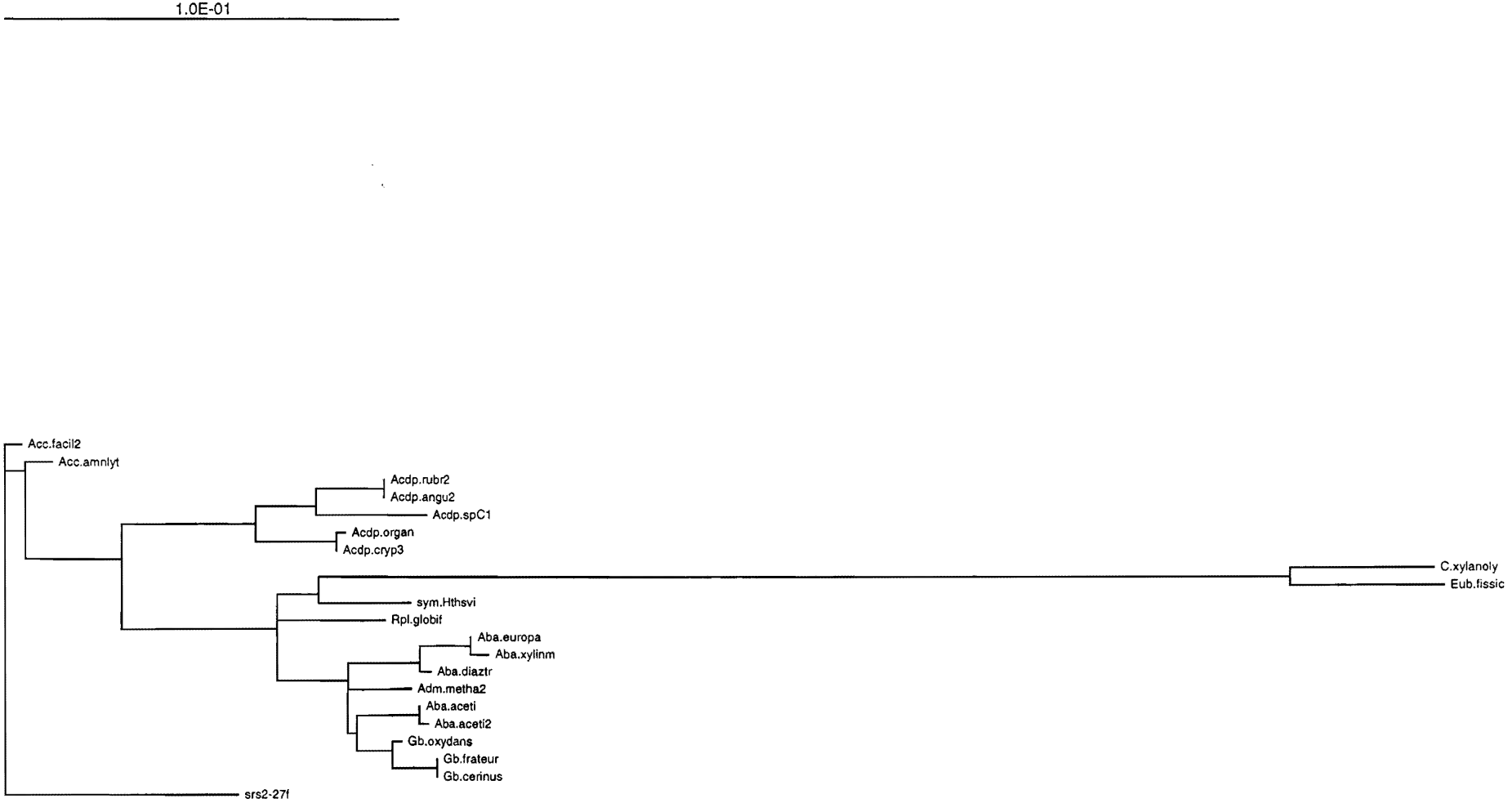
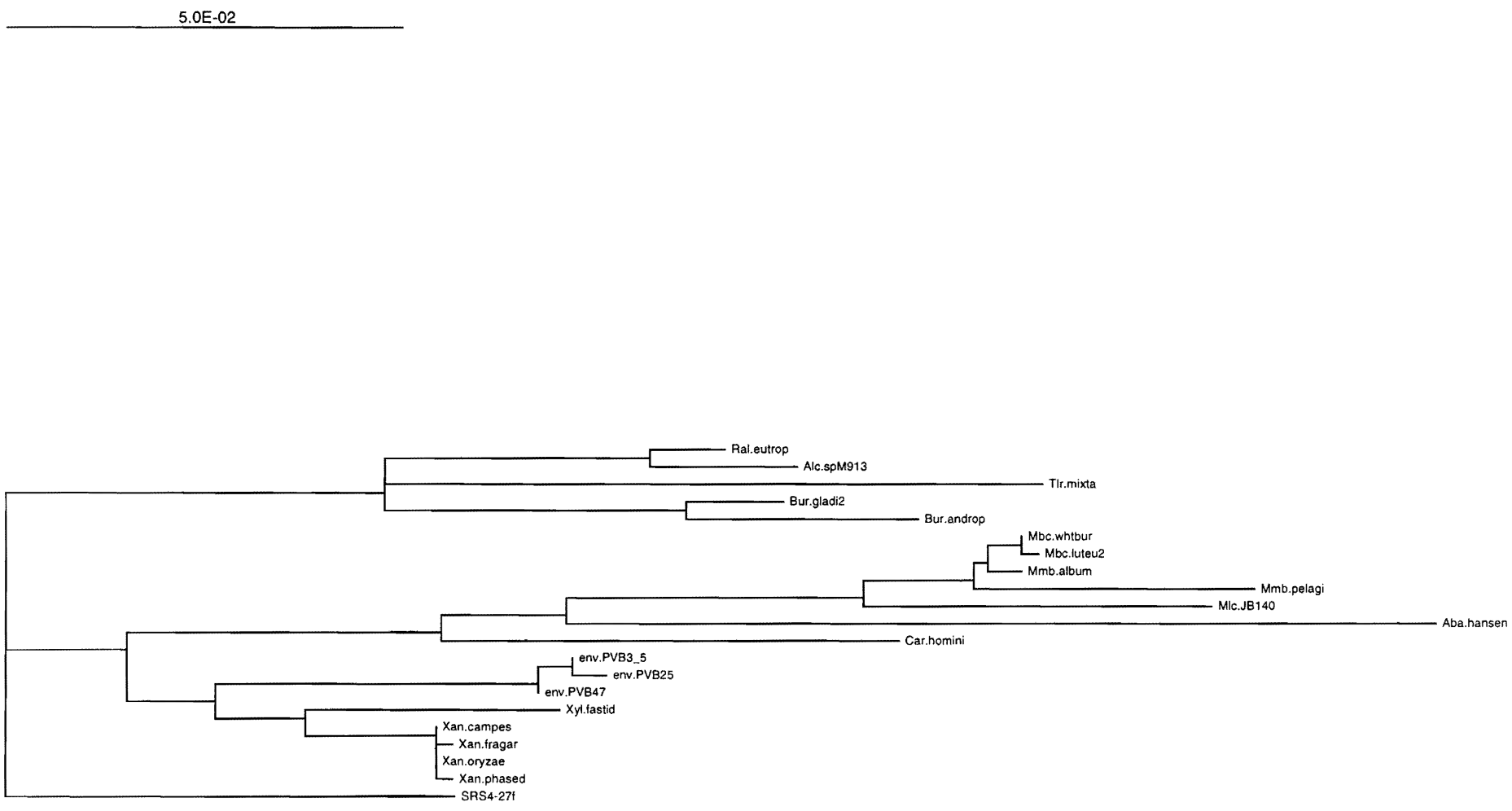


Figure 8: Phylogenetic Comparison of SRS4 with Closely Related Microorganisms



DISCUSSION

The results obtained from the molecular study of acidophilic bacteria conducted for this thesis, support partially all three parts of the hypothesis. The researcher was able to show that two of the three bacterial isolates acquired from the larger study grow best on acidic media in the laboratory. Thus all were not acidophiles. One organism, SRS4, grew best at neutral pH of 7.0 and grouped with the neutrophiles (Wells 1987). The second part of the hypothesis was also supported. All of the isolates could grow on acidic media, even if the isolate was not an acidophilic microorganism. The isolates also shared other common physiological characteristics like a very slow growth rate. The DNA recovery and analysis showed that these microorganisms were, however, different. The last part of the hypothesis stated that a majority of the isolates would group into the new phyla described by Ludwig, et al in 1997. This was supported because two of the three isolates studied were identified by computer analysis to belong to this branch of bacteria.

SRS1 and SRS2 were identified by the BLAST search as members of the *Acidophilium* genus. They are also a part of the phylum *Holophaga/ Acidobacterium* (Ludwig 1997). This was an expected result because the microorganisms in this phylum are widespread, live in acidic pH environments, and have much uncharacterized biochemical potential. The microorganisms recovered and studied in this thesis may be determined to be novel members of the genus and phylum after the complete 16S rRNA sequence is determined.

SRS4 was identified to be a member of the *Xylella* genus. This genus was described in a 1987 paper by Wells, et al as having the following characteristics: gram-negative; Eubacteria; Proteobacteria; gamma subdivision; *Lysobactera Xanthomonas*

group. This was a surprise because this microorganism is a neutrophile and grows best at neutral pH. After further consideration of this surprise, the researcher determined that the presence of this microorganism fit the known data. The *Xylella* representative may be part of the PAH degradation consortia and may exist because the pH of its microenvironment is kept at a neutral pH rather than the macroenvironmental acidic pH of 3.0. Not a lot is known about *Xylella* as a genus other than the characteristics already reported. In future work, it will be useful to obtain the entire 16S rRNA sequence and to further examine its biochemical potential to determine if it is part of the consortia.

Conclusions

This study has conclusively shown that microorganisms can live and flourish at very acidic pHs of 3.0 and below. Further, it has helped to elucidate and characterize a specific group of these microorganisms. The representative members of the microbial community that this work studied were determined to be related to genera that are already known and characterized using computer analysis.

This thesis has only begun to characterize the true diversity of the extremely acidic site. It has proven the robustness of the 16S rRNA molecular phylogeny techniques to identify members of complex microbial communities existing under these conditions. Future research should be targeted at finding ways to use the members of the acidophilic phyla to return the site to its former condition of neutral pH and no PAH contamination.

ACKNOWLEDGMENTS

I would especially like to thank Dr. Gary Stacey and Dr. Raymond Stapleton, my faculty mentors. I would also like to thank everyone else in the Center for Environmental Biotechnology at the University of Tennessee. They have been a constant resource of help, support, and guidance on this project.

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APPENDIX

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DNA Extraction from Pure Culture

2-11-98

Procedure:

1. resuspend frozen pure culture pellet in 10mL TE buffer
2. Treat w/ 1mL lysozyme solution (10mg/mL TE) at 60° for 1
3. add 1mL 5% SDS to lyse cells for 5min at 37°C
4. add 100µL of proteinase K solution (10mg/mL TE buffer) at 37°C for 15-20min
5. add 2.4mL of 5M sodium perchlorate; Shake vigorously
6. add ~7mL of phenol and ~7mL of chloroform. shake to mix
7. centrifuge at 10000rpm for 7min
8. take off aqueous layer and transfer to a new tube (top layer in this case)
9. add ~13mL chloroform, mix
10. centrifuge at 10000 rpm for ~7min
11. transfer aqueous layer to a new tube (top layer again)
12. add approximately 30mL of cold pure ethanol to recovered aqueous layer. mix well. freeze and allow to precipitate overnight

- we worked w/ 4 different pure culture samples:
  1 methanotroph, 1 from soil site, 2 others

Finish DNA Extraction / PCR Set up / pour gels (1%) 2-12-98

13. Spin tubes at 10,000 rpm for ~25 minutes
14. Vacuum & heat dry tubes for 15 minutes
15. rinse DNA from tubes with 100 to 200 µL of Sterile water and put in new PCR tubes

PCR procedure:

1. dilute DNA from soil sample (Downstream SRS) ②
   a. add 9 µL of Sterile H_2O to 5 PCR tubes
   b. add 1 µL of sample to 1st tube
   c. dilute 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}
2. prepare stock PCR reaction mixture for 10 RXN tubes:
   - 25 µl primer 1 27f (2.5 µl)
   - 25 µl primer 2 1492r (2.5 µl)
   - 20 µl dATP (2.0 µl)
   - 20 µl dTTP (2.0 µl)
   - 20 µl dCTP (2.0 µl)
   - 20 µl dGTP (2.0 µl)
   - 100 µl 10X PCR buffer from kit (1.5 mM $MgCl_2$) (10 µl)
   - 760 µl Sterile dH_2O (76.0 µl)
3. add 99 µl of stock reaction mixture to each PCR tube; one tube will be a control (*)
4. add 1 µl of each template: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}; ~~#6~~ ~~#10~~ SRS4, A1, A2
5. boil the tubes for 5 minutes — this breaks down 2° & 3° structures
6. add .5 µl of Taq polymerase
7. put in PCR machine overnight

   (94° 1 min
   42° 1 min
   72° 2 min) 38 cycles
   10 min final extension

Gel procedure:

1. put 100 mL of TBE buffer in flask
2. add 1 g of agarose
3. microwave until clear
4. add 4 µl (2 µl per gel) to flask
5. Pour 2 50 mL gels and chill; cover w/ Aluminum foil

Run PCR products on gel (1% Agarose)

1. set up gels w/ 1% TBE buffer
2. make 1kb ladder for comparisons:
   - 20µL of ladder (1µg/µL)
   - 60µL of loading dye
   - 40µL of universal buffer (10x)
   - 280µL of d H_2O

   400µL total
3. mix 2.5µL of loading dye w/ 20µL of PCR product for each sample on parafilm
4. for gel 1 load 1kb ladder in lane 1; for gel 2 load 1kb ladder in lane 2 — helps tell them apart
5. Gel 1:

Lane	Sample
1	1kb ladder
2	A1
3	A2
4	SRS4

6. Gel 2:

Lane	Sample
1	10^{-5}
2	1kb ladder
3	10^{-4}
4	10^{-3}
5	10^{-2}
6	10^{-1}
7	* control

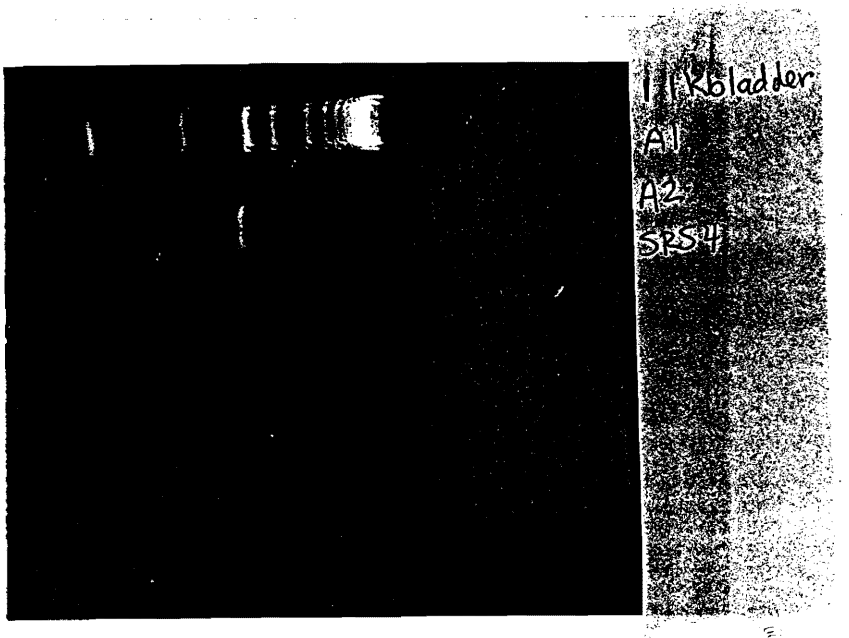
7. Run gels at 80 volts for about 1.5 hours
8. Visualize w/ UV and photograph
   - Gel 1 Lane 3 worked
   - Gel 2 — control worked*

Set up PCR Reactions / pour 1% gel

1. dilute SRS4 DNA 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}
2. prepare stock PCR reaction mixture (refer to page 2) for 6 PCR reactions
3. add 99mL of stock mixture to each PCR tube
4. add 1μL of template to each tube
5. boil for 5 minutes
6. add .5μL of taq polymerase
7. put in PCR machine overnight (refer to page 2)

Gel Procedure

1. measure .5g of Agarose
2. add to 50mL of 1% TBE buffer
3. heat to mix
4. add 2μL of EtBr
5. pour gel and let set up



Run PCR products on gel

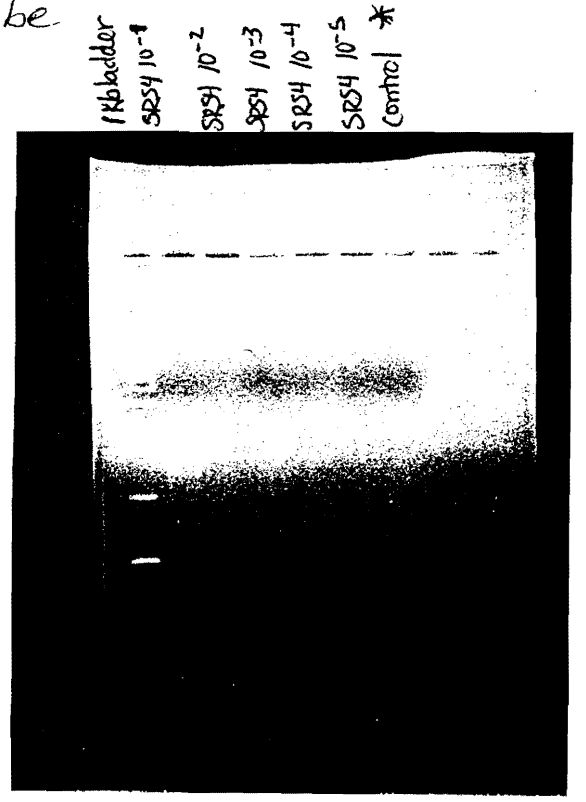
1. Set up gel w/ 1% TBE buffer
2. mix 2.5uL of dye w/ each PCR product (20 µl)
3. load gel:

Lane	Product Sample
1	1kb ladder
2	SRS4 10^{-1}
3	SRS4 10^{-2}
4	SRS4 10^{-3}
5	SRS4 10^{-4}
6	SRS4 10^{-5}
7	* Control

4. Run gels at 80 volts for ~1.5 hours (B→R)
5. visualize w/ UV and photograph

Set up PCR on soil sample

1. Set up a series of dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) on soil sample; make control; use undiluted sample as well
2. prepare stock buffer for 8 samples (refer to page 2)
3. add stock solution to each tube
4. add 1µL of template to each tube
5. boil
6. add .5uL of Taq polymerase
7. put in machine overnight



Make gel (1%+TBE)

1. see page 2 for procedure

Run PCR products (2-17-98) on gel

1. See page 3 for procedure
2. gel loaded the following way:

Lane	Sample
1	1Kb ladder
2	10^{0} Downstream
3	10^{-1} "
4	10^{-2} "
5	10^{-3} "
6	10^{-4} "
7	10^{-5} "
8	10^{-6} "
9	* Control

3. Run gels for 1.5 hours at 80 volts
4. Visualize w/ UV and photograph
5. Nothing worked

Set up Ligations

1. Make up the ligation Reaction (per tube)

Sterile water	5μl
10X ligation buffer	1μl
Vector (25ng/μl)	2μl
PCR product	1μl
T4 DNA ligase	1μl

