Investigating the Microbial Ecology of Yellowstone Lake

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Abstract

Yellowstone National Park is well known for its geothermal features. Among microbiologists it is equally well known for its unique microbial ecology and extreme habitats associated with terrestrial hot springs, geysers, and fumaroles. Yellowstone Lake has also been shown to contain geothermal activity, and the presence of hydrothermal vents with water temperatures up to 120˚C have been reported. The vents emit a number of compounds which are important to microorganisms as nutrients for growth or substrates for energy. Thus, similar to the terrestrial habitats, Yellowstone Lake presents unique systems to assess microbial diversity and ecology. In order to examine the microbial ecology of the lake and its hydrothermal features, we have used both traditional culture and enrichment techniques to isolate bacteria, and modern molecular methods to assess the microbial diversity. For example, enrichment and cultural methods have yielded the characterization of a new genus and species of thermophilic sulfate-reducing bacteria, *Thermodesulfovibrio yellowstonii*, isolated from a hydrothermal vent in Sedge Bay.

Introduction

Microbial ecology is the study of microorganisms in relation to their biotic and abiotic environment. In practice, it has been described in a graduate student motto as "the study of physiology under the worst possible conditions" (Brock 1966). More recently, microbial ecology has also been indicated to be the link between all branches of microbiology (Zinder and Salyers 2001). In any case, similar to traditional ecology, microbial ecologists study individual organisms, populations (of individuals), communities (of populations), and ecosystems. This is done this with a variety of approaches and tools, including microscopy, culturing, molecular biology, and biochemistry. Much of what is studied by microbial ecologists revolves around three questions: (1) Who is out there? (2) How many are there? and (3) What are they doing?

Yellowstone Lake has been considered to be oligotrophic (e.g., Remsen et al. 1990; Gresswell et al. 1994). In other words, it has a low amount of productivity and is nutrient-poor. However, recent reports have suggested that the levels of nutrients indicate it should be considered more mesotrophic, or have a higher level of productivity than previously believed (Kilham et al. 1996; Theriot et al. 1997). When applying the above questions to Yellowstone Lake, the task of answering them might appear to be somewhat daunting. The sheer size of the

lake makes it difficult to know just where a microbial ecologist should begin (Table 1). It gets even more complex if one considers that there are around a million bacteria per milliliter of water. In Yellowstone Lake, our focus has been on

Table 1. Characteristics of Yellowstone Lake. Data compiled from Pierce (1987), Kaeding et al. (1996), and Kilham et al. (1996).

Altitude above mean sea level	2,356.0 m
Surface area	341.0 km^2
Shore line length	239.0 km
Mean depth	48.5 m
Maximum depth	107.0 m
Estimated capacity	1.517×10^{13}

the geothermal activity exhibited by sublacustrine (i.e., at the bottom of lakes) hydrothermal vents and geysers (Marocchi et al. 2001; Remsen et al., this volume). However, even considering these locations presents some difficulties. The water and gases emanating from vents and geysers have influences that can extend some distance away from their origin (Figure 1). Water coming out of a vent forms a plume which mixes with the bulk water and transports vent material throughout the water column. The influence and the size of the plume depends upon the amount and periodicity of flow coming out of the vent orifice. Gas bubbles from a vent adsorb microorganisms and carry them to the water surface, where, after the bubble bursts, bacteria can be deposited at the air–water interface on what are called *film drops*, or transported into the atmosphere on what are known as *jet drops* (Maki and Hermansson 1994). Solid objects, such as rocks or aquatic plants that intersect the plume or gas flow, can also develop microbial communities directly influenced by vent emanations. In addition, there are also influences on the sediments that surround the vent, starting at the tube leading to the vent orifice and extending outwards. Thus, to get a complete picture, a variety of factors must be examined.

