

Exploring Biodiversity:
Identification of a Unique Bacterium
Found in Georgia Marine Sediment

Maria W. Anderson

MARS 3450L

April 26, 2002

Introduction

“Biodiversity” has become a new media buzzword in recent months, and the term often brings to mind images of exotic plants and strange endangered animals. However, microbial biodiversity—diversity among microorganisms such as bacteria and archaea—is just as important, if less noticed. Only about 5000 non-eukaryotic species have been identified and described, compared to over 500,000 insect species; yet, every insect plays host to thousands of microscopic guests (Pace, 1997).

Prokaryotes house a large portion of the biosphere’s nutrients. The amount of carbon contained in these organisms equals 60-100% of the total carbon in plants; enormous amounts of nitrogen and phosphorus are also thought to be stored in bacteria and archaea. With prokaryotes totaling between 415 and 640×10^{28} cells, they may be responsible for as much as 353-546 Pg of carbon, and including these amounts in global estimates will increase greatly the calculated amount of carbon stored in living organisms (Whitman *et al.*, 1998).

Despite their lack of publicity, prokaryotic organisms play a huge role in almost every ecosystem, especially saltwater communities. Photosynthetic cyanobacteria and diatoms in the water column and surface sediment are responsible for generating oxygen (O_2) during carbon dioxide (CO_2) fixation. Purple and green sulfur bacteria—anoxygenic photosynthesizers—use hydrogen sulfide (H_2S) rather than H_2O when fixing CO_2 and thus do not generate O_2 . Other bacteria act as decomposers: they break down particulate organic carbon (POC) from detritus and carcasses and recycle it back into the food web for use by other organisms. Still others thrive in extreme conditions—high salinity, high temperatures, etc.—live as symbionts with some of the higher life forms (such as

polychaete worms), and provide the foundation of the food webs for these environments (Nybakken, 2001).

Because of the significant role that these microorganisms play in marine food webs and many other ecosystems, sequencing, identifying, and cataloging individual species is a vitally important, though somewhat daunting task. Modern techniques in molecular biology have made this project easier in recent years (DeLong, 2001). The most often used method involves the 16S rRNA gene, a gene encoding an RNA molecule present in archaeal and bacterial ribosomes. This gene's highly conserved sequences can be used to bind PCR primers, while its highly variable regions can be used for identification (DeLong and Pace, 2001).

In this work, we have isolated an organism from a marine sediment sample, obtained its 16S rRNA sequence, and used that sequence to identify the bacterium. By comparing our sequence with those of similar bacteria, we have attempted to place it on a phylogenetic tree and determine if it is, in fact, a new species.

Methods and Procedures

Isolation of bacterial colony – We first created a dilution series: A 1-mL sample of marine sediment (mud collected from a salt marsh near Sapelo Island, GA) was added to a 9-mL sample of sterile seawater. From this sample, 1 mL was removed and added to a second 9-mL sample of seawater. This was repeated 9 times for a total of 10 tubes (10^{-1} through 10^{-10}), each having 1/10 the sediment concentration of the previous tube.

From each of the ten tubes, a small amount of material was spread onto an agar plate. The ten plates were placed in a 25°C incubator and left for three days. Then, two

single (large and well-separated) colonies were chosen and streaked onto two new agar plates using the quadrant streaking method. These plates were left in a 25°C incubator for four days; again, two single colonies were chosen and streaked onto fresh plates. After three more days of growth in a 25°C incubator, the plates were removed and placed in a refrigerator. At this point, we had isolated a pure culture: only one organism was growing on the plates.

Sequencing of the bacterial 16S rRNA gene – Next, we had to extract the bacterial DNA. A small amount of material was removed from one of the plates and placed in a microcentrifuge tube containing 500 µL of sterile water. The tube was placed in a boiling water bath for 10 minutes then centrifuged at 13,000 rpm for 10 minutes. Approximately half of the supernatant was pipetted into a new microcentrifuge tube—this material served as the DNA template for our PCR reaction.