2. put in ~~Fridge~~ Refrigerator for 2 days

Transformation w/ ligation Products from 2-18-98

Samples

1	SRS4	4	eb-4
2	A2	5	CAFB
3	eb-3		

1. Thaw 5 vials of One Shot cells on ice (from kit).
2. add 2 µL of .5 M β-ME into each vial and mix gently
3. add 2 µL of ligation reaction to each vial and mix gently (refer to above)
4. incubate on ice for 30 minutes
5. heat shock for 30 seconds at 42° C — DO NOT MIX
6. put vials on ice for 2 minutes
7. add 250 µL of SOC medium to each vial ~~and~~
8. incubate at 37° with 225 rpm of shaking for 1 hour; put vials back on ice

Label plates

1. 5 of 50 µL, 100 µL, Rest each
2. name, date, LB-AMP, ligation/transformation
3. incubate

— select white colonies (over weekend) and plate again (poor transformation %'s)

Mini-Preps

1. Select colonies (2 gels of 8, 1 gel w/7)
2. CAFB eb-4 : 1, 2, 4, 5, 12] gel A
   SRS4 16S : 3, 10, 11
   CAFB [redacted] 16S : 1, 2, 3, 4, 7, 8, 10, 11 gel B
   A2 16S : 1, 4, 5, 6
   CAFB 16 eb-3 : 1, 2, 4] gel C
3. Labeling
   A1: CAFB-eben-4 TA clones 16S ; 1, 2, 4, 5, 12
   A2: SRS4 16S ; 3, 10, 11
   B: CAFB-tol-4 16S ; 1, 2, 3, 4, 7, 8, 10, 11
   C1: Arthrobacter 16S ; 1, 4, 5, 6
   C2: CAFB-eben-3 16S ; 1, 2, 4

Procedure:

4. Suspend colony in 40μL STET buffer
   STET = (50mM EDTA
   50mM Tris pH 8.0
   5% Triton
   8% sucrose)
5. Add 4μL of 10mg/mL lysozyme
6. Vortex
7. boil for 1minute in waterbath
8. Spin for 10minutes at 14,000rpm
9. remove pellet w/sterile toothpick
10. add 200μL of .3M KAc and 500μL of cold EtOH → mix well
11. Vortex
12. Store at -20°C for 1 hour
13. Spin for 15minutes at 14,000rpm
14. Decant supernatent ; wash w/ 70% EtOH and spin again
15. Vacuum dry
16. Resuspend in digestion buffer
17. Cut w/ EcoRI (1hr digestion at 37°C) → 2-24-98

(Prepare 3 gels for tomorrow ; 1% TBE agarose + EtB

- Digestion RXN: 2.5μL of React Buffer #3
  .5μL of EcoRI
  22μL of dH_2O

2-24-98

9

Run digests from 2-23-98 on gels

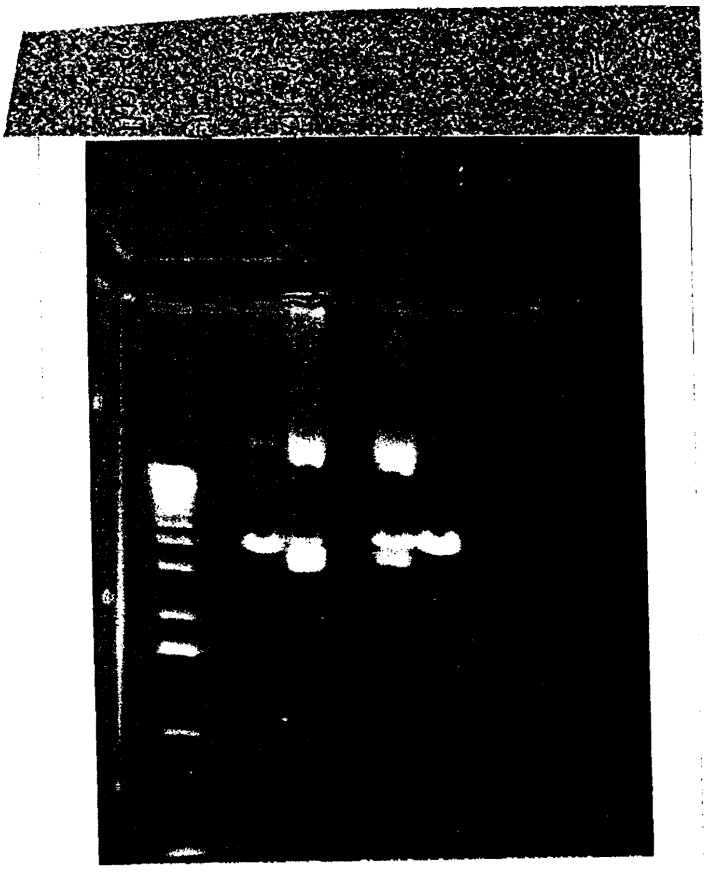
~~Gel A: Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9~~
~~Sample | 1kb ladder | A11 | A12 | A14 | A15 | A112 | A23 | A210 | A211~~

Gel A:

Lane #	1	2	3	4	5	6	7	8	9
Sample	1Kb ladder	A1 1	A1 2	A1 4	A1 5	A1 12	A2 3	A2 10	A2 11

- only plasmid visible

- only plasmid 2-26-98



2-24-98

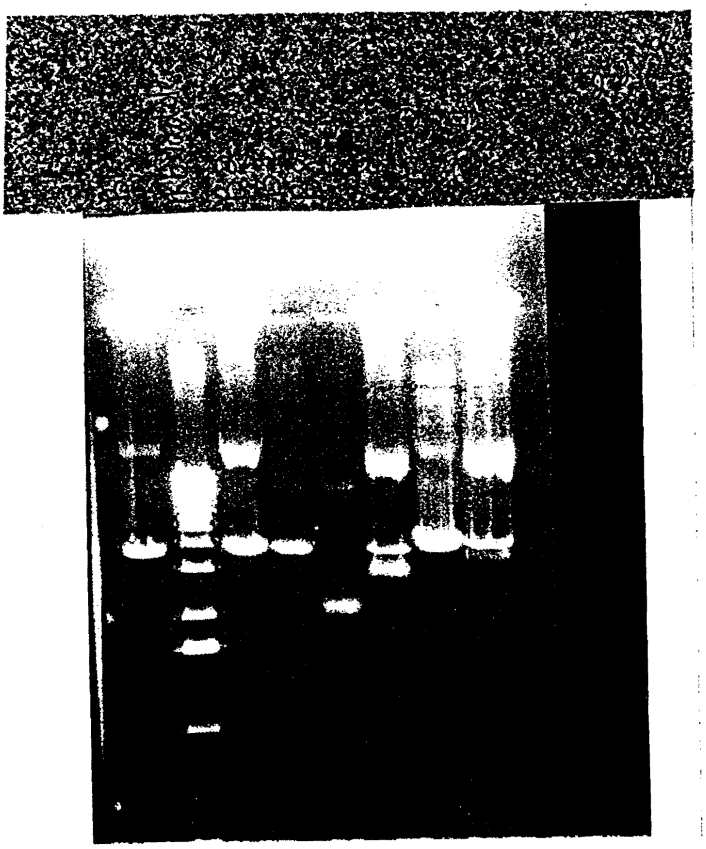
10

Gel B:

Lane#	1	2	3	4	5	6	7	8	9
Sample	B 1	1 Kb ladder	B 2	B 3	B 4	B 7	B 8	B 10	B 11

-only plasmid visible

-only plasmid 2-20-98



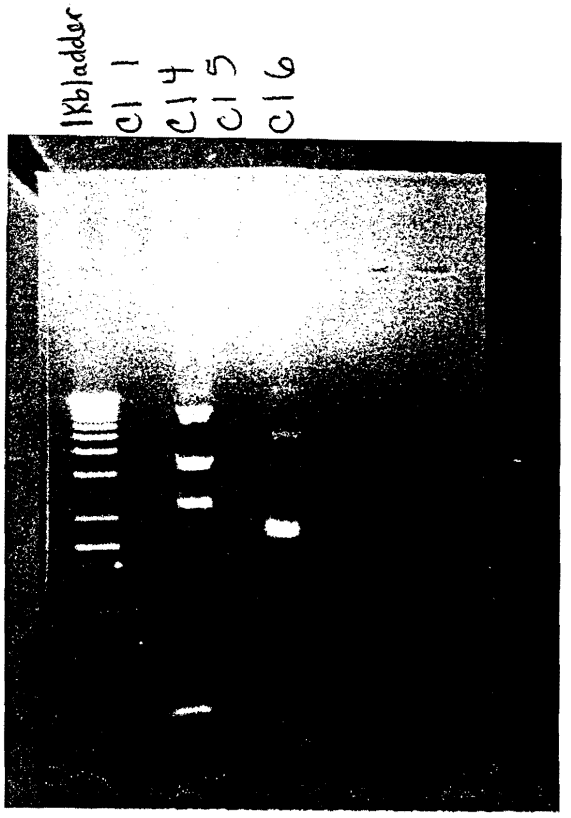
Gel C:

Lane #	1	2	3	4	5	6	7	8
Sample	C1 1	C1 4	1Kb ladder	C1 5	C1 6	C2 1	C2 2	C2 4

-only plasmid visible.

Run on Gel 2-25-98

C1 1
C1 4
★
C1 5
C1 6



Mini-Prep (again)

1. Re-prep Arthrobacter C1 plate : 1, 4, 5, 6
   see procedure page 8
2. Set up Digestion Reaction buffer (5 RXNS)

React 3	(2.5 µL) x 5	= 12.5 µL
Eco RI	(1.0 µL) x 5	= 5.0 µL
dH_2O	(22.0 µL) x 5	= 110.0 µL
		25.5 µL per tube

3. Digest 2 hours
4. add dye & run on 1% agarose gel
   C1 6 worked! (page 11 picture)

2-26-10

13

Mini-Prep / Digests / Subculture

1. Mini-Prep

A1	1, 2, 4
B	1, 2, 3, 8
A2	3, 10, 11
C2	1, 2, 4

See procedure page 8

2. Digest for 15 RXNS
See page 12

3. Subculture C1 6 sample onto 2 plates
-use streak method

4. Prepare 2 1% TBE + Agarose + EtBr gels

Ligation Reaction / PCR optimization

1. See page 6 for procedure
   Samples: ~~A1: CAFB eben-4 TA clones 16s~~
   ~~B: CAFB-101-4 16s~~
   C2: CAFB-eben3 16s
   A2: SRS4 16s
2. refrigerate

PCR procedure

1. Reaction mixture (for each sample)
   - ✓5µl dNTP
   - ✓5µl Buffer (A, B, C, D, J, F, N)
   - ✓2µl 27f primer
   - ✓2µl 1492 primer
   - ✓1µl template
   - ✓ 35µl d H_2O
2. boil 5minutes
3. add Taq (.5 µL)
4. put in PCR machine overnight

PCR samples:
SRS Downstream DNA
10^0 ~~10^{-2}~~
10^{-1} ~~10^{-3}~~

3-3-98

15

Run PCR products on gel

1. Gel 1:

Lane	1	2	3	4	5	6	7	8
Sample	1 Kb ladder	10^{0} A	10^{0} B	10^{0} C	10^{0} D	10^{0} F	10^{0} J	10^{0} N

Gel 2:

Lane	1	2	3	4	5	6	7	8
Sample	10^{-1} A	1 Kb ladder	10^{-1} B	10^{-1} C	10^{-1} D	10^{-1} F	10^{-1} J	10^{-1} N

- NOTHING WORKED
  → try PCR on another soil sample (Coal pile) (Source sample)

3-4-10

16

Transformation of 3-298 Ligation Products/PCR

1. procedure from page 7
2. Samples CAFB eb-3 Lig ("C2")
   SRS4 Lig ("A2")

PCR RXN

1. Samples SRS source 1
   SRS source 2
2. dilute each sample 10^{-1}, 10^{-2}, 10^{-3}
3. set up reactions for 10^{0}, 10^{-1}, 10^{-2}, 10^{-3} for each
4. procedure page 8
5. place in machine overnight

Spread plates w/transformants

1. add 40µL of X-gal to each & spread
2. label 50µL, 100µL, Rest
3. spread plates (incubate 37°C)

Make gel

1. See page 2

3-5-98

Run PCR (3-4-98) on gel / Subculture transformation 17

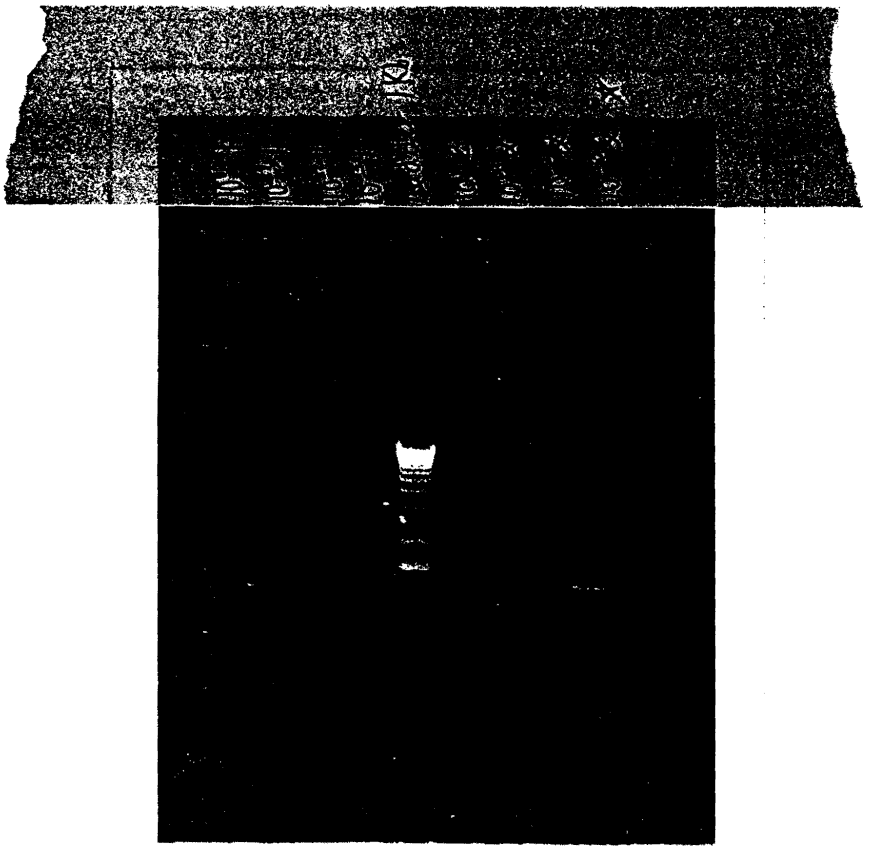
1. Sample	10^{-1}	10^{-1}	10^{-2}	10^{-3}	Ladder 1kb	10^{-2}	10^{-2}	10^{-2}	10^{-3}
Lane #	1	2	3	4	5	6	7	8	9

10^{-3} Source 2 was amplified

Subculture Transformations from 3-4-98

1. use toothpick to pick white colonies
2. restreak on grided plate
3. incubate overnight

– Both plates appeared to have worked



3-6-98

18

Mini-Prep from 3-5-98 Subculture / Ligation

1. Samples
   - CEb-3 14 — labeled CE 1-14
   - SRS4 14 — labeled SR 1-14
2. Procedure
   - see page 8

Ligation

1. Sample Source 2 10^{-3} PCR product from 3-4-98
2. See procedure page 6

Mini-Prep. Cont'd / Transformation

1. Finish mini-preps, procedure page 8
2. Samples CE 1-14 ; SR 1-14

Transformation w/ 3-6-98 Lig. products

1. Procedure page 7
2. Samples Eb-4 / S2

Digestion Reactions

1. procedure page 7
2. Samples CE 1-14 ; SR 1-14
   (Stock reaction for 30 samples?)
   660 µl dH_2O
   75 µL buffer
   15 µL EcoRI

Make 1% gels + EtBr

3-10-98

20

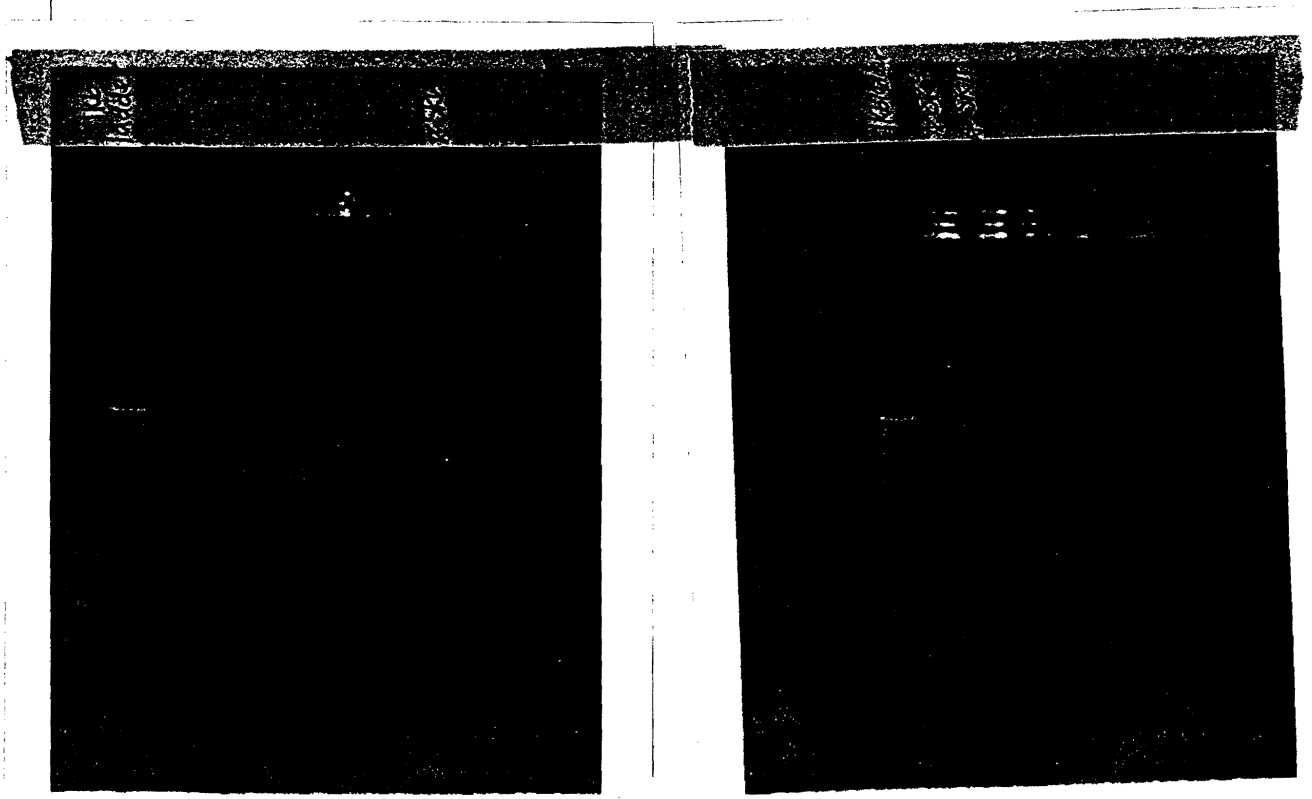
Run Mini-Preps

1. Gel 1:

Lane	1	2	3	4	5	6	7 ✱	8
Sample	~~SR1~~ 1kb ladder	~~ladder~~ SR1	SR2	SR3	SR4	SR5	SR6	SR7