The presence of the hydrothermal vents provides another factor to consider for a microbial ecologist: temperature. The lake contains a range of temperatures that extend into the extreme. The lake generally becomes stratified in July and the thermocline may exist through mid-September with surface temperatures very seldom going above 18°C (Gresswell et al. 1994; Kaeding et al. 1996). Ice cover occurs from mid-December through May or even June, providing plenty of low temperatures (e.g., <4°C). On the other end of the temperature range, the hydrothermal vents have waters that reach up to 120°C (Buchholz et al. 1995; Klump et al. 1995). This allows for the presence of the entire range of optimal-growth temperature categories of microorganisms (Table 2) in Yellowstone Lake. Some microbes in the domain *Eucarya* can grow up into the thermophilic range, but most have lower (mesophilic) temperature requirements. Of the procaryotes, members of the domain *Bacteria* are found in all categories. Procaryotes that fall into the hyperthermophile category belong primarily in the domain *Archaea* (Brock 1994).

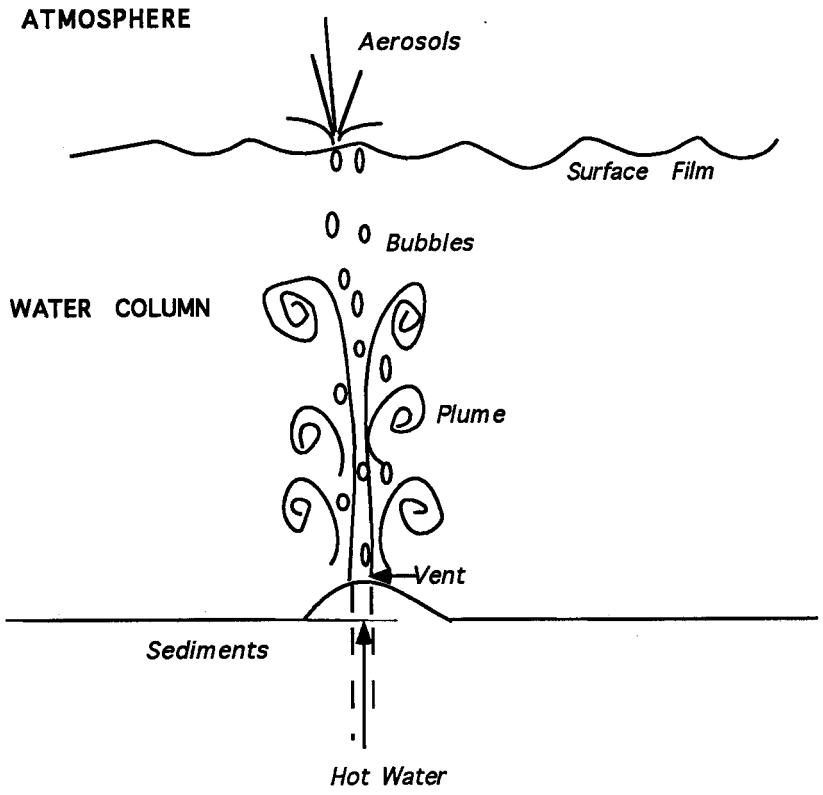

Figure 1. Schematic of the influence a hydrothermal vent may have on the water body into which it flows.

Table 2. Categories of growth temperature optima for microorganisms.

Description	*Growth temperature optima*
Hyperthermophiles	>80°C
Thermophiles	45-80°C
Mesophiles	15-45°C
Psychrophiles	<15°C

Of the three questions listed above, "What are they doing?" has been addressed elsewhere (Cuhel, Aguilar, Anderson et al., this volume), so the focus here will be on some of our work to determine "Who is out there?" and "How many are there?" in Yellowstone Lake. Our interest has been primarily on the procaryotic microorganisms of the domains *Bacteria* and *Archaea*, although it will be clear that our work did not exclude the *Eucarya*.