In order to set up our PCR, we obtained three fresh microcentrifuge tubes, each with a reaction bead in the bottom. All three tubes contained sterile water (dH₂O), a forward primer (27F), and a reverse primer (1522R); two tubes contained our DNA preparation material, while one contained dH₂O and served as a negative control. The three tubes were loaded into a thermal cycler and allowed to cycle through three temperatures: at 94°C, the dsDNA was converted to ssDNA, at 55°C the primers bound to the complementary regions of the 16S rRNA gene, and at 72°C the *Taq* polymerase inserted the correct nucleotides between the primer binding sites. This cycle was repeated 35 times in order to amplify our sequence. At the end, the cycler cooled to 4°C, and the three tubes were placed in the refrigerator.

To examine our PCR products, we ran them in an agarose gel. From each of the three tubes, 7 μL of material were added to 1 μL of blue loading dye, and these three samples were pipetted into the wells of an agarose gel. In a fourth well, we added 5 μL of DNA marker, which provided a DNA 'ladder' with which we could compare our samples. The gel ran for 40 minutes.

After verifying on the gel that our PCR product was the correct size (~1500 bp) and concentration, we took the remaining DNA preparation to the UGA Molecular Genetics Instrumentation Facility (MGIF) to be sequenced.

Identification of organism and submission of sequence – To compare our organism to others like it, we performed a BLAST search on GenBank using a trimmed version of its 16S rRNA gene sequence and obtained a list of the closest possible matches (attached).

Next we submitted the 16S rRNA gene sequences of our organism (trimmed version), of three of its closest relatives, and of five unrelated bacteria (Figure 1), to the Ribosomal Database Project (RDP) at Michigan State University's Center for Microbial Ecology. This information was used to create a phylogenetic tree (attached) illustrating how closely related our bacterium is to the other eight species. The tree also allowed us to place our organism in its correct Division and Subdivision.

Finally we submitted our bacterium's trimmed 16S rRNA gene sequence (attached) to the Sapelo Island Microbial Observatory (SIMO) and to GenBank.

Results and Discussion

Sequence analysis – When our trimmed 16S rRNA sequence was searched on GenBank, we obtained almost 50 matches with 96-97% sequence similarities; 10 of these matches are listed in our data sheet. According to the phylogenetic tree generated by RDP, the bacterium that we isolated from the marine sediment belongs to Division *Bacillus*, Subdivision *fusiformis*. Our sequence was only 525 base pairs long, shorter than the normal 1500 bp, because it was trimmed before being submitted to SIMO and GenBank. Its SIMO isolate number is IS-S74-230; its GenBank bankit number is 462817.

This study allowed me to see how easily information can be submitted to online genetic databases and reminded me how little of our world we know anything about. New species are being discovered every day, but we have no idea how many species we are losing simultaneously. We have a long way to go before we will ever comprehend the full extent of our planet's biodiversity.

Related Species	Other Species
<i>Bacillus sphaericus</i> , strain 8	<i>Pyrochlorococcus marinus</i> , strain SSW5
<i>Bacillus sphaericus</i> , strain 2	<i>Bacillus subtilis</i> , strain 168
<i>Bacillus fusiformis</i>	<i>Erythrobacter longus</i> , strain Och101
	<i>Stappia stellulata</i> , IAM 12621
	<i>Pseudomonas putida</i> , strain mt-2
	<i>Escherichia coli</i> , strain K-12

Figure 1. Bacterial species used in constructing phylogenetic tree.

Literature Cited

DeLong, Edward F. "Microbial seascapes revisited," *Current Opinion in Microbiology*, 4: 290-295, 2001.

DeLong, Edward F., and Norman R. Pace. "Environmental Diversity of Bacteria and Archaea," *Systematic Biology*, 50(4): 470-478, 2001.

Nybakken, James W. *Marine Biology: An Ecological Approach*. Fifth Edition. Benjamin Cummings: San Francisco, 2001.

Pace, Norman R. "A Molecular View of Microbial Diversity and the Biosphere," *Science*, Vol. 276, May 2, 1997.

Whitman, William B. et al., "Prokaryotes: The unseen majority," *Proceedings of the National Academy of Sciences*, Vol. 95 (June 1998), pp. 6578-6583.