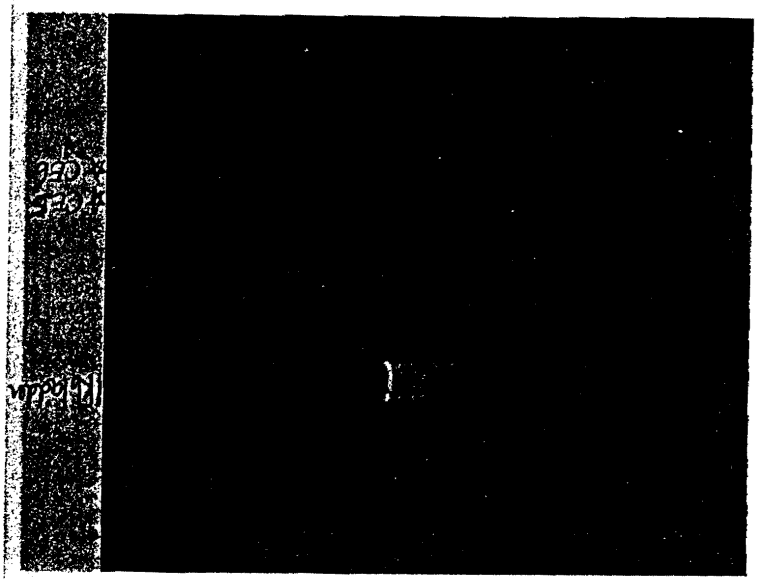
Gel 2:

Lane	1	2	3 ✱	4 ✱	5	6	7	8
Sample	SR8	1kb ladder	SR9	SR10	SR11	SR12	SR13	SR14



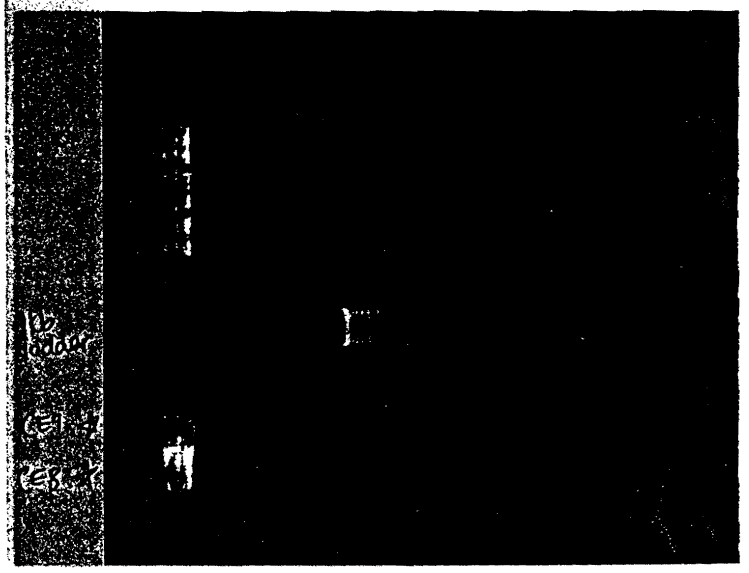
Gel 3:

Lane	1	2	3	4	5	6 ☆	7 ☆	8
Sample	CE 1	CE 2	1Kb ladder	CE 3	CE 4	CE 5	CE 6	CE 7



Gel 4:

Lane	1 ☆	2 ☆	3	4	5	6	7	8	
Sample	CE 8	CE 9	CE 10	1Kb ladder	CE 11	CE 12	CE 13	CE 14	C0 3



Subculture 3-9-98 Transformations

1. See page 17 for procedure

3-11-98

22

Subculture positive Mini-Preps

1. Restreak positive cultures on LB-Amp plates

PCR Source 2 again

1. Samples 10^{0}, 10^{-1}, 10^{-2}, 10^{-3} control (★) Source 1 / Source 2
2. procedure page 2

3-12-98

23

Check PCR (3-11-98) / Mini-Prep Pure Subcultures

1. Gel : Nothing Worked

Mini-Prep
1. Procedure page 8
2. Samples:
C16A, B CE 5, 6, 8, 9 SR 6, 9, 10

3-13-98

24

Run Mini-Preps on gel from 3-12-98 Mini-Preps cont'd.

1. Gel 1:

Lane	1	2	3	4 ★	5 ★	6 ★	7 ★
Sample	1kb ladder	C16 A	C16 B	CE5	CE6	CE8	CE9

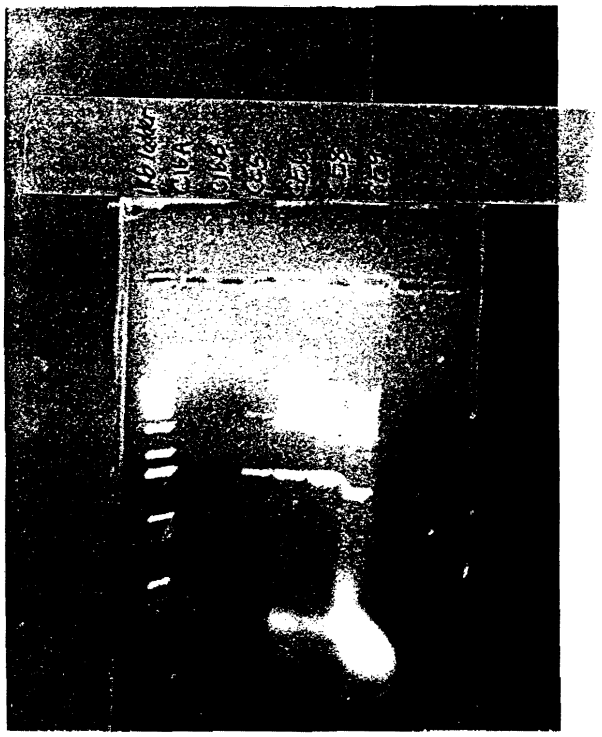
Gel 2:

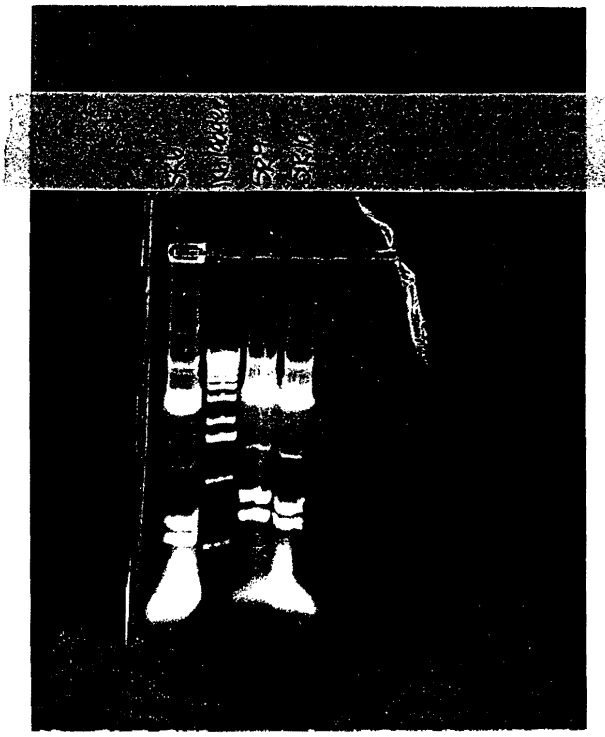
Lane	1 ★	2	3 ★	4 ★
Sample	SR6	1kb ladder	SR9	SR10

★ Worked → plasmid + Gene Visible

Mini-Preps

1. See Procedure page 8
2. Samples: E4 1-24





3-16-98

25

Finish Mini-Preps / Pour Gels / Run Gels

1. Finish procedure page 8
2. Digestion Buffer:

.5 µL	EcoRI	X 25	=	12.5 µL
2.5 µL	Buffer H	X 25	=	62.5 µL
22 µL	dH_2O	X 25	=	550 µL

Gel Procedure

1. See page 2
2. Made 3 gels 1% agarose + Et-Br

Gel 1:

Lane	1	2	3	4	5	6	7	
Sample	1kb ladder	E41	E42	E43	E44	E45	E46	E47

Gel 2:

Lane	1	2	3	4	5	6	7	8	9
Sample	E48	1kb ladder	E49	E410	E411	E412	E413	E414	E415

Gel 3:

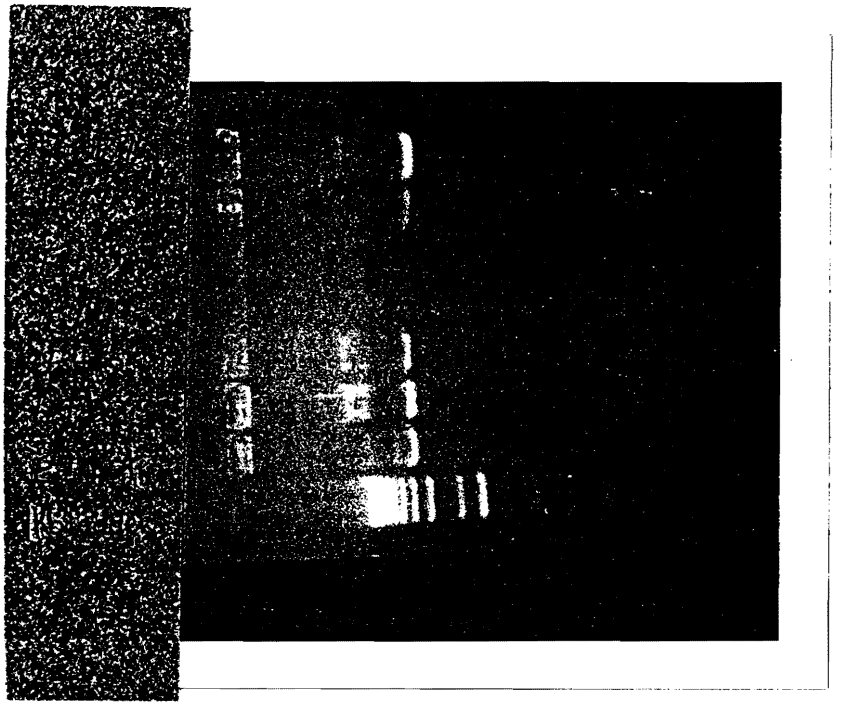
Lane	1	2	3	4	5	6	7	8	
Sample	E416	E417	1kb ladder	E418	E419	E420	E421	E422	E4

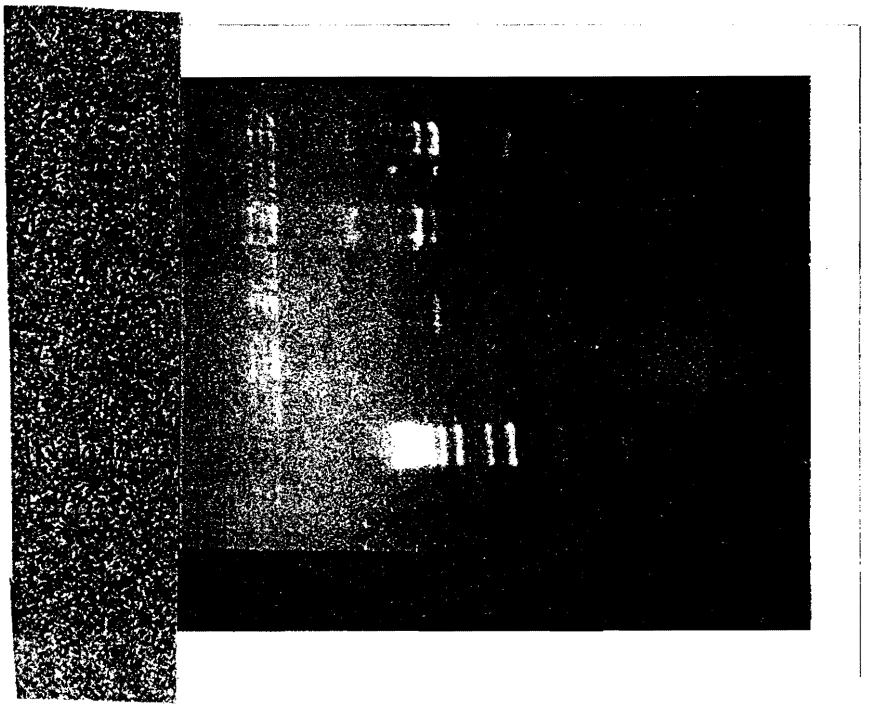
3-17-98

96

Run gels

See page 25 for lane designations





2. Samples E41, E413, E415 worked.
3. Restreak for cloning prep.

3-18-98

27

Plasmid Prep. for Sequencing / Mini-Prep / Makegels

1. Make LB-broth for culture
2. add antibiotic, ampicillin, @50 μL to each

Mini-Prep

1. Procedure page 8
2. Samples CAFB-eben-4 clone #14 / Clone #15
3. digest

Gel Procedure

1. See page 2

Run gel

1. E495 was verified to have insert on gel

3-19-98

equipment broken

3-20-98
28

Midi-Plasmid Prep for sequencing

1. Grow bacteria in 250ml of LB+ampicillin broth with shaking at 37°C
2. remove 1ml and freeze for further use
3. Centrifuge the remaining broth in 250mL centrifuge bottle for 10minutes at 8k
4. Resuspend cells in 3mL of cold lysis buffer and transfer to 30mL centrifuge tube
5. Add 6ml of .2N NaOH, 1% SDS (double recipie)
   2 pellets NaOH (1)
   22.50 ml H_2O (11.25)
   2.5ml 10% SDS (1.25)
   mix well but do not vortex
6. add 3.7mL of 5mKAC
7. Centrifuge for 30 minutes at 10K and 4°C
8. collect supernatent filtered through cheesecloth in a clean, sterile 30mL tube. Add an equal volume of isopropanol. ~~centrifuge~~
9. Centrifuge for 30minutes at 10K and 4°C
10. pour off supernatent; wash with 70% EtOH
    (70% EtOH : 15mL EtOH 35mL dH_2O)
11. vacuum dry pellet
12. add 500µL H_2O and resuspend; add 5µL RNAase
13. put in 37°C water bath with shaking
14. place solution in clean eppendorf tube
    - add 500µL of phenol: chloroform: isoamyl
    - centrifuge 5minutes
    - ~~dd~~ - remove supernatent and place in clean tube
    - repeat this until very little cellular debris
15. transfer supernatent to clean tube; add 600µL isopropanol
16. centrifuge 10minutes
17. wash pellet with 70% EtOH and vacuumdryp
18. resuspend pellet in 200µL TE

Biodegradation of Aromatic Hydrocarbons in an Extremely Acidic Environment

Raymond D. Stapleton[1,4], Dwayne C. Savage[3], Gary S. Sayler[1,3,4] and Gary Stacey[1,2,3,4*],

Center for Environmental Biotechnology[1], Center for Legume Research[2], Department of Microbiology[3], and Department of Ecology and Evolutionary Biology[4]
The University of Tennessee-Knoxville
Knoxville, TN 37996

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Running Title: Biodegradation in Acidic Environments

*Correspondent Footnote: Gary Stacey, PhD
Center for Environmental Biotechnology
676 Dabney Hall
The University of Tennessee-Knoxville
Knoxville, TN 37996
ph:(423) 974-4041; fax:(423) 974-4007; email:GSTACEY@utk.edu

Key Words: extremophiles, bioremediation, naphthalene, coal microbiology

Abstract

The potential for biodegradation of aromatic hydrocarbons was evaluated in soil samples recovered along both contaminant and pH gradients existing downstream of a long-term coal pile storage basin. pH values for areas greatly impacted by runoff from the storage basin were 2.02. Even at such a reduced pH, the indigenous microbial community showed signs of remaining metabolically active, showing the ability to oxidize more than 40 percent of the parent hydrocarbons naphthalene and toluene to carbon dioxide and water. Treatment of the soil samples with cylcohexamide inhibited mineralization of the aromatic substrates. DNA hybridization analysis indicated whole community nucleic acids recovered from these samples did not hybridize with common enzymes from neutrophilic bacteria, including *nahA, nahG, nahH, todC1C2* and *tomA*. Since these data suggested the degradation of aromatic compounds may involve a microbial consortia instead of individual acidiphilic bacteria, experiments using microorganisms isolated from these samples were initiated. While no self-constructed mixed cultures were able to evolve $^{14}CO_2$ from labeled substrates in these mineralization experiments, an undefined mixed culture including a fungus, a yeast, and several bacteria successfully metabolized approximately 27 percent of naphthalene after one week. This study shows that biodegradation of aromatic hydrocarbons can occur in environments suffering from reduced pH values.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) occur as common constituents of petroleum, coal tar, and shale oil, but are most frequently formed by incomplete combustion of fossil fuels (21). These contaminants represent a class of compounds that are widely distributed in nature (31) and generally considered to have genotoxic or carcinogenic potential (21; 31). Several species of algae, bacteria, and fungi are known to degrade PAHs (31). Lower molecular weight PAH compounds, including naphthalene, phenanthrene, and anthracene, have been previously shown to be mineralized by bacteria (Cerniglia, 1992). The capacity of microorganisms to degrade specific PAHs in nature depends on the physical and chemical properties of the contaminants, the environment, and activity of indigenous organisms.

In spite of the wide ranges of environments suffering from PAH contamination, most investigations have largely focused on species of microorganisms that grow on common laboratory media at room temperature (mesophiles) at neutral pH (neutrophiles). Many environments in which PAH contamination occur, such as mine drainage basins, are acidic (acidiphilic) and occasionally at temperatures well above room temperature (thermophilic). Significant numbers of acidiphilic bacteria have been found in these environments (10, 20,32). Many of these isolates are autotrophic or mixotrophic acidiphiles. However, heterotrophic acidiphiles belonging to the genus *Acidiphilium* have been isolated from coal mine drainage (10). These bacteria are mesophilic, gram-negative, aerobic rods that utilize citrate and other simple organic compounds as energy sources. Most members of this genus grow at pH 2-3, but none grow above pH 6.0.

This paper describes initial investigations into the biodegradation of aromatic compounds under acidiphilic conditions. Surface water and soil samples were strategically recovered from a coal pile storage area, the downstream drainage basin, and a nearby creek. These areas provide samples with different degrees of PAH concentrations and pH values.

Materials and Methods

Site and Sample Description: Soil and surface water samples were recovered from a coal runoff basin on the Westinghouse Savannah River Laboratory site in Aiken, SC. Surface runoff from the coal storage piles was discharged to surface streams until 1977, when new regulatory requirements were initiated. At that time, unlined earthen containment basins were constructed to intercept, stabilize, and treat surface runoff from the coal storage area. Water leaking from these basins has contaminated nearby soil and surface water with heavy metals and PAH compounds, as well as threatened local groundwater supplies. Microbiological analyses were performed on samples representing three distinct levels of PAH concentration and pH values. The samples were taken from a source coal pile (identified as source), downstream from the pile in the catch basin (identified as downstream), and from an outfall creek located near the catch basin but unaffected by drainage from the coal pile (identified as creek). Each sediment sample was collected and individually sealed in sterile, plastic sample bags, placed on ice, and immediately shipped to our laboratory. Corresponding water samples were collected in sterile 1L Nalgene bottles, placed on ice, and immediately shipped to our laboratory.