Sampling

Most of our collection of hydrothermal vent and bulk waters on the lake was accomplished using the National Park Service research vessel *Cutthroat.* Both SCUBA divers (in shallow waters) and a remotely operated vehicle (ROV; in deeper waters) have been used to collect the vent water samples (e.g., Klump et al. 1992; Buchholz et al. 1995). Over the years, we have been on a learning curve using the ROV; after each sampling season, discussions with Dave Lovalvo (Eastern Oceanics, West Redding, Connecticut), who operates the ROV for us in the lake, have resulted in modifications to enable better collection of water and other samples. Some idea of the changes involved have been presented elsewhere (Marocchi et al. 2001; Remsen et al., this volume) and will not be discussed in detail.

Who is Out There? How Many are There? Quantitative Analyses

Analysis of hydrothermal vent water chemistry reveals that not only are the vents in various regions of the lake different, but vents within the same region appear distinct from each other (Klump et al. 1988; Remsen et al. 1990; Klump et al. 1992; Buchholz et al. 1995). The chemistry data suggest that each of these vents could represent a different microbial habitat, and thus should have different microbial communities. Initially, some of our research examined these communities using quantitative methods.

We assessed microbial communities quantitatively by two means. First, we used multiple staining techniques and fluorescence microscopy to count microbial cells directly (e.g., Sherr and Sherr 1983). Second, we used culture methods where a water sample is serially diluted and each dilution is used to inoculate a solid growth medium that is incubated, and after a certain amount of time the colonies that arise (called *colony-forming units,* or CFU) are counted. In the latter case, the medium we have used is Castenholz TYE (Castenholz 1969) and is solidified using agar for mesophiles or Gelrite for thermophiles and hyperthermophiles (see Table 2 for temperature ranges involved). Using these methods to compare samples from different vents in Sedge Bay revealed that the numbers of distinct types of microorganisms determined by direct counts and CFU vary between vents and are different from those in the bulk waters (Figure 2). These data support the idea of each vent being able to maintain different microbial communities. Some types of microorganisms (e.g., phototrophs, algae excluding the cyanobacteria) were only visible in the bulk water samples. All other types were present in all samples examined. One important type of microbe present everywhere was the heteroflagellates. These are eucaryotic microorganisms that feed upon the bacteria and provide the beginning link from procaryotes to larger organisms in the food chain, eventually leading to zooplankton and fish.

However, the data presented in Figure 2 also illustrate the major problem associated with using only a culture approach for isolating bacteria and other microorganisms—or with examining any form of microbial diversity. As can be seen when comparing the number of bacterial CFU and the total counts of bacteria in the different vent samples, the number of CFU is around two orders of

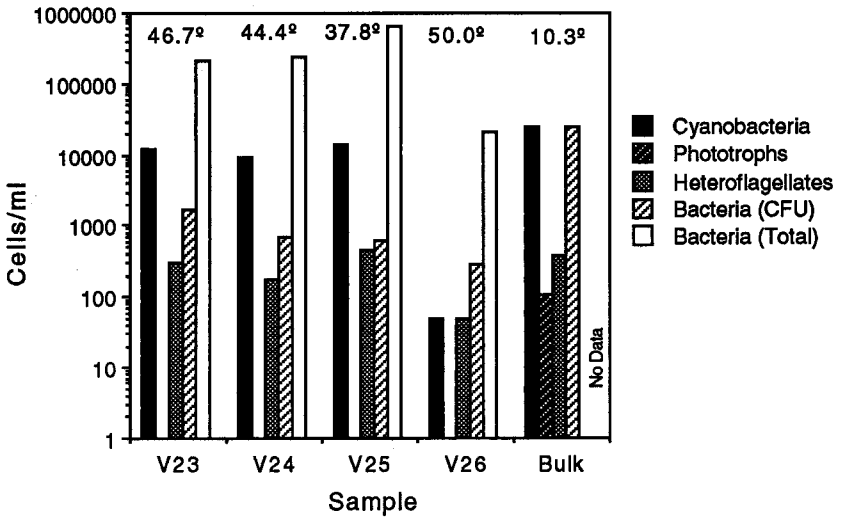

Figure 2. Comparison of the microbial communities of four separate hydrothermal vents in Sedge