Upon receipt of the soil and water samples in the laboratory, microbial enumerations, enrichments with aromatic hydrocarbons, mineralization assays utilizing ^{14}C-labeled compounds, and DNA extractions were immediately initiated. The pH values of each soil and water sample was determined. Soil from the source area had a pH of 6.09, with the pH of the corresponding water sample 2.0. Soil from the downstream sample had a pH of 3.35, with the pH of the corresponding water sample 2.02. Soil from the creek sample had a pH of 6.59, with the pH of the corresponding water sample 5.42. Additional sediment and water samples were stored at 4°C.

Microbial Enumerations: Neutrophilic and acidiphilic heterotrophic bacteria were enumerated using traditional spread plate analysis on solid agar media. Total viable counts (TVC) for neutrophilic, heterotrophic microorganisms were determined in triplicate by adding 1.0 gm of sediment to 9.0 ml of sterile 0.1 % (w/v) sodium pyrophosphate dilution buffer (pH 7.0) (4), then vortexing vigorously for 30 s on, 30 s off, and 30 s on. Appropriate dilutions were plated from each series on 1/4th strength YEPG (YEPG = g/L dH_20: yeast extract, 0.2; polypeptone, 2.0; D-glucose, 1.0; ammonium nitrate, 0.2; agar, 17.0; pH 7.0) and incubated at 25°C for one week before determining colony forming units (cfu) (25). Acidiphilic bacteria were enumerated using the method of Harrison (1981). Again, serial dilutions were performed on triplicate samples and appropriate dilutions were plated from each dilution series on acidiphile medium as described by Harrison (9). Acidiphile plates were incubated for 2 weeks at 25°C before determining colony forming units.

Biodegradation Analysis: All radiochemicals were purchased from Sigma (St. Louis, MO). The specific activities (mCi / mmol) were as follows: toluene-ring-UL-^{14}C, 60.0; naphthalene-UL-^{14}C, 49.8; phenanthrene-9-^{14}C, 13.3; anthracene-UL-^{14}C, 15.0; and salicylic-acid-ring-UL-^{14}, 10.0. Mineralization assays, using the method of Sanseverino et al. (24), were set up on the soil samples in order to determine the biodegradation potential of toluene, naphthalene, phenanthrene and anthracene in each soil sample. Biodegradation was defined as the evolution of $^{14}CO_2$, which was determined by liquid scintillation counting. Briefly, 2 gm samples were slurried with 1 mL of the corresponding filter-sterilized (0.45 μm filter apparatus; Corning Costar Corporation, Cambridge, MA) water sample in 40 mL EPA certified vials (Eagle-Picher, Miami, OK). Sterility of the water samples used for mineralization experiments was determined by the lack of colony forming units appearing on either 1/4th strength YEPG (pH 7.0) or acidiphile plate medium (pH 3.0) after 1 week incubation. Carbon dioxide traps were created by inserting an 8 mL glass vial containing 0.5 mL of a 0.5 M sodium hydroxide solution into the 40 mL mineralization vial. Approximately 100,000 dpm of the appropriate ^{14}C-labeled substrate was added and the vials sealed with screw caps and teflon lined septa. In experiments with cyloclohexamide treatment, a solution of 10 mg/mL of the appropriate filter sterilized water sample was freshly prepared and 2 mL of the solution was added.

For mineralization experiments with pure cultures, isolates were grown for 48 hrs in 1 L liquid acidiphile medium (9). Individual organisms were concentrated by

centrifugation at 5500 x g, the resulting cell pellet washed 2X in minimal salts medium (pH 3.0), and the final cell pellet suspended in 50 mL fresh minimal salts medium (resulting optical density of 1.8 to 2.0 at 543 nm). Each mineralization vial received 1 mL culture and 4 mL either acidiphile medium, minimal salts medium, or filter-sterilized water sample from the downstream area. Radiolabeled salicylic acid, toluene, naphthalene, and phenanthrene were used. In all mineralization experiments, active assays and controls were killed by the addition of 1 mL 37% formaldehyde (Fisher Chemicals, Fair Lawn, NJ).

Molecular Diagnostics: Duplicate 50 gm samples were used for DNA extraction studies using the method of Ogram et al. (14, 19, 28). Cell lysis was initiated by a heat-SDS treatment and completed by ballistic disintegration using bead-mill homogenization. After concentrating nucleic acids from the aqueous phase by isopropanol precipitation, the samples were dialyzed against TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 7.5) overnight. The samples were then extracted 1X with Tris-saturated phenol (pH 7.5), followed by extraction 1X with Chloroform:Isoamyl alcohol (24:1). The final aqueous phase was recovered and ethanol precipitated overnight at -20°C. DNA was collected by centrifugation and dried under vacuum. The final pellet was suspended in 1 mL sterile TE buffer (pH 7.5) and stored at -20°C until used for hybridization studies.

DNA recovered from each soil sample was vacuum blotted onto 0.22 μm Biotrans nylon membranes (ICN Biomedical, Inc., Costa Mesa, CA) and fixed by baking at 80°C for 1 hour, then rinsing in 2X SSC buffer (g / L dH_2O: sodium chloride, 17.53; sodium

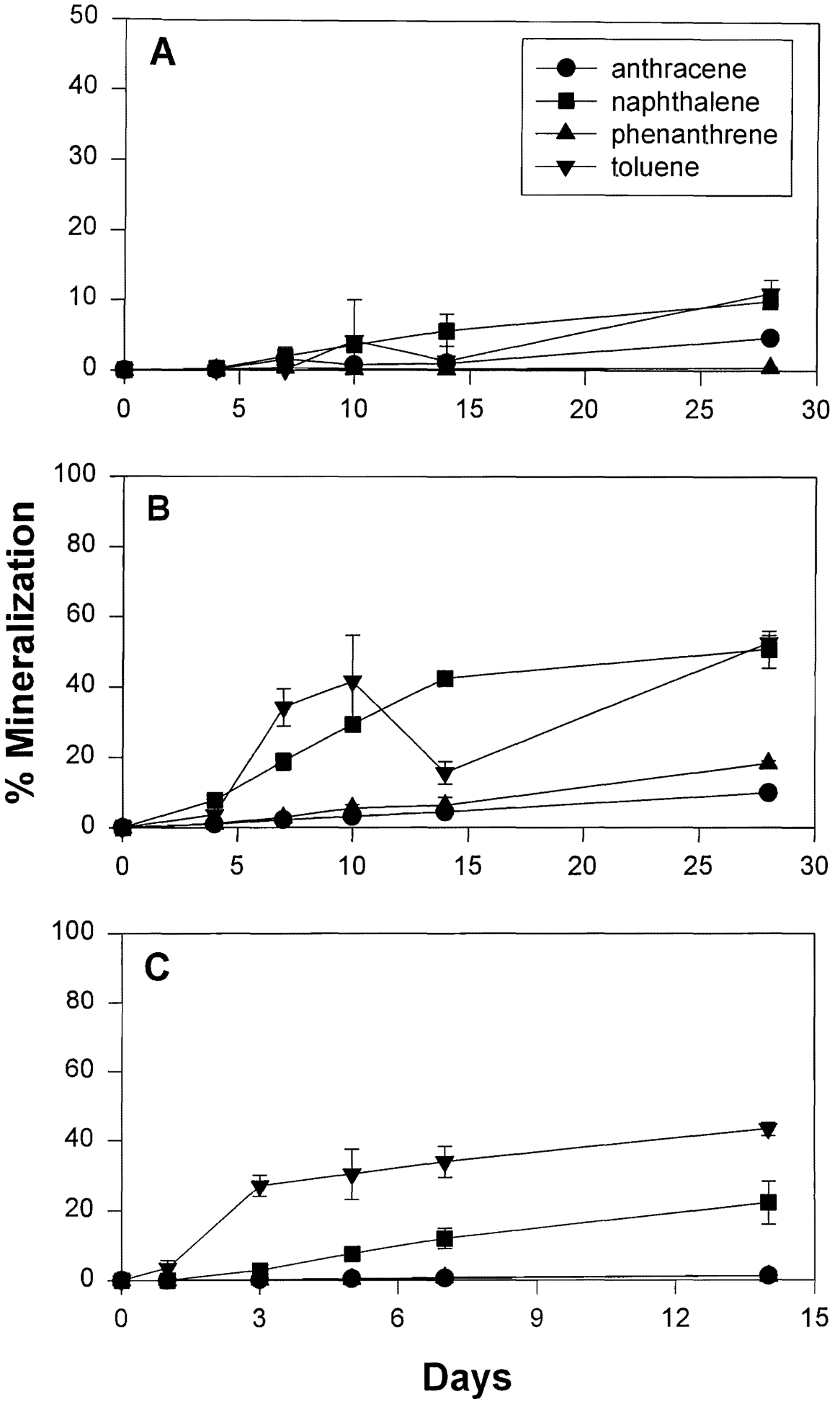
citrate, 8.82; pH 7.0), followed by baking at 80°C for another hour. Membranes were pre-hybridized 12-18 hrs, then hybridized an additional 12-18 hrs with the appropriate probe as previously described (28). The membranes were washed 3X with high stringency wash buffer (g / L: sodium chloride, 0.59; Tris-base, 2.42; EDTA, 0.37, SDS, 5.00; pH 7.5) and positive hybridization signals determined by autoradiography. DNA was recovered from the isolate organisms using the method of Marmur (1962).

PCR, Cloning, and Sequencing of 16S rDNA from Isolates: PCR amplification of 16S rDNA from soil isolates was performed using primers 27f and 1492r (*Eschericia coli* numbering system) as previously described (16, 26). The 1.5 kb PCR products were cloned using the TA cloning kit (Invitrogen, San Diego, CA) following the manufacturer's instructions. Bacterial colonies were screened for plasmids containing the correct insert using the rapid-boiling plasmid mini-preparation technique of Holmes and Quigley (12), followed by restriction digestion with *Eco*R1. Plasmid DNA containing the correct insert for DNA sequence analysis was prepared (22) and the amount of plasmid DNA recovered was determined using a Hoefer DyNA Quant 2 fluorometer (San Francisco, CA). DNA sequencing was conducted using an Applied Biosystems Division of Perkin Elmer (Foster City, CA) automated DNA sequencer in the Molecular Biology Resource Facility at The University of Tennessee-Knoxville using the sequencing primer 27f (16). Tentative phylogenetic identification of microorganisms using DNA sequence analysis of cloned small subunit ribosomal RNA was performed with existing GenBank databases using the FASTA format in the BLAST program (2).

Results

Enumeration of microorganisms from acidic soil samples was performed by traditional agar spread plate analysis. Neutrophilic heterotrophs were present at values of 1.1×10^4 ($\pm 4.1 \times 10^3$) cfu / gm source soil, 1.0×10^3 ($\pm 4.7 \times 10^2$) cfu / gm downstream soil, and 2.2×10^6 ($\pm 8.2 \times 10^5$) cfu / gm creek soil. Estimates of acidiphilic bacteria ranged form 10^2 - 10^3 cfu / gm of each sample, but statistical analysis was confounded because the plates yielded few colonies and were contaminated with fungi.

Results from the ^{14}C-labeled substrate mineralization experiments indicate that natural samples of acidic pH maintain the capacity for significant biodegradation of aromatic hydrocarbons (Figure 1). Each of the three soil samples tested possessed the ability to mineralize one or more of the aromatic hydrocarbons tested. Figure 1A shows mineralization of aromatic hydrocarbons by the source soil sample. The potential for biodegradation on a per gram of soil basis is probably underestimated because this sample was collected directly from the storage pile and contained significant amounts of solid coal. Nevertheless, mineralization of toluene and naphthalene reached approximately 10% at 28 days. The downstream soil sample showed mineralization of each compound tested (Figure 1B). Toluene and naphthalene mineralization approached 50% degradation, while phenanthrene and anthracene showed 10-20% mineralization. Soil samples from the creek area showed significant toluene mineralization (40%) at 14 days (Figure 1C). Naphthalene mineralization reached 20% mineralization at 2 weeks, while the other PAH compounds, phenanthrene and anthracene, showed little degradation.


A
anthracene
naphthalene
phenanthrene
toluene
B
C
% Mineralization
Days

Microbial enumeration studies revealed representation of both fungi and yeast in the soil microbial communities in each sample. A mineralization experiment was designed to test the impact of these micro-eukaryotes on biodegradation of the test aromatic hydrocarbons. Cyclohexamide was used to repress micro-eukaryotic metabolism during a 14 day mineralization experiment. Cyclohexamide treatment of the downstream samples eliminated aromatic hydrocarbon metabolism, but did not eliminate mineralization in the creek samples (Table 1). This indicates the mineralization of aromatic hydrocarbons in the highly acidic downstream sample involves eukaryotic organisms, probably either yeast, fungi, or both.

Whole community DNA was extracted from each soil sample and hybridized with molecular probes targeting genes commonly associated with aromatic hydrocarbon degradation under neutrophilic conditions (data not shown). Hybridization analyses with the gene probes *nahA* (naphthalene dioxygenase), *nahH* (catechol-2,3-dioxygenase), *nahG* (salicylate hydroxylase), *todC1C2* (toluene dioxygenase), and *tomA* (toluene monooxygenase) did not produce positive signals above the calculated detection limit of 0.003 ng of target sequence. The extracted DNA did hybridize positively with a Universal 16S rDNA oligonucleotide (27, 28), indicating that DNA of sufficient quality for analysis was successfully recovered from the samples. The strongest hybridization signal with the Universal 16S rDNA oligonucleotide was seen with DNA recovered from the creek sample.

In order to explore the possibility of isolating a pure culture of an obligately acidiphilic microorganism capable of growth on PAHs, enrichments were prepared using 10 gm downstream soil sample and 40 mL filter-sterilized corresponding water sample

Table 1. Effects of cyclohexamide (10 mg/ml) on mineralization of naphthalene and toluene after 14 days.

sample	% naphthalene mineralization	% toluene mineralization
downstream	31.74 ± 6.47	5.19 ± 1.62
downstream with cyclohexamide	0.00 ± 0.00	0.00 ± 0.00
creek	74.77 ± 1.16	45.54 ± 15.25
creek with cyclohexamide	56.57 ± 4.35	30.58 ± 19.14

```
     1321 caactcgact ccatgaagtc ggaatcgcta gtaatcgcag atcagattgc tgcgg
     1381 acgttcccgg gnnttgtaca caccgcncgt cacaccatgg gagtttgttg cacca
     1441 aggtagctga accgcgagga gggcgctnnc agcggtgtgg cngatgactg ggg
//
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UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

Name: Jerrie Caroline Haney

College: Agriculture Department: Microbiology

Faculty Mentor: Dr. Gary Stacey

PROJECT TITLE: "Characterization of Bacterial Isolates from an Acidic Soil Environment Using 16S r RNA Phylogeny Techniques"

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: _____, Faculty Mentor

Date: 5/11/98

Comments (Optional):

Excellent job.

Caroline obtained some useful practical experience through the lab work she did for this project. This work has now landed her a job for the summer.

It was a pleasure working w. the lab.

(pH 2.0) saturated with naphthalene. These microcosm enrichments were allowed to shake for 2 weeks at 25°C at 100 rpm and then 10 μL of the aqueous phase was spread plated on acidiphile medium agar plates (9). The acidiphile plates were incubated at 25°C for 1 week before individual colonies were re-streaked. After 2 consecutive re-streaks, the cultures appeared to be of a single colony type. Each isolate was re-streaked twice more to ensure the culture was axenic. At this point, three distinct isolates were identified based on colony morphology and growth characteristics. Two isolates were microscopically identified as bacteria, designated SRS 1 and SRS 2, and the third isolate identified as a yeast, designated SRS 3. Furthermore, the two bacterial isolates were defined as obligate acidiphiles by their ability to grow on acidiphile medium plates (pH 3.0), but not on 1/4th strength YEPG medium plates (pH 7.0). The yeast was able to grow on both types of medium.

Partial sequence analysis of small subunit ribosomal DNA provided tentative identification of the bacterial isolates (Table 2). SRS 1 showed 96% sequence similarity with both *Acidocella sp.* (11) and *Acidiphilium facilis* (14). SRS 2 showed 89% sequence homology with both *Acidocella sp.* (11) and *Acidiphilium facilis* (14) . The heterotrophic yeast was microscopically designated as *Pichia sp.* SRS 4 showed 88% sequence homology with a neutrphilic, nitrate-reducing iron-oxidizing bacteria isolated from a German sediment sample and 87% sequence homology with a neutrohilic, iron-oxidizing bacteria isolated from groundwater in Michigan.

Growth assays on simple carbon substrates and mineralization studies were performed on the isolates SRS 1, SRS 2, and SRS 3. Growth assays were scored as

Table 2. Identification of soil isolates

Isolate	Closest Sequence Match	% Sequence Similarity	Reference
SRS1	*Acidocella sp.*	96	11
	Acidiphilium facilis	96	14
SRS2	*Acidocella sp.*	89	11
	Acidiphilium facilis	89	14
SRS3[a]	*Pichia sp.*	n/a	n/a
SRS4	Denitrifying Iron-Oxidizing Bacteria	88	28
	Lithotrophic Iron-Oxidizing Bacteria	87	8

[a] Isolate SRS3 was identified as a yeast microscopically, not using DNA sequence analysis.

positive if they showed signs of turbidity (Table 3). The isolate designated SRS 1 grew to turbidity in minimal salts medium plus each of the substrates tested except lactose, arginine, and phenylalanine. The isolate designated SRS 2 showed turbidity on only citrate, catechol, and yeast extract. The isolate designated SRS 3 grew on every carbon source except lactose. Biodegradation of toluene, naphthalene, or phenanthrene was at background levels, with no more than 3% mineralization under any of the conditions tested by any of the isolates (Table 4). However, salicylic acid was mineralized to varying degrees by all three isolates. SRS 1 showed a marked increase in mineralization of salicylic acid when incubated with filter-sterilized water from the downstream area. SRS 2 also showed an increase in salicylic acid mineralization when incubated with the natural water sample. SRS 3 mineralized salicylic acid under all conditions tested, with the highest amount of ^{14}C-labeled carbon dioxide occurring in minimal salts medium (pH 3.0).

The ability of model mixed microbial cultures to biodegrade aromatic hydrocarbons at pH 2.0 was evaluated using the previously mentioned isolates, along with 5 fungal isolates, slurried in filter-sterilized water from the downstream drainage basin area (Table 5). Microorganisms isolated from the downstream sample were used for the mixed community mineralizations included two obligate acidiphilic bacteria (SRS 1 and SRS 2), a heterotrophic yeast (SRS 3), and a fungus (Fungus 3). Initial work with SRS 1, SRS 2, SRS 3, and Fungus 3 revealed the ability of this mixed culture to mineralize naphthalene. However, microscopic analysis of the Fungus3 enrichment culture revealed it was contaminated with bacteria. A bacterium designated as SRS 4 was recovered from the contaminated fungal culture, but proved unimportant in the

Table 3. Carbon source utilization spectrum for soil isolates.

carbon source	SRS1	SRS2	SRS3
maltose	+	-	+
galactose	+	-	+
glucose	+	-	+
lactose	-	-	-
citrate	+	+	+
acetate	+	-	+
succinate	+	-	+
salicylate	+	+	+
catechol	+	+	+
yeast extract	+	+	+
arginine	-	-	+
phenylalanine	-	-	+
acidophile media	+	+	+
1/4 YEPG	-	-	+

Table 4. Mineralization of aromatic hydrocarbons by soil isolates.

isolate	compound	acidophile medium	minimal salts medium	natural water sample
SRS1	salicylic acid	2.29	5.12	51.02
	toluene	0.00	0.01	0.50
	naphthalene	0.01	1.76	1.73
	phenanthrene	1.64	0.51	0.83
SRS2	salicylic acid	4.67	13.0	35.10
	toluene	0.03	1.30	0.73
	naphthalene	0.02	1.45	2.36
	phenanthrene	2.27	0.95	0.68
SRS3	salicylic acid	26.40	67.30	36.87
	toluene	0.00	0.88	0.00
	naphthalene	1.40	1.50	0.88
	phenanthrene	1.50	1.20	0.98

mineralization of naphthalene either individually, or in mixed culture. SRS 4, showing similar sequence homology to several neutrophilic, iron-oxidizing bacteria, grew rapidly on 1/4th YEPG at pH 7.0, but very slowly on acidiphile medium at pH 3.0. No other organisms have been isolated from the contaminated fungal culture. Only the microbial consortia including the contaminated Fungus 3 and unidentified, uncultured organisms were able to mineralize naphthalene to carbon dioxide. Fungus 3 was isolated in pure culture by successive transfers in liquid acidiphile medium containing the antibiotics streptomycin, kanomycin, and rifampicin. The fungal culture was determined to be axenic by microscopic analysis. When SRS 1, SRS 2, SRS 3, SRS 4, and uncontaminated Fungus 3 were mixed together, naphthalene mineralization did not occur. This supports the hypothesis that other uncultured organisms exist in the undefined microbial consortium capable of evolving mineralizing naphthalene. While the model microbial mixed cultures constructed using pure cultures were unsuccessful in mineralization of naphthalene, salicylic acid was mineralized to a greater extent (Table 5). Mineralization approached 100% after 1 week in mixed cultures, but remained under 52% by any individual isolate.

Discussion

The data presented conclusively shows that biodegradation of aromatic hydrocarbons can occur in extremely acidic environments. While involvement of individual heterotrophic, prokaryotic acidiphiles has not been eliminated, biodegradation in the downstream area of this particular drainage basin likely requires micro-eukaryotic organisms, such as fungi and yeast. Since $^{14}CO_2$ evolution from fungi has been shown to

Table 5. Mineralization of aromatic hydrocarbons by soil microorganisms[a].

microorganism (s)	salicylate	naphthalene
SRS1	51.02 ± 25.99	1.73 ± 1.22
SRS2	35.10 ± 6.84	2.36 ± 0.90
SRS3	36.87 ± 4.82	0.88 ± 0.33
SRS4	41.31 ± 6.02	0.00 ± 0.00
Fungus3	38.67 ± 0.37	0.00 ± 0.00
SRS1 / SRS2	82.60 ± 4.49	0.00 ± 0.00
SRS1 / SRS3	79.78 ± 1.48	0.84 ± 0.02
SRS2 / SRS3	64.89 ± 3.40	0.19 ± 0.10
SRS1 / SRS2 / SRS3	97.55 ± 3.87	0.43 ± 0.01
SRS1 / SRS2 / SRS3 / fungus1	93.88 ± 11.33	1.45 ± 0.66
SRS1 / SRS2 / SRS3 / fungus2	90.04 ± 0.89	0.35 ± 0.07
SRS1 / SRS2 / SRS3 / fungus3	32.08 ± 1.72	0.00 ± 0.00
SRS1 / SRS2 / SRS3 / fungus4	73.57 ± 4.21	0.47 ± 0.52
SRS1 / SRS2 / SRS3 / fungus5	86.59 ± 6.75	0.45 ± 0.44
undefined mixed culture[b]	93.34 ± 4.44	27.13 ± 15.73

[a] Microorganisms suspended in filter sterilized water sample from the downstream drainage basin area (pH2.0).

[b] The undefined mixed culture consisted of SRS1, SRS2, SRS3, fungus 3 and unidentified bacterial contaminants of the original fungus3 culture.

be rare (6), complete mineralization of the aromatic contaminants may involve a complex interaction between several distinct groups of microorganisms.

It is well understood that the biotransformation of PAH compounds by fungi primarily exists as a detoxification mechanism, with the secondary metabolites formed having lower toxicity than the parent compound (30). Moreover, In der Wiesch et al. (13) showed that the addition of soil microorganisms increased mineralization of the PAH pyrene. This study suggested that initial fungal, extracellular enzymatic attacks on the PAH produced intermediates that were available for further degradation by soil microorganisms. We hypothesize that a similar situation may explain the aromatic hydrocarbon mineralization activity detected under extremely acidic conditions. This hypothesis is supported by the demonstration that cyclohexamide treatment eliminates mineralization of both naphthalene and toluene in the sediment sample recovered form the downstream drainage basin area. The ability of organisms present in the soil samples to degrade these compounds but not hybridize to gene probes targeting biochemical pathways commonly associated with prokaryotic aromatic hydrocarbon degradation further supports the involvement of micro-eukaryotic organisms. Attempts to isolate pure cultures of aromatic hydrocarbon degrading acidiphilic microorganisms are ongoing. However, 16S rRNA sequence analysis clearly indicate the presence of known groups of acidiphilic bacteria in these samples.

Metabolism of salicylic acid was shown to be greatly affected by addition of the water sample from the downstream area. These data suggest that an unknown co-factor or nutrient may enhance levels of salicylic acid metabolism by acidiphilic bacteria.

Increased mineralization of salicylic acid was seen by consortia of microorganims when compared with single organisms.

Additional support on the potential for biodegradation of aromatic contaminants by acidiphilic bacteria comes from the work of Quentmeier and Freidrich (23). These experiments showed that plasmids encoding either phenol degradation or antibiotic resistance from neutrophilic bacteria could be acquired by *Acidiphilium cryptum* by conjugation. Once the genes were successfully transferred into the acidiphile, the encoded proteins were shown to be functional. This directly supports the hypothesis that genes encoding enzymes involved in degrading aromatic compounds can be acquired and expressed in heterotrophic acidiphiles.

To date, the great majority of studies focusing on aromatic hydrocarbon biodegradation have been performed using organisms isolated from non-extreme environments. Considering that aromatic hydrocarbon contamination has been documented within extreme environments, clearly research is needed to ascertain the ability of these environments to biodegrade such compounds. This study lends credence to the hypothesis that microbially-based degradation mechanisms are at work in extreme acidic ecosystems. Furthermore, this study suggests that instead of a single organism being responsible for complete mineralization of aromatic contaminants to carbon dioxide and water, biodegradation in these environments may be the result of complex interactions within the microbial community. Such consortium-based systems have recently been reported for compounds generally considered to be recalcitrant in nature (1, 3, 7, 17).

Acknowledgements

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Figure Legends

Figure 1. Mineralization of aromatic hydrocarbons by acidic soil samples. Panel A shows biodegradation by the source coal pile sample, panel B shows biodegradation by the downstream drainage basin sample, and panel C shows biodegradation by the control creek sample. Each soil sample was slurried with its corresponding water sample.

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SRS1 Sequence Data

TCTTACCTTGGCCNCATGNTTAACACATGCAAGTCGCACGGTCAGCAATGGCA

GTGGCGGACGGGTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGACAAC

AGCGGGAAACTGCTGCTAATACCGCATGATACCTGAGGGTCAAAGGCGCAAG

TCGCCTTGGGAGGAGCCTGCGTCTGATTAGCTTGTTGGTGGGGTAAAGGCCTA

CCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGAC

TGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAA

TGGGGGAAACCCTGATCCAGCAATGCCGCGTGTGTGTAGAAGGTCTTCGGATT

GTAAAGCACTTTTGGCAGGGACGATGATGACGGTACCTGCAGAATAACCCCG

GCTAACTTCCTGCCAGCAGCCGCGGTAATACGAANGGGGCTACCTTTGCTCGG

AATGACTGGGCGTAAAAGGCGCCTTAAGCCGCTTACACATCAAAATTAAATTC

CTGGGCTCAACCTGGGACTGCTTTTGATACTTTTTTCTAAATTAGAAAAAGTTT

GTTGAATTTCCATTTTAAAGTTNAAATCCGTAATATTGAAAGAACCCGGTTGC

AAAGGCGCACCTTGTCCCTTTACTGACCCT

[redacted]

Your query has been submitted, please wait for results...

BLASTN 1.4.11 [24-Nov-97] [Build 24-Nov-97]

Reference: Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-10.

Notice: this program and its default parameter settings are optimized to find nearly identical sequences rapidly. To identify weak similari encoded in nucleic acid, use BLASTX, TBLASTN or TBLASTX.

Query= tmpseq_1
(742 letters)

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
333,892 sequences; 664,089,867 total letters.
Searching.....done

Sequences producing High-scoring Segment Pairs:		High Score	Pr P(
emb|X91797|ASPGS19H	Acidocella sp. 16S ribosomal RNA gene	1942	4.
dbj|D86510|D86510	Acidocella sp. DNA for 16S rRNA	1969	8.
dbj|D30771|ACD16SRNAD	Acidiphilium aminolytica gene for .95	1936	1.
dbj|D30774|ACD16SRNAG	Acidiphilium facilis gene for 16S .96	1960	7.
gb|AF047643|	Uncultured eubacterium TRB25 16S r...	1584	2.
dbj|D30772|ACD16SRNAE	Acidiphilium angustum gene for 16S .83	1584	3.
dbj|D30768|ABC16SRNAA	Acetobacter aceti gene for 16S rib...	1624	1.
dbj|D86508|D86508	Acidiphilium sp. DNA for 16S rRNA ...	1584	1.
dbj|D30775|ACD16SRNAH	Acidiphilium organovorum gene for .85	1572	3.
dbj|D30773|ACD16SRNAF	Acidiphilium cryptum gene for 16S .85	1572	3.
emb|X91796|ASPGS18H	Acidiphilium sp. 16S ribosomal RNA...	1572	4.
emb|X74066|AE16SRRN	A.aceti gene for 16S rRNA	1615	9.
dbj|D86512|D86512	Acidosphaera rubrifaciens DNA for ...	1576	1.
emb|X86771|BS16SRRN3	Bacterial species 16S rRNA gene, c...	1563	1.
dbj|AB006712|AB006712	Acidiphilium multivorum gene for 1...	1572	2.
dbj|AB006711|AB006711	Acidiphilium multivorum gene for 1...	1572	2.
emb|X75265|AC16SRR	A.cryptum (B-Het4) gene for 16S ri...	1563	4.
emb|X71863|APRNA	A.pasteurianus gene for 16S riboso...	1588	1.
emb|AJ001632|APLTH16SR	Acetobacter pomorum 16S ribosomal ...	1588	1.
emb|X80775|GC16SRRNA	G.cerinus 16S rRNA gene	1566	1.
emb|X82290|GF16SRRNA	G.frateurii 16S rRNA gene	1561	3.
emb|X73820|GO16S	Gluconobacter oxydans gene for 16S...	1561	8.
emb|X77468|AM16SRNA1	A.methanolicus 16S rRNA gene	1552	4.
emb|X75618|AD16SRR	A.diazotrophicus gene for 16S ribo...	1525	7.
dbj|D30770|ADM16SRNAC	Acidomonas methanolica gene for 16...	1552	2.
emb|Y15289|AEJK216SR	Acetobacter europaeus 16S rRNA gene	1525	4.

emb|Z21936|AE16SRNAA	A.europaeus 16S rRNA gene	1525	4.
emb|X75617|AL16SRR	A.liquefaciens gene for 16S riboso...	1516	4.
dbj|D86511|D86511	Thiobacillus acidophilus DNA for 1...	1157	3.
emb|X75619|AX16SRR	A.xylinum gene for 16S ribosomal RNA	1516	5.
emb|Y14694|AI16RRNA	Acetobacter intermedius 16S rRNA gene	1534	8.
gb|L22481|HVT16S	Heliothis virescens testis endosym...	1097	9.
emb|X72908|RT16SRNA	R.thiosulatophilus gene for 16S rRNA	1581	1.
emb|AJ001631|AOLTH16SR	Acetobacter oboediens 16S ribosoma...	1525	4.
emb|X68471|UPBMC106	Unknown Proteobacterium, alpha-1 s...	1560	5.
emb|X80165|GA16SRRNA	G.asaii 16S rRNA gene	1530	1.
emb|Y07583|BSY07583	Bacterial species 16S rRNA gene (c...	1570	4.
gb|AF047648|	Uncultured eubacterium TRB3 16S ri...	1139	5.
emb|X75620|AH16SRR	A.hansenii gene for 16S ribosomal RNA	1498	8.
dbj|D86513|D86513	Rhodopila globiformis DNA for 16S ...	1153	2.
dbj|D30769|ACD16SRNAB	Acidiphilium sp. gene for 16S ribo...	1121	3.
gb|M59066|RBLRR16S	Rhodopseudomonas globiformis 16S r...	1144	4.
emb|X68474|UPBMC24	Unknown Proteobacterium, alpha-1 s...	1464	2.
emb|X68472|UPBMC74	Unknown Proteobacterium, alpha-1 s...	1076	2.
gb|AF050533|	Uncultured eubacterium WCHB1-87 16...	1103	1.
emb|X97085|APBTM120	Alpha-proteobacterium species 16S ...	1588	3.
emb|Y12351|RSPY12351	Rhizobium sp. 16S rRNA gene, parti...	1018	4.
dbj|D14432|RSPNCIMBG	Rhodospirillum salinarum (strain N...	1076	7.
emb|Y12596|UE16SR111	Unidentified eubacterium 16S rRNA ...	1076	7.
gb|AF007948|	Rasbo bacterium 16S ribosomal RNA ...	1018	7.
emb|Y12361|RGY12361	Rhizobium galegae 16S rRNA gene, p...	1014	2.
emb|Y12360|RGY12360	Rhizobium galegae 16S rRNA gene, p...	1014	2.
emb|Y12359|RGY12359	Rhizobium galegae 16S rRNA gene, p...	1014	2.
emb|Y12357|RGY12357	Rhizobium galegae 16S rRNA gene, p...	1014	2.
gb|M34127|RMVRRDA	R.vannielii 16S ribosomal RNA.	1024	3.
dbj|D14433|RSPNCIMBH	Rhodospirillum fulvum (strain NCIM...	1009	7.
emb|Y13212|MCY13212	Magnetic coccus small subunit rRNA...	1282	8.
gb|AF025853|AF025853	Rhizobium galegae strain 59A2 16S ...	1014	9.
gb|M59069|RSPRR16SD	Rhodospirillum salinarum 16S ribos...	1055	9.
emb|Z29637|AS16SRRN0	A.sp. (DSM 4835) 16S ribosomal RNA	1009	9.
dbj|D25305|MTB16SRR1	Methylobacterium sp. 16S rRNA gene	1053	2.
emb|X81188|PSCS40816	Magnetic coccus 16S rRNA gene (CS408)	991	4.
dbj|D45202|D45202	Eubacteria DNA for 16S rRNA	1018	5.
emb|X89815|RP16SRRN1	R.palustris DNA for 16S ribosomal RNA	1018	7.
emb|Y12355|RGY12355	Rhizobium galegae 16S rRNA gene, p...	1014	8.
emb|Y12358|RGY12358	Rhizobium galegae 16S rRNA gene, p...	1014	8.
emb|Y12356|RGY12356	Rhizobium galegae 16S rRNA gene, p...	1014	8.
emb|Y12352|RSPY12352	Rhizobium sp. 16S rRNA gene, parti...	996	8.
gb|U58014|UEU58014	Unidentified eubacterium MHP17 16S...	1045	9.
gb|AF050531|	Uncultured eubacterium WCHB1-55 16...	1018	1.
dbj|D25311|RHE16SRD1	Rhodoplanes elegans 16S rRNA gene	1009	1.
dbj|D32230|MTBJCMA07	Methylobacterium zatmanii gene for...	1044	1.
gb|M59072|RSPRR16SX	Rhodospirillum sodomense 16S ribos...	1046	1.
dbj|D14503|ATU16SRDI	Agrobacterium rubi gene for 16S ri...	996	1.
dbj|D25312|RBL16SRD2	Rhodopseudomonas palustris 16S rRN...	1027	2.
emb|X97089|APBTM212	Alpha-proteobacterium species 16S ...	1469	2.
gb|U87771|AGU87771	Afipia genosp. 5 16S ribosomal RNA...	1018	2.
emb|X87279|RP16S126S	R.palustris 16S rRNA gene	1027	2.
gb|M59068|RBLRR16SC	Rhodopseudomonas palustris 16S rib...	1027	2.

dbj|D12793|RHM16SRRNE	Rhizobium galegae 16S rRNA gene	1014	2.
emb|X67228|ARU16SRNA	A.rubi (LMG 156) gene for 16S rRNA	996	2.
dbj|D12787|ATU16S	Agrobacterium rubi 16S rRNA gene	996	2.
gb|AF025852|AF025852	Rhizobium huautlense 16S ribosomal...	996	2.
dbj|D14510|RHM16SRDN	Rhizobium sp. gene for 16S ribosom...	996	2.
dbj|D25306|MTB16SRR2	Methylobacterium sp. 16S rRNA gene	1053	3.
gb|M59067|RSPRR16S	Rhodospirillum molischianum 16S ri...	994	3.
dbj|AB004808|AB004808	Bradyrhizobium japonicum 16S rRNA,...	1036	3.
dbj|AB004807|AB004807	Bradyrhizobium japonicum 16S rRNA,...	1036	3.
dbj|D12700|RBLRRNA	Rhodopseudomonas palustris 16S rRN...	1021	5.
gb|L25709|SGBRRNA	Solemya reidi gill symbiont riboso...	1273	6.
emb|Z23156|MSP16RRNA	Metylobacterium sp. 16S rRNA gene	1044	8.
dbj|D11343|RHM16SRDB	Rhizobium galegae gene for 16S rib...	1014	8.
dbj|D32234|MTBF37A11	Methylobacterium sp. (strain F37) ...	1044	9.
emb|X81186|PSCS10516	Magnetic coccus 16S rRNA gene (CS105)	991	1.
gb|U89827|RLU89827	Rhizobium leguminosarum bv. viciae...	987	1.
gb|U89824|RLU89824	Rhizobium leguminosarum bv. viciae...	987	1.
gb|U73209|RLU73209	Rhizobium leguminosarum 16S riboso...	987	1.
gb|U73208|RLU73208	Rhizobium leguminosarum 16S riboso...	987	1.
emb|X67226|RG16SRRNA	R.galegae (LMG 6214) gene for 16S ...	1005	1.
emb|X67227|RL16SRRNA	R.leguminosarum (LMG 8820) gene fo...	987	1.

emb|X91797|ASPGS19H Acidocella sp. 16S ribosomal RNA gene
Length = 1442

Plus Strand HSPs:

Score = 1942 (536.6 bits), Expect = 4.6e-179, Sum P(5) = 4.6e-179
Identities = 400/416 (96%), Positives = 400/416 (96%), Strand = Plus

```
Query:     5 ACCTTGGCCNCATGNTTAACACATGCAAGTCGCACGGTCAGCAATGGCAGTGGCGGA
             ||  ||||  |||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct:     8 ACGCTGGCGGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGGCAGTGGCGGA

Query:    65 GTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGACAACAGCGGGAAACTGCTGC
             ||||||||||||||||||||||||   ||||||||||||||||||||||||||||||
Sbjct:    68 GTGAGTAACACGTAGGAATCTATCCAGAGGTGGGGGACAACAGCGGGAAACTGCTGC

Query:   125 TACCGCATGATACCTGAGGGTCAAAGGCGCAAGTCGCCTTGGGAGGAGCCTGCGTCT
             |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct:   128 TACCGCATGATACCTGAGGGTCAAAGGCGCAAGTCGCCTTTGGAGGAGCCTGCGTCT

Query:   185 TAGCTTGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAG
             |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct:   188 TAGCTTGTTGGTGGGGTAAAGGCTTACCAAGGCGACGATCAGTAGCTGGTCTGAGAG

Query:   245 GATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   248 GATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGG

Query:   305 TATTGGACAATGGGGGAAACCCTGATCCAGCAATGCCGCGTGTGTGTAGAAGGTCTT
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                ||||||||||||||| | || ||||||||||||||||||||||||| |||||||||
Sbjct:    308 TATTGGACAATGGGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTCTT

Query:    365 ATTGTAAAGCACTTTTGGCAGGGACGATGATGACGGTACCTGCAGAATAACCCCGG
              |||||||||||||||||||||||||||||||||||||||||||||||||| ||| |
Sbjct:    368 ATTGTAAAGCACTTTTGGCAGGGACGATGATGACGGTACCTGCAGAATAAGCCCCG

 Score = 354 (97.8 bits), Expect = 4.6e-179, Sum P(5) = 4.6e-179
 Identities = 75/81 (92%), Positives = 75/81 (92%), Strand = Plus / Pl

Query:    413 AACCCCGGCTAACTTCCTGCCAGCAGCCGCGGTAATACGAANGGGGCTACCTTTGCT
              | |||||||||||||| |||||||||||||||||||||| ||||||| | |||||
Sbjct:    417 AGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCT

Query:    473 AATGACTGGGCGTAAAAGGCG 493
              ||||||||||||||||| ||||
Sbjct:    477 AATGACTGGGCGTAAAGGGCG 497

 Score = 117 (32.3 bits), Expect = 4.6e-179, Sum P(5) = 4.6e-179
 Identities = 25/27 (92%), Positives = 25/27 (92%), Strand = Plus / Pl

Query:    515 AAATTAAATTCCTGGGCTCAACCTGGG 541
              || | ||||||||||||||||||||||
Sbjct:    520 AAGTGAAATTCCTGGGCTCAACCTGGG 546

 Score = 86 (23.8 bits), Expect = 4.6e-179, Sum P(5) = 4.6e-179
 Identities = 18/19 (94%), Positives = 18/19 (94%), Strand = Plus / Pl

Query:    539 GGGACTGCTTTTGATACTT 557
              ||||||||||||||||| |
Sbjct:    545 GGGACTGCTTTTGATACGT 563

 Score = 105 (29.0 bits), Expect = 4.6e-179, Sum P(5) = 4.6e-179
 Identities = 35/54 (64%), Positives = 35/54 (64%), Strand = Plus / Pl

Query:    633 AAAGGCGCACCTTGTCCCTTTACTGACCCTNAGCCCAAAANCTTTGGGAACCAA 68
              || |  ||| | ||  |||||||||| || || |   ||    |||| | |||
Sbjct:    646 AAGGCGGCAACCTGGTCCTTTACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAA 69


dbj|D86510|D86510 Acidocella sp. DNA for 16S rRNA
           Length = 1407

  Plus Strand HSPs:

 Score = 1969 (544.1 bits), Expect = 8.5e-179, Sum P(4) = 8.5e-179
 Identities = 403/416 (96%), Positives = 403/416 (96%), Strand = Plus

Query:      5 ACCTTGGCCNCATGNTTAACACATGCAAGTCGCACGGTCAGCAATGGCAGTGGCGGA
              ||  |||| |||| |||||||||||||||| |||||||||||||| ||||||
Sbjct:      6 ACGCTGGCGGCATGCTTAACACATGCAAGTCGCGCGGTCAGCAATGGCAGCGGCGGA
```

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Query:    65 GTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGACAACAGCGGGAAACTGCTGC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:    66 GTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGACAACAGCGGGAAACTGCTGC

Query:   125 TACCGCATGATACCTGAGGGTCAAAGGCGCAAGTCGCCTTGGGAGGAGCCTGCGTCT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   126 TACCGCATGATACCTGAGGGTCAAAGGCGCAAGTCGCCTTGGGAGGAGCCTGCGTCT

Query:   185 TAGCTTGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   186 TAGCTTGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAG

Query:   245 GATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   246 GATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGG

Query:   305 TATTGGACAATGGGGGAAACCCTGATCCAGCAATGCCGCGTGTGTGTAGAAGGTCTT
             ||||||||||||||  | ||  ||||||||||||||||||||||||  ||||||||||
Sbjct:   306 TATTGGACAATGGGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTCTT

Query:   365 ATTGTAAAGCACTTTTGGCAGGGACGATGATGACGGTACCTGCAGAATAACCCCGG
             ||||||||||||||||||||||||||||||||||||||||||||||||||| ||| |
Sbjct:   366 ATTGTAAAGCACTTTTGGCAGGGACGATGATGACGGTACCTGCAGAATAAGCCCCG

 Score = 360 (99.5 bits), Expect = 8.5e-179, Sum P(4) = 8.5e-179
 Identities = 77/84 (91%), Positives = 77/84 (91%), Strand = Plus / Pl

Query:   413 AACCCCGGCTAACTTCCTGCCAGCAGCCGCGGTAATACGAANGGGGCTACCTTTGCT
             | ||||||||||||||| ||||||||||||||||||||||| ||||||| | |||||
Sbjct:   415 AGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCT

Query:   473 AATGACTGGGCGTAAAAGGCGCCT 496
             |||||||||||||||| ||||| |
Sbjct:   475 AATGACTGGGCGTAAAGGGCGCGT 498

 Score = 111 (30.7 bits), Expect = 8.5e-179, Sum P(4) = 8.5e-179
 Identities = 24/27 (88%), Positives = 24/27 (88%), Strand = Plus / Pl

Query:   515 AAATTAAATTCCTGGGCTCAACCTGGG 541
             || | |||| ||||||||||||||||
Sbjct:   518 AAGTGAAATNCCTGGGCTCAACCTGGG 544

 Score = 86 (23.8 bits), Expect = 8.5e-179, Sum P(4) = 8.5e-179
 Identities = 18/19 (94%), Positives = 18/19 (94%), Strand = Plus / Pl

Query:   539 GGGACTGCTTTTGATACTT 557
             |||||||||||||||| |
Sbjct:   543 GGGACTGCTTTTGATACGT 561
```

dbj|D30771|ACD16SRNAD Acidiphilium aminolytica gene for 16S ribosomal
Length = 1406

SRS2 Sequence Data

TACGACCTGGCCGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGGGC

AGTGNGCGGACGGGTGAGTAACACGTNGGAATCTATCCCAGGGTGGGGGGAC

ACAGCNGGGAAAACTGCTGCTAATNCCGCATGATACCTGAAGGGTCAAAGGC

GCCAGTCCCCTTNGGANGAACCTGCCTCTGATTAACNTGTTGGTGGGGTTNAN

GCCNACCCAAGCGACCATCANTTTCTGGTCNGANAAGATNATCNNCCNCCTTG

GGACTGANACACNGCCCCAACTCCNNCCGGANGCAACNGTGGGGAATATTGG

ACCATGGGGGAAACCNGATTCAGCAATNCCNCNTGTNTNAANAAAGTCTTCC

GATTGTNAANCACTTTTGGCANGGACGATNATNACNGTACCTGCAATANNCC

CCCCCGGCTTACTTCCTNCCANCAACCNCNGTTAATNCCAAAGGGGCNAGCCT

TTGCTCCCGAAATGACTGGGCCTTTAAAGGGCCCTTNTGGCGGCGTTTCACCN

TTCCGAAATTTAAAATTCCCTGGGGCTCCACCCTGGGGGACTGCCTTTTTGAA

AACCTTTTTTTTTCTAAAATAGAAAAGGGTTTTTTNNAAAANNNTTCCCCCCC

CTTTTNTAAAGGTT

[redacted]

Your query has been submitted, please wait for results...

BLASTN 1.4.11 [24-Nov-97] [Build 24-Nov-97]

Reference: Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. Biol. 215:403-10.

Notice: this program and its default parameter settings are optimized to find nearly identical sequences rapidly. To identify weak similari encoded in nucleic acid, use BLASTX, TBLASTN or TBLASTX.

Query= tmpseq_1
(904 letters)

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
333,892 sequences; 664,089,867 total letters.
Searching.....done

G: Gluconobacter

Sequences producing High-scoring Segment Pairs:		High Score	Pr P(
dbj|D86510|D86510	Acidocella sp. DNA for 16S rRNA	631	8.
dbj|D30774|ACD16SRNAG	Acidiphilium facilis gene for 16S ...	631	8.
emb|X91797|ASPGS19H	Acidocella sp. 16S ribosomal RNA gene	622	1.
dbj|D30771|ACD16SRNAD	Acidiphilium aminolytica gene for ...	616	4.
dbj|D30768|ABC16SRNAA	Acetobacter aceti gene for 16S rib...	604	8.
emb|X74066|AE16SRRN	A.aceti gene for 16S rRNA	595	5.
emb|Y07647|BS16SR3C	Bacterial species 16S rRNA gene (p...	651	7.
emb|X82290|GF16SRRNA	G.frateurii 16S rRNA gene	568	1.
emb|X80775|GC16SRRNA	G.cerinus 16S rRNA gene	568	1.
emb|X73820|GO16S	Gluconobacter oxydans gene for 16S...	568	4.
emb|Y07583|BSY07583	Bacterial species 16S rRNA gene (c...	559	1.
emb|X80165|GA16SRRNA	G.asaii 16S rRNA gene	550	1.
emb|Y12362|RGY12362	Rhizobium galegae 16S rRNA gene, p...	673	2.
emb|X97089|APBTM212	Alpha-proteobacterium species 16S ...	595	3.
gb|U09829|UAU09829	Unknown alpha proteobacterium clon...	616	5.
emb|X71863|APRNA	A.pasteurianus gene for 16S riboso...	586	1.
emb|Y14694|AI16RRNA	Acetobacter intermedius 16S rRNA gene	568	1.
emb|AJ001631|AOLTH16SR	Acetobacter oboediens 16S ribosoma...	568	1.
emb|X75618|AD16SRR	A.diazotrophicus gene for 16S ribo...	568	1.
emb|AJ001632|APLTH16SR	Acetobacter pomorum 16S ribosomal ...	586	1.
emb|X77468|AM16SRNA1	A.methanolicus 16S rRNA gene	577	6.
emb|X75620|AH16SRR	A.hansenii gene for 16S ribosomal RNA	568	6.
emb|Y15289|AEJK216SR	Acetobacter europaeus 16S rRNA gene	568	6.
emb|Z21936|AE16SRNAA	A.europaeus 16S rRNA gene	568	6.
emb|X97085|APBTM120	Alpha-proteobacterium species 16S ...	586	1.
dbj|D86512|D86512	Acidosphaera rubrifaciens DNA for ...	581	2.

dbj|D30770|ADM16SRNAC	Acidomonas methanolica gene for 16...	568	3.
emb|X75619|AX16SRR	A.xylinum gene for 16S ribosomal RNA	559	3.
emb|X75617|AL16SRR	A.liquefaciens gene for 16S riboso...	550	3.
emb|X93475|PBBN9902R	Phototrophic bacterium 16S rRNA ge...	646	4.
dbj|AB006037|AB006037	Agrobacterium sp. gene for 16S rRNA	623	5.
gb|L22481|HVT16S	Heliothis virescens testis endosym...	482	1.
gb|AF047643|	Uncultured eubacterium TRB25 16S r...	559	1.
dbj|D30772|ACD16SRNAE	Acidiphilium angustum gene for 16S...	559	2.
emb|X86771|BS16SRRN3	Bacterial species 16S rRNA gene, c...	550	2.
dbj|AB006712|AB006712	Acidiphilium multivorum gene for 1...	550	2.
dbj|D30775|ACD16SRNAH	Acidiphilium organovorum gene for ...	550	2.
dbj|D30773|ACD16SRNAF	Acidiphilium cryptum gene for 16S ...	550	2.
emb|X91796|ASPGS18H	Acidiphilium sp. 16S ribosomal RNA...	550	2.
dbj|AB006711|AB006711	Acidiphilium multivorum gene for 1...	550	3.
dbj|D86508|D86508	Acidiphilium sp. DNA for 16S rRNA ...	559	1.
emb|X75265|AC16SRR	A.cryptum (B-Het4) gene for 16S ri...	550	9.
gb|U71005|BSU71005	Brucella sp. A4/I 16S ribosomal RN...	616	2.
emb|X93482|PBDSM4180	Phototrophic bacterium 16S rRNA ge...	634	2.
emb|X81840|CPRRNA16S	C.paradoxa gene for 16S small subu...	583	6.
emb|X68474|UPBMC24	Unknown Proteobacterium, alpha-1 s...	523	1.
emb|Y10825|RE18516SR	Ralstonia eutropha 16S rRNA gene, ...	639	2.
dbj|D84429|D84429	Porphyrobacter tepidarius gene for...	608	4.
emb|Y11637|UEY11637	Unidentified eubacterium 16S rRNA ...	625	1.
gb|AF022403|	Shewanella sp. ANG.309 16S ribosom...	600	3.
gb|L35468|GPSRGDE	Gamma Proteobacterial sp. (clone S...	626	3.
dbj|D86513|D86513	Rhodopila globiformis DNA for 16S ...	492	6.
dbj|D86511|D86511	Thiobacillus acidophilus DNA for 1...	508	1.
emb|X75045|SSRRNTRN	Spirulina sp. genes for 16S rRNA, ...	566	1.
gb|U03777|CRU03777	Cowdria ruminantium 16S rRNA gene.	593	1.
dbj|D84009|PSEIAM08	Pseudomonas azotoformans 16S rRNA ...	604	2.
gb|M59066|RBLRR16S	Rhodopseudomonas globiformis 16S r...	483	3.
gb|AF047648|	Uncultured eubacterium TRB3 16S ri...	499	5.
emb|X68471|UPBMC106	Unknown Proteobacterium, alpha-1 s...	547	6.
gb|AF008130|AF008130	Rhizobium gallicum strain R602sp 1...	619	7.
gb|AF008129|AF008129	Rhizobium gallicum strain FL27 16S...	619	7.
gb|AF008128|AF008128	Rhizobium gallicum strain CbS-18 1...	619	7.
gb|AF008127|AF008127	Rhizobium gallicum strain CbS-17 1...	619	7.
gb|AF008126|AF008126	Rhizobium gallicum strain CbS-1 16...	619	7.
gb|U54805|ESU54805	Ehrlichia sp. strain Germishuys 16...	593	1.
emb|X84609|UNSBR2078	Unknown organism, partial 16S rRNA...	510	2.
gb|U37762|PLU37762	Prionitis lanceolata gall symbiont...	555	5.
dbj|AB004806|AB004806	Bradyrhizobium japonicum 16S rRNA,...	591	5.
emb|X97114|PBRRTM234	Proteobacterium species 16S rRNA g...	477	1.
gb|AF039055|AF039055	Shewanella oneidensis strain SP-22...	604	1.
emb|X97113|PBRRTM20	Proteobacterium species 16S rRNA g...	535	1.
gb|U41049|SOU41049	Solemya occidentalis gill symbiont...	485	2.
gb|M27534|RBLRRSS	R.marina ribosomal RNA small subunit.	501	3.
emb|X72908|RT16SRNA	R.thiosulatophilus gene for 16S rRNA	487	5.
dbj|D30769|ACD16SRNAB	Acidiphilium sp. gene for 16S ribo...	481	6.
gb|AF001730|AF001730	Unidentified rumen bacterium RCP6 ...	617	8.
dbj|D88000|D88000	Ralstonia eutropha DNA 16S ribosom...	565	1.
dbj|D30790|RDM16SRNAA	Rhodobium marinum 16S rRNA gene, c...	495	1.
gb|AF018560|	Unidentified rumen bacterium 12-74...	565	2.

gb|U22537|SSSSURNAS1	Sphingomonas sp. small subunit rib...	483	2.
dbj|AB002660|AB002660	Unidentified gamma proteobacterium...	601	4.
gb|AF050533|	Uncultured eubacterium WCHB1-87 16...	451	8.
gb|AF001711|AF001711	Unidentified rumen bacterium RC21 ...	595	1.
dbj|D50614|D50614	Synechococcus sp. gene for 16S rRN...	562	1.
gb|U64022|UEU64022	Unidentified eubacterium SCB45 16S...	498	1.
emb|Z25522|PB16SRNAA	Purple bacteria, gamma subdivision...	537	1.
gb|M63814|CYARGDB	Cyanobacterium sp. 16S ribosomal RNA.	571	1.
emb|X68472|UPBMC74	Unknown Proteobacterium, alpha-1 s...	460	1.
gb|U63956|SSU63956	Sphingomonas sp. 16S ribosomal RNA...	483	1.
emb|X87164|SF16SRRN1	S.flava 16S rRNA gene, strain 'Pse...	483	1.
emb|X97115|PBRRTM205	Proteobacterium species 16S rRNA g...	450	2.
emb|X87277|LM16S2157	L.mucor 16S rRNA gene	480	2.
gb|AF009994|	Unidentified eubacterium 16S ribos...	508	2.
gb|U63937|SSU63937	Sphingomonas sp. 16S ribosomal RNA...	483	2.
emb|Z49716|LADELSSRN	L.adelaidensis gene for ribosomal ...	500	4.
emb|X84630|UNSBR2101	Unknown organism, partial 16S rRNA...	489	4.
gb|U10876|MCU10876	Moraxella catarrhalis ATCC 25238 1...	532	4.
emb|X87163|SF16SRRNA	S.flava 16S rRNA gene, strain 'Fla...	483	4.
gb|U40338|MAU40338	Microcystis aeruginosa PCC7005 16S...	508	5.
emb|Z73631|SSPRB2256	Sphingomonas sp. 16S rRNA gene	483	5.

dbj|D86510|D86510 Acidocella sp. DNA for 16S rRNA
Length = 1407

Plus Strand HSPs:

Score = 202 (55.8 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
Identities = 42/44 (95%), Positives = 42/44 (95%), Strand = Plus / Pl

```
Query:    32 CTGGCCGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGG 75
             ||||| ||||||||||||||||||||||| ||||||||||||
Sbjct:     9 CTGGCGGCATGCTTAACACATGCAAGTCGCGCGGTCAGCAATGG 52
```

Score = 242 (66.9 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
Identities = 52/58 (89%), Positives = 52/58 (89%), Strand = Plus / Pl

```
Query:    83 GCGGACGGGTGAGTAACACGTNGGAATCTATCCCAGGGTGGGGGGACACAGCNGGGA
             |||||||||||||||||||| |||||||||||||||||||||   ||||| || |
Sbjct:    58 GCGGACGGGTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGGACAACAGCGGGAA
```

Score = 156 (43.1 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
Identities = 33/36 (91%), Positives = 33/36 (91%), Strand = Plus / Pl

```
Query:   137 GGAAAACTGCTGCTAATNCCGCATGATACCTGAAGG 172
             || |||||||||||||| |||||||||||||| ||
Sbjct:   110 GGGAAACTGCTGCTAATACCGCATGATACCTGAGGG 145
```

Score = 631 (174.4 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
Identities = 146/185 (78%), Positives = 146/185 (78%), Strand = Plus

```
Query:   170 AGGGTCAAAGGCGCCAGTCCCCTTNGGANGAACCTGCCTCTGATTAACNTGTTGGTG
             ||||||||||||||| |||| |||| ||| || ||||| |||||||| | ||||||||
Sbjct:   142 AGGGTCAAAGGCGCAAGTCGCCTTGGGAGGAGCCTGCGTCTGATTAGCTTGTTGGTG

Query:   230 TTNANGCCNACCCAAGCGACCATCANTTTCTGGTCNGANAAGATNATCNNCCNCCTT
             |  | ||| ||| | ||||| |||| |  |||||| || | ||| |||  || | ||
Sbjct:   202 TAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATT

Query:   290 ACTGANACACNGCCCCAACTCCNNCCGGANGCAACNGTGGGGAATATTGGACCATGG
             ||||| |||| |||| ||||||  | ||| ||| | |||||||||||||||| ||||
Sbjct:   262 ACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG

Query:   350 AAACC 354
              || |
Sbjct:   322 CAAGC 326

 Score = 328 (90.6 bits), Expect = 9.7e-17, P = 9.7e-17
 Identities = 73/92 (79%), Positives = 73/92 (79%), Strand = Plus / Pl

Query:   345 GGGGGAAACCNGATTCAGCAATNCCNCNTGTNTNAANAAAGTCTTCCGATTGTNAAN
             || | || || ||| ||||||| || | ||| | || || |||||| |||||| ||
Sbjct:   318 GGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAG

Query:   405 TTTTGGCANGGACGATNATNACNGTACCTGCA 436
             |||||||| ||||||| || || |||||||||
Sbjct:   378 TTTTGGCAGGGACGATGATGACGGTACCTGCA 409
```

dbj|D30774|ACD16SRNAG Acidiphilium facilis gene for 16S ribosomal RNA
Length = 1407

Plus Strand HSPs:

```
 Score = 202 (55.8 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
 Identities = 42/44 (95%), Positives = 42/44 (95%), Strand = Plus / Pl

Query:    32 CTGGCCGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGG 75
             ||||| ||||||||||||||||||||||| ||||||||||||
Sbjct:     9 CTGGCGGCATGCTTAACACATGCAAGTCGCGCGGTCAGCAATGG 52

 Score = 242 (66.9 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
 Identities = 52/58 (89%), Positives = 52/58 (89%), Strand = Plus / Pl

Query:    83 GCGGACGGGTGAGTAACACGTNGGAATCTATCCCAGGGTGGGGGGACACAGCNGGGA
             |||||||||||||||||||| |||||||||||||||||||||   ||||| || |
Sbjct:    58 GCGGACGGGTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGACAACAGCGGGAA

 Score = 156 (43.1 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
 Identities = 33/36 (91%), Positives = 33/36 (91%), Strand = Plus / Pl

Query:   137 GGAAAACTGCTGCTAATNCCGCATGATACCTGAAGG 172
             || |||||||||||||| |||||||||||||| ||
```

```
Sbjct:   110 GGGAAACTGCTGCTAATACCGCATGATACCTGAGGG 145

 Score = 631 (174.4 bits), Expect = 8.6e-72, Sum P(4) = 8.6e-72
 Identities = 146/185 (78%), Positives = 146/185 (78%), Strand = Plus

Query:   170 AGGGTCAAAGGCGCCAGTCCCCTTNGGANGAACCTGCCTCTGATTAACNTGTTGGTG
             ||||||||||||||| |||| |||| ||| || ||||| |||||||| | ||||||||
Sbjct:   142 AGGGTCAAAGGCGCGAGTCGCCTTGGGAGGAGCCTGCGTCTGATTAGCTTGTTGGTG

Query:   230 TTNANGCCNACCCAAGCGACCATCANTTTCTGGTCNGANAAGATNATCNNCCNCCTT
             |  | ||| ||| | ||||| |||| |  |||||| || | ||| |||  || | ||
Sbjct:   202 TAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATT

Query:   290 ACTGANACACNGCCCCAACTCCNNCCGGANGCAACNGTGGGGAATATTGGACCATGG
             ||||| |||| |||| ||||||  | ||| ||| | ||||||||||||||| ||||
Sbjct:   262 ACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG

Query:   350 AAACC 354
              || |
Sbjct:   322 CAAGC 326

 Score = 328 (90.6 bits), Expect = 9.7e-17, P = 9.7e-17
 Identities = 73/92 (79%), Positives = 73/92 (79%), Strand = Plus / Pl

Query:   345 GGGGGAAACCNGATTCAGCAATNCCNCNTGTNTNAANAAAGTCTTCCGATTGTNAAN
             || | || || ||| ||||||| || | ||| | || || |||||| |||||| ||
Sbjct:   318 GGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAG

Query:   405 TTTTGGCANGGACGATNATNACNGTACCTGCA 436
             |||||||| ||||||| || || |||||||||
Sbjct:   378 TTTTGGCAGGGACGATGATGACGGTACCTGCA 409


emb|X91797|ASPGS19H Acidocella sp. 16S ribosomal RNA gene
           Length = 1442

  Plus Strand HSPs:

 Score = 211 (58.3 bits), Expect = 1.5e-69, Sum P(4) = 1.5e-69
 Identities = 43/44 (97%), Positives = 43/44 (97%), Strand = Plus / Pl

Query:    32 CTGGCCGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGG 75
             ||||| ||||||||||||||||||||||||||||||||||||||
Sbjct:    11 CTGGCGGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGG 54

 Score = 215 (59.4 bits), Expect = 1.5e-69, Sum P(4) = 1.5e-69
 Identities = 49/58 (84%), Positives = 49/58 (84%), Strand = Plus / Pl

Query:    83 GCGGACGGGTGAGTAACACGTNGGAATCTATCCCAGGGTGGGGGGACACAGCNGGGA
             |||||||||||||||||||| |||||||||||   ||||||||   ||||| || |
Sbjct:    60 GCGGACGGGTGAGTAACACGTAGGAATCTATCCAGAGGTGGGGGACAACAGCGGGAA
```

```
 Score = 156 (43.1 bits), Expect = 1.5e-69, Sum P(4) = 1.5e-69
 Identities = 33/36 (91%), Positives = 33/36 (91%), Strand = Plus / Pl

Query:   137 GGAAAACTGCTGCTAATNCCGCATGATACCTGAAGG 172
             || ||||||||||||||| ||||||||||||||| ||
Sbjct:   112 GGGAAACTGCTGCTAATACCGCATGATACCTGAGGG 147

 Score = 622 (171.9 bits), Expect = 1.5e-69, Sum P(4) = 1.5e-69
 Identities = 145/185 (78%), Positives = 145/185 (78%), Strand = Plus

Query:   170 AGGGTCAAAGGCGCCAGTCCCCTTNGGANGAACCTGCCTCTGATTAACNTGTTGGTG
             ||||||||||||||| |||| |||| ||| || ||||| |||||||| | ||||||||
Sbjct:   144 AGGGTCAAAGGCGCAAGTCGCCTTTGGAGGAGCCTGCGTCTGATTAGCTTGTTGGTG

Query:   230 TTNANGCCNACCCAAGCGACCATCANTTTCTGGTCNGANAAGATNATCNNCCNCCTT
             |  | ||  ||| | ||||| |||| |  |||||| || | ||| |||  || | ||
Sbjct:   204 TAAAGGCTTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATT

Query:   290 ACTGANACACNGCCCCAACTCCNNCCGGANGCAACNGTGGGGAATATTGGACCATGG
             ||||| |||| |||| ||||||  | ||| ||| | ||||||||||||||||| ||||
Sbjct:   264 ACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG

Query:   350 AAACC 354
              || |
Sbjct:   324 CAAGC 328

 Score = 328 (90.6 bits), Expect = 9.7e-17, P = 9.7e-17
 Identities = 73/92 (79%), Positives = 73/92 (79%), Strand = Plus / Pl

Query:   345 GGGGGAAACCNGATTCAGCAATNCCNCNTGTNTNAANAAAGTCTTCCGATTGTNAAN
             || | || || ||| ||||||| || | ||| | || || |||||| |||||| ||
Sbjct:   320 GGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAG

Query:   405 TTTTGGCANGGACGATNATNACNGTACCTGCA 436
             |||||||| ||||||| || || |||||||||
Sbjct:   380 TTTTGGCAGGGACGATGATGACGGTACCTGCA 411
```

dbj|D30771|ACD16SRNAD Acidiphilium aminolytica gene for 16S ribosomal
Length = 1406

```
  Plus Strand HSPs:

 Score = 211 (58.3 bits), Expect = 4.3e-69, Sum P(4) = 4.3e-69
 Identities = 43/44 (97%), Positives = 43/44 (97%), Strand = Plus / Pl

Query:    32 CTGGCCGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGG 75
             ||||| ||||||||||||||||||||||||||||||||||||||
Sbjct:     9 CTGGCGGCATGCTTAACACATGCAAGTCGCACGGTCAGCAATGG 52

 Score = 215 (59.4 bits), Expect = 4.3e-69, Sum P(4) = 4.3e-69
 Identities = 49/58 (84%), Positives = 49/58 (84%), Strand = Plus / Pl
```

SRS4 Sequence Data

TGACGCTGGCGGCATGCTTAACACATGCAAGTCGAACGGCAGCACAGCA

GTAGCAATACTGTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGG

ACCTGCCCAGACGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATA

CGTCCTACGGGAGAAAGCGGGGGATCGCAAGACCTCGCGCGGTTGGATGGAC

CGATGTTCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGA

TAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGA

CTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGAT

CCAGCAATGCCGCGTTGTTGTTGAAGAAAGGCCTTCGGGTTGTTAAAGCACTT

TTATCCAGGAGCCGAAACGCTGTCGGCTAATACCCCGGCGGAACTTGACGGTA

CCTTGAAGAAATAAGCACCNGCTAACTTCCGTGCCCACCACCGCGGTAATACA

AAGGTTGCAACCGTTTAATCCGAATTACTGGCCGTTANCCTTGCGTTTAGCGG

TTGAATTTANTTCTGCTNGTGAAATCCCCCNGGCTCCACCCTTGGAAATGGCA

GTTGAAAACTGGATTCCCTAAATT

Data Collection

File: Sample 20
Sample: SRS-4/27f
Comment: 610 ng/ul - 0.74 ul/rxn
Lane Number: 20
Channel Number: 102
Number of Scans: 8350
Length: 755
Run started at: 6/4/1998, 16:13
Run stopped at: 7/4/1998, 06:11
Gel: New Gel File
Dyeset/Primer: DT4%Ac{A Set-AnyPrimer}
Comb: 36-well sharks-tooth
Instrument Name: 94112221S.MatrixFile
Collect Vers.: N/A

E2

Data Analysis

Base Call Start: 975
Base Call End: 8350
Primer Peak Loc.:975
Signal: G (189), A (418), T (176), C (105)
Matrix Name: 94112221S.MatrixFile
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 11.51 - SemiAdaptive

```
  1 NTGACGCTGG CGGCATGCTT AACACATGCA AGTCGAACGG CAGCACAGCA GTAGCAATAC TGTGGGTGGC GAGTGGCGGA CGGGTGAGGA ATACATCGGG ACCTGCCCAG 110
111 ACGTGGGGGA TAACGTAGGG AAACTTACGC TAATACCGCA TACGTCCTAC GGGAGAAAGC GGGGGATCGC AAGACCTCGC GCGGTTGGAT GGACCGATGT TCGATTAGCT 220
221 AGTTGGTGAG GTAATGGCTC ACCAAGGCGA CGATCGATAG CTGGTCTGAG AGGATGATCA GCCACACTGG GACTGAGACA CGGCCCAGAC TCCTACGGGA GGCAGCAGTG 330
331 GGGAATATTG GACAATGGGC GCAAGCCTGA TCCAGCAATG CCGCGTTGTT GTTGAAGAAA GGCCTTCGGG TTGTTAAAGC ACTTTTATCC AGGAGCCGAA ACGCTGTCGG 440
441 CTAATACCCC GGCGGAACTT GACGGTACCT TGAAGAAATA AGCACCNGCT AACTTCCGTG CCCACCACCG CGGTAATACA AAGGTTGCAA CCGTTTAATC CGAATTACTG 550
551 GCCGTTANCC TTGCGTTTAG CGGTTGAATT TANTTCTGCT NGTGAAATCC CCCNGGCTCC ACCCTTGGAA ATGGCAGTTG AAAACTGGAT TCCCTAAATT NTTANAAAGA 660
661 NGGTTNGGNA AATTCCCCGG TTTTTTACCG TTNAATGCCT TTAAAAAATT CGGGNAAGNA AACTCCCNTT NGCNAAAAGG CNGCCNTNCT GGNTN 770
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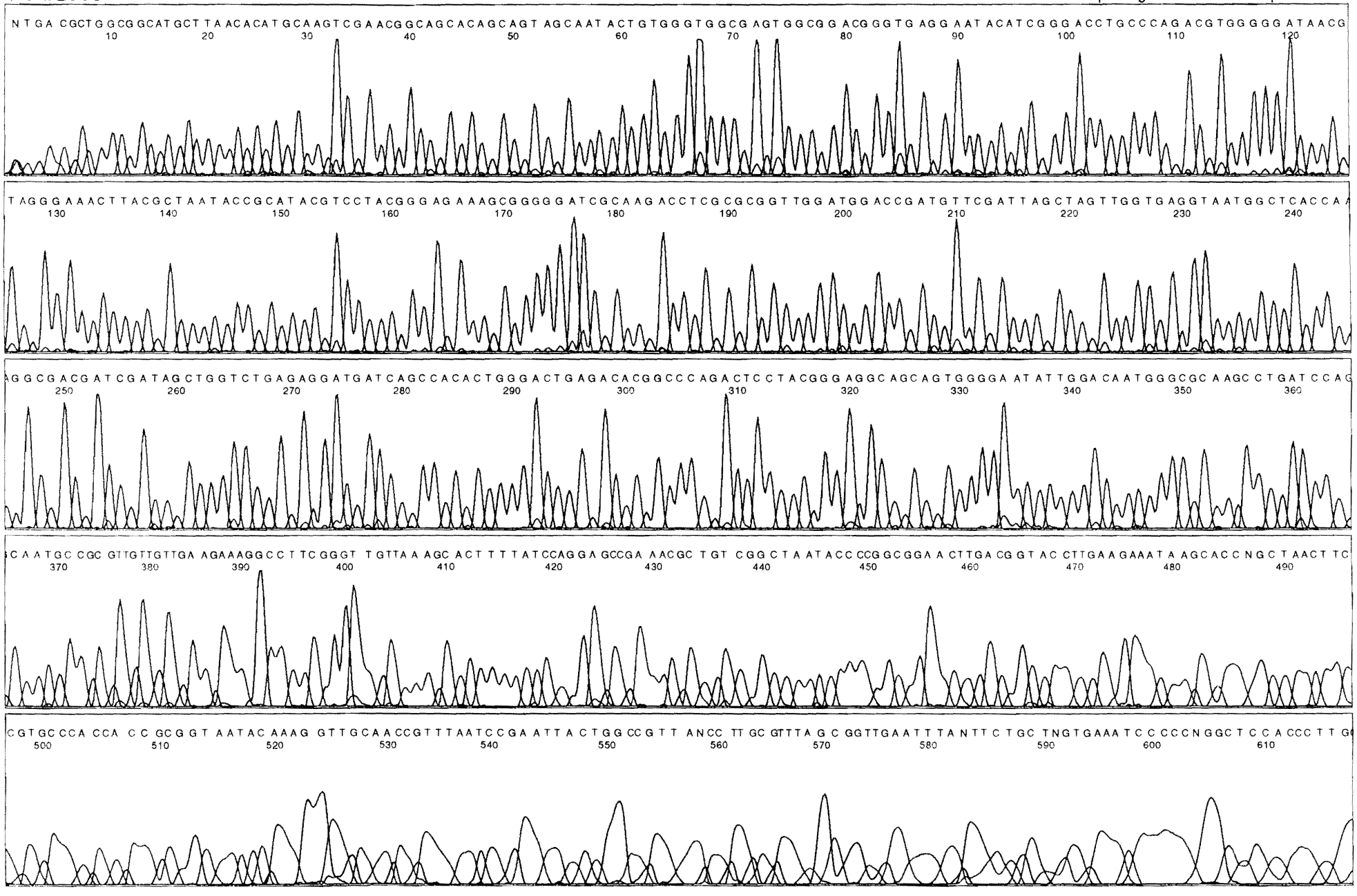
ABI PRISM

Model
Version 2.1.1

Sample 20
610 ng/ul - 0.74 ul/rxn
SRS-4/27f
Lane 20

Signal G:189 A:418 T:176 C:105
DT4%Ac{A Set-AnyPrimer}
94112221S.MatrixFile
Points 975 to 8350 Base 1: 975

Apr, 7, 1998 6:36 AM
Apr, 6, 1998 4:13 PM
Spacing: 11.50 SemiAdaptive



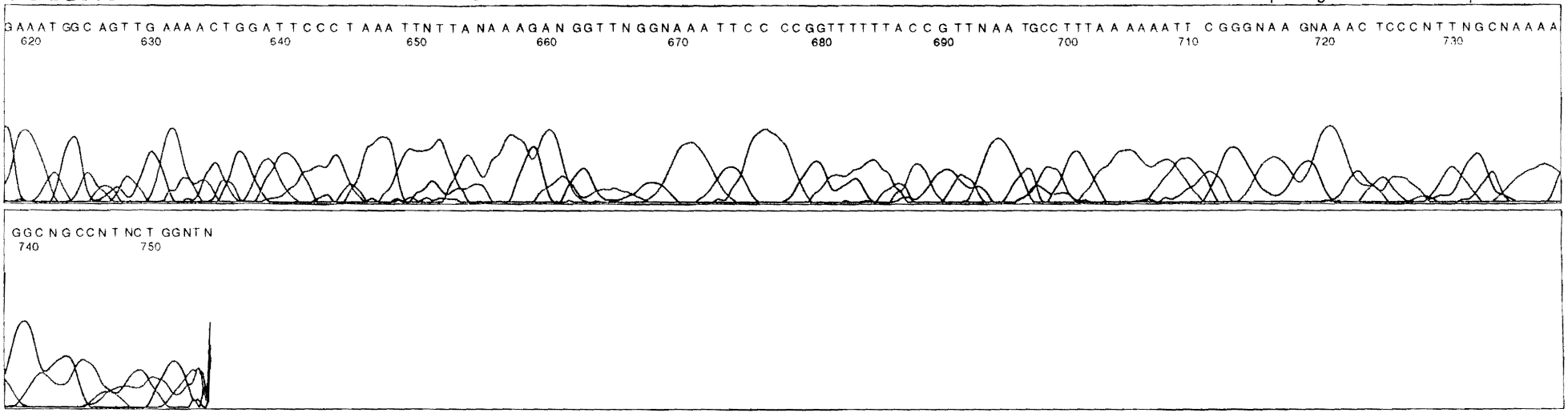
ABI PRISM

Model
Version 2.1.1

Sample 20
610 ng/ul - 0.74 ul/rxn
SRS-4/27f
Lane 20

Signal G:189 A:418 T:176 C:105
DT4%Ac{A Set-AnyPrimer}
94112221S.MatrixFile
Points 975 to 8350 Base 1: 975

Apr, 7, 1998 6:36 AM
Apr, 6, 1998 4:13 PM
Spacing: 11.50 SemiAdaptive



E4

NCBI BLAST Search Results Entrez ?

Your query has been submitted, please wait for results...

BLASTN 1.4.11 [24-Nov-97] [Build 24-Nov-97]

Reference: Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. Biol. 215:403-10.

Notice: this program and its default parameter settings are optimized to find nearly identical sequences rapidly. To identify weak similari encoded in nucleic acid, use BLASTX, TBLASTN or TBLASTX.

Query= tmpseq_1
(755 letters)

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
338,156 sequences; 680,895,594 total letters.
Searching.....done

Sequences producing High-scoring Segment Pairs:		High Score	Pr P(	S
gb|M26601|XYLSSRNA	X.fastidiosa small subunit ribosom...	1552	5.	
emb|X95918|XARR494	X.albilineans 16S rRNA gene	1533	2.	
emb|Y10756|XMY10756	X.melonis 16SrRNA gene	1524	1.	
emb|X99298|XC16S726	X.campestris 16S rRNA gene, strain...	1515	6.	
emb|Y10754|XHY10754	X.hyacinthi 16S rRNA gene	1509	1.	
emb|AJ000921|PSJ000921	Proteobacterium sp. partial 16S rR...	1253	2.	
gb|U51103|DFU51103	Denitrifying Fe/II/-oxidizing bact...	1513	4.	
emb|AJ002807|SSPAJ2807	Stenotrophomonas sp. 16S rRNA gene...	1456	4.	
emb|X95923|SMRR958	S.maltophilia 16S rRNA gene, strai...	1479	5.	
gb|AF012541|	Iron-oxidizing lithotroph ES-1 16S...	1479	1.	
emb|Y10766|XSY10766	X.sacchari 16S rRNA gene	1465	8.	
emb|AJ002806|SSPAJ2806	Stenotrophomonas sp. 16S rRNA gene...	1455	5.	
emb|Y10755|XVY10755	X.vasicola 16S rRNA gene	1311	1.	
emb|X95919|XARR538	X.axonopodis 16S rRNA gene	1311	1.	
emb|X84636|UNSBR2112	Unknown organism, partial 16S rRNA...	1495	2.	
emb|Y10761|XVY10761	X.vesicatoria 16S rRNA gene	1302	7.	
emb|Y10763|XTY10763	X.theicola 16S rRNA gene	1302	7.	
emb|Y10759|XHY10759	X.hortorum 16S rRNA gene /emb|Y107...	1302	7.	
emb|X95921|XORR5047	X.oryzae 16S rRNA gene	1302	7.	
emb|X95917|XCRR568	X.campestris 16S rRNA gene	1302	7.	
gb|AF000946|AF000946	Xanthomonas campestris pv. campest...	1302	8.	
gb|AF058381|AF058381	Uncultured proteobacterium clone M...	1296	1.	
emb|Y10758|XPY10758	X.pisi 16S rRNA gene	1297	2.	
emb|Y10760|XCY10760	X.cucurbitae 16S rRNA gene	1297	2.	
emb|Y10765|XCY10765	X.codiaei 16S rRNA gene	1305	4.	
emb|Y10762|XCY10762	X.cassavae 16S rRNA gene	1292	5.	

gb|AF017749|AF017749	Stenotrophomonas maltophilia 16S r...	1435	1.
emb|Y10764|XBY10764	X.bromi 16S rRNA gene	1302	1.
emb|AJ002814|SSPAJ2814	Stenotrophomonas sp. 16S rRNA gene...	1296	1.
emb|X95922|XPRR5743	X.populi 16S rRNA gene	1284	2.
emb|X95920|XFRR708	X.fragariae 16S rRNA gene	1293	2.
gb|U64004|XSU64004	Xanthomonas sp. 16S ribosomal RNA ...	1461	1.
emb|X95924|SMRR11087	S.maltophilia 16S rRNA gene, strai...	1284	1.
gb|AF047645|	Uncultured eubacterium TRA5-3 16S ...	1301	2.
emb|Z93955|UBZ93955	Unidentified bacterium DNA for 16S...	1300	2.
emb|Y13836|XS16SRRNA	Xanthomonas sp. 16S ribosomal RNA	1275	7.
gb|U15113|HVU15113	Hydrothermal vent eubacterium PVB_...	1399	4.
gb|U15112|HVU15112	hydrothermal vent eubacterium PVB_...	1399	4.
gb|AF058383|AF058383	Uncultured proteobacterium clone M...	1273	8.
emb|X95925|SMRR11114	S.maltophilia 16S rRNA gene, strai...	1442	1.
gb|U15111|HVU15111	Hydrothermal vent eubacterium PVB_...	1390	2.
gb|U78786|HOU78786	Haererehalobacter ostenderis 16S r...	1273	2.
dbj|D16212|RHYFR3E	R.fermentans gene for 16S ribosoma...	1264	3.
dbj|D16211|RHYFR2D	R.fermentans gene for 16S ribosoma...	1264	3.
gb|U89956|GPU89956	Gamma proteobacterium N4-7 16S rib...	801	3.
emb|X87219|CM16SATCC	C.marismortui 16S rRNA gene, ATCC ...	1273	5.
emb|X92555|PL16SR220	P.lemoignei 16S ribosomal RNA (str...	1259	1.
emb|X84627|UNSBR2098	Unknown organism, partial 16S rRNA...	1300	4.
emb|Y09967|BACSP16SR	denitrifying bacterium 72Chol 16S ...	1380	4.
emb|X84603|UNSBR2071	Unknown organism, partial 16S rRNA...	1300	7.
gb|U27839|TTU27839	Thiobacillus thermosulfatus 16S ri...	1201	1.
emb|X84477|UNSBR1034	Unknown organism, partial 16S rRNA...	1312	2.
gb|U78719|CMU78719	Chromohalobacter marismortui 16S r...	1273	2.
emb|X87220|CM16SRA65	C.marismortui 16S rRNA gene, A-65 ...	1273	3.
emb|X87222|CM16SA492	C.marismortui 16S rRNA gene, A-492...	1273	3.
emb|X84504|UNSBR1072	Unknown organism, partial 16S rRNA...	1300	4.
emb|X92554|PL16SRA62	P.lemoignei 16S ribosomal RNA (str...	1255	4.
emb|X84593|UNSBR2059	Unknown organism, partial 16S rRNA...	1309	4.
dbj|D14555|ZYP16SRNA	Zymobacter palmae 16S rRNA gene	1273	4.
emb|X84556|UNSBR2012	Unknown organism, partial 16S rRNA...	1294	7.
emb|X84502|UNSBR1070	Unknown organism, partial 16S rRNA...	1300	9.
gb|U89297|MTU89297	Methylocaldum tepidum 16S ribosoma...	1217	1.
emb|X87221|CM16SA100	C.marismortui 16S rRNA gene, A-100...	1273	1.
emb|Z99998|BSPZ99998	Environmental sample; bacterial 16...	1237	2.
emb|X84511|UNSBR1079	Unknown organism, partial 16S rRNA...	1291	2.
emb|X84471|UNSBR1027	Unknown organism, partial 16S rRNA...	1282	2.
gb|U64000|CSU64000	Chromohalobacter sp. 16S ribosomal...	1273	2.
emb|X95837|BS16ST3	Bacteria species 16S rRNA gene, st...	1219	3.
emb|X65589|TM16SR	T.mixta 16S rRNA	1240	3.
gb|U62131|STU62131	Solemya terraeregina gill symbiont...	1344	4.
gb|U85123|UBU85123	Unidentified beta proteobacterium ...	1224	4.
emb|X84523|UNSBR1095	Unknown organism, partial 16S rRNA...	1294	7.
gb|AF011345|AF011345	Azoarcus indigens 16S ribosomal RN...	1231	1.
emb|X84538|UNSBR1112	Unknown organism, partial 16S rRNA...	1291	1.
emb|X92417|HSRRN16S	H.desiderata 16S ribosomal RNA	1237	1.
gb|AF005252|AF005252	Shewanella sp. MR-4 16S ribosomal ...	1344	2.
gb|AF025563|	Gamma proteobacterium MED20 16S ri...	1259	3.
gb|U81871|PSU81871	Pseudomonas sp. strain BI*8 16S ri...	1185	3.
gb|U89300|MSU89300	Methylocaldum szegediense 16S ribo...	1190	4.

dbj|D88006|D88006	Variovorax paradoxus DNA for 16S r...	1213	5.
emb|X92142|BSPRR65B4	Bacterial sp. 16S rRNA gene (Lake ...	1249	5.
gb|AF011344|AF011344	Azoarcus sp. BH72 16S ribosomal RN...	1222	5.
emb|X84491|UNSBR1055	Unknown organism, partial 16S rRNA...	1312	8.
gb|S55004|S55004S1	16S rRNA [Burkholderia pickettii, ...	1335	9.
gb|AF019073|	Hydrogenophaga palleronii 16S ribo...	1223	9.
emb|AJ002815|ASPAJ2815	Alcaligenes sp. 16S rRNA gene, iso...	1320	1.
gb|U70707|U70707	Unidentified beta proteobacterium ...	1192	1.
gb|AF027407|AF027407	Ralstonia eutropha 16S ribosomal R...	1210	3.
gb|L25707|LGSRRNA	Lucina floridana gill symbiont rib...	1215	5.
dbj|D16209|RHYDSM110B	R.tenuis gene for 16S ribosomal RNA	1201	5.
dbj|D88001|D88001	Ralstonia sp. DNA for 16S ribosoma...	1201	5.
gb|L15531|AZORR16SB	Azoarcus indigens (strain VB32) 16...	1210	5.
gb|AF037271|AF037271	Aeromonas sp. RC50 16S ribosomal R...	1191	5.
gb|L25712|CGTRRNA	Codakia costata gill symbiont ribo...	1215	6.
emb|X73406|LF16SRNC	L.feeleii sgp2 (ATCC 35849) gene f...	1317	6.
emb|AJ002302|RBSJ2302	Ralstonia basilensis 16S rRNA gene	1219	7.
dbj|D88008|D88008	Alcaligenes faecalis DNA for 16S r...	1320	7.
gb|U81870|PSU81870	Pseudomonas sp. strain BI*7 16S ri...	1183	8.
emb|Z93956|UBZ93956	Unidentified bacterium DNA for 16S...	1234	8.
dbj|D30793|VIX16SRNA1	Variovorax paradoxus gene for 16S ...	1198	9.

gb|M26601|XYLSSRNA X.fastidiosa small subunit ribosomal RNA.
Length = 1493

Plus Strand HSPs:

Score = 1552 (428.8 bits), Expect = 5.1e-122, Sum P(3) = 5.1e-122
Identities = 342/383 (89%), Positives = 342/383 (89%), Strand = Plus

```
Query:     4 ACGCTGGCGGCATGCTTAACACATGCAAGTCGAACGGCAGCACAGCAGTAGCAATAC
             |||||||||||| || ||||||||||||||| ||||||||||   |||| |||||
Sbjct:    35 ACGCTGGCGGCAGGCCTAACACATGCAAGTCGGACGGCAGCACATTGGTAGTAATAC

Query:    64 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGGACCTGCCCAGACGTGGGGGA
             |||||||||||||||||||||||||||||||||||| | || ||    ||||||||
Sbjct:    95 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTATCGTGGGGGA

Query:   124 CGTAGGGAAACTTACGCTAATACCGCATACGTCCTACGGGAGAAAGCGGGGGATCGC
             |||||||||||||||||||||||||||||| |||||||| |||||| ||||| |
Sbjct:   155 CGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTT

Query:   184 ACCTCGCGCGGTTGGATGGACCGATGTTCGATTAGCTAGTTGGTGAGGTAATGGCTC
              ||| | ||| |||||||  |||||||||||||||||||||||||||||| |||||
Sbjct:   215 GCCTTGTGCGATTGGATGAGCCGATGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTC

Query:   244 AAGGCGACGATCGATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACA
             |||||||||||| |||||||||||||||||||||||||||||||| |||||||||
Sbjct:   275 NAGGCGACGATCGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACA

Query:   304 CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA
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                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:    335 TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA

Query:    364 AGCAATGCCGCGTTGTTGTTGAA 386
              ||| |||||||||| | ||  |||
Sbjct:    395 AGCTATGCCGCGTGGGTGAAGAA 417

 Score = 84 (23.2 bits), Expect = 5.1e-122, Sum P(3) = 5.1e-122
 Identities = 21/27 (77%), Positives = 21/27 (77%), Strand = Plus / Pl

Query:    389 AAGGCCTTCGGGTTGTTAAAGCACTTT 415
              || |||||||||||||| ||  |  |||
Sbjct:    416 AATGCCTTCGGGTTGTAAAGCCCNTTT 442

 Score = 127 (35.1 bits), Expect = 5.1e-122, Sum P(3) = 5.1e-122
 Identities = 32/41 (78%), Positives = 32/41 (78%), Strand = Plus / Pl

Query:    495 TCCGTGCCCACCACCGCGGTAATACAAAGGTTGCAACCGTT 535
              | ||||||  |  |||||||||||| |||| ||| | ||||
Sbjct:    514 TTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCNAGCGTT 554
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emb|X95918|XARR494 X.albilineans 16S rRNA gene
Length = 1500

Plus Strand HSPs:

```
 Score = 1533 (423.6 bits), Expect = 2.0e-121, Sum P(3) = 2.0e-121
 Identities = 341/384 (88%), Positives = 341/384 (88%), Strand = Plus

Query:      4 ACGCTGGCGGCATGCTTAACACATGCAAGTCGAACGGCAGCACAGCAGTAGCAATAC
              |||||||||||| || ||||||||||||||||||||||||||||  ||||||||||
Sbjct:      6 ACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGCAGCACAGTGGTAGCAATAC

Query:     64 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGGACCTGCCCAGACGTGGGGGA
              |||||||||||||||||||||||||||||||||||| | || ||    |||||||||
Sbjct:     66 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGA

Query:    124 CGTAGGGAAACTTACGCTAATACCGCATACGTCCTACGGGAGAAAGCGGGGGATCGC
              |||||||||||||||||||||||||||||| |||  ||| |||||||| ||| |
Sbjct:    126 CGTAGGGAAACTTACGCTAATACCGCATACGACCTTAGGGTGAAAGCGGAGGACCTT

Query:    184 ACCTCGCGCGGTTGGATGGACCGATGTTCGATTAGCTAGTTGGTGAGGTAATGGCTC
               | |||||||| | ||||  |||||||  |||||||||||||||| ||||| ||| |
Sbjct:    186 GCTTCGCGCGGATAGATGAGCCGATGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCC

Query:    244 AAGGCGACGATCGATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACA
              ||||||||||||  ||||||||||||||||||||||||||||||| |||||||||
Sbjct:    246 AAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACA

Query:    304 CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct:   306 TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA

Query:   364 AGCAATGCCGCGTTGTTGTTGAAG 387
             ||| |||||||||| | ||  ||||
Sbjct:   366 AGCCATGCCGCGTGGGTGAAGAAG 389

 Score = 90 (24.9 bits), Expect = 2.0e-121, Sum P(3) = 2.0e-121
 Identities = 22/27 (81%), Positives = 22/27 (81%), Strand = Plus / Pl

Query:   389 AAGGCCTTCGGGTTGTTAAAGCACTTT 415
             |||||||||||||||| ||  |  |||
Sbjct:   387 AAGGCCTTCGGGTTGTAAAGCCCTTTT 413

 Score = 133 (36.8 bits), Expect = 2.0e-121, Sum P(3) = 2.0e-121
 Identities = 33/41 (80%), Positives = 33/41 (80%), Strand = Plus / Pl

Query:   495 TCCGTGCCCACCACCGCGGTAATACAAAGGTTGCAACCGTT 535
             | ||||||  |  ||||||||||| |||| ||||| ||||
Sbjct:   485 TTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTT 525


emb|Y10756|XMY10756 X.melonis 16SrRNA gene
             Length = 1500

  Plus Strand HSPs:

 Score = 1524 (421.1 bits), Expect = 1.1e-120, Sum P(3) = 1.1e-120
 Identities = 340/384 (88%), Positives = 340/384 (88%), Strand = Plus

Query:     4 ACGCTGGCGGCATGCTTAACACATGCAAGTCGAACGGCAGCACAGCAGTAGCAATAC
             |||||||||||| || ||||||||||||||||||||||||||||  ||||||||||
Sbjct:     6 ACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGCAGCACAGTGGTAGCAATAC

Query:    64 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGGACCTGCCCAGACGTGGGGGA
             |||||||||||||||||||||||||||||||||| | || ||    |||||||||
Sbjct:    66 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGA

Query:   124 CGTAGGGAAACTTACGCTAATACCGCATACGTCCTACGGGAGAAAGCGGGGGATCGC
             |||||||||||||||||||||||||||||| |||  ||| |||||||| ||| |
Sbjct:   126 CGTAGGGAAACTTACGCTAATACCGCATACGACCTTAGGGTGAAAGCGGAGGACCTT

Query:   184 ACCTCGCGCGGTTGGATGGACCGATGTTCGATTAGCTAGTTGGTGAGGTAATGGCTC
             | |||||||| | ||||  |||||||  |||||||||||||| | ||||| ||| |
Sbjct:   186 GCTTCGCGCGGATAGATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCC

Query:   244 AAGGCGACGATCGATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACA
             ||||||||||||  ||||||||||||||||||||||||||||||| |||||||||
Sbjct:   246 AAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACA

Query:   304 CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   306 TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA
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```
Query:   364 AGCAATGCCGCGTTGTTGTTGAAG 387
             ||| ||||||||| | ||  ||||
Sbjct:   366 AGCCATGCCGCGTGGGTGAAGAAG 389

 Score = 90 (24.9 bits), Expect = 1.1e-120, Sum P(3) = 1.1e-120
 Identities = 22/27 (81%), Positives = 22/27 (81%), Strand = Plus / Pl

Query:   389 AAGGCCTTCGGGTTGTTAAAGCACTTT 415
             |||||||||||||||| ||  |  |||
Sbjct:   387 AAGGCCTTCGGGTTGTAAAGCCCTTTT 413

 Score = 133 (36.8 bits), Expect = 1.1e-120, Sum P(3) = 1.1e-120
 Identities = 33/41 (80%), Positives = 33/41 (80%), Strand = Plus / Pl

Query:   495 TCCGTGCCCACCACCGCGGTAATACAAAGGTTGCAACCGTT 535
             | ||||||  |  |||||||||||| |||| ||||| ||||
Sbjct:   485 TTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTT 525
```

emb|X99298|XC16S726 X.campestris 16S rRNA gene, strain LMG 726
Length = 1500

Plus Strand HSPs:

```
 Score = 1515 (418.6 bits), Expect = 6.0e-120, Sum P(3) = 6.0e-120
 Identities = 339/384 (88%), Positives = 339/384 (88%), Strand = Plus

Query:     4 ACGCTGGCGGCATGCTTAACACATGCAAGTCGAACGGCAGCACAGCAGTAGCAATAC
             |||||||||||| || ||||||||||||||||||||||||||||  ||||||||||
Sbjct:     6 ACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGCAGCACAGTGGTAGCAATAC

Query:    64 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGGACCTGCCCAGACGTGGGGGA
             |||||||||||||||||||||||||||||||||||| | || ||    |||||||||
Sbjct:    66 GGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGA

Query:   124 CGTAGGGAAACTTACGCTAATACCGCATACGTCCTACGGGAGAAAGCGGGGGATCGC
             |||||||||||||||||||||||||||||| |||  ||| |||||||| ||| |
Sbjct:   126 CGTAGGGAAACTTACGCTAATACCGCATACGACCTTAGGGTGAAAGCGGAGGACCTT

Query:   184 ACCTCGCGCGGTTGGATGGACCGATGTTCGATTAGCTAGTTGGTGAGGTAATGGCTC
              | ||||||||  | ||||  |||||||  |||||||||||||| | ||||| ||| |
Sbjct:   186 GCTTCGCGCGGATAGATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCC

Query:   244 AAGGCGACGATCGATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACA
             |||||  ||||||  ||||||||||||||||||||||||||||||  |||||||||
Sbjct:   246 AAGGCAACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACA

Query:   304 CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   306 TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA
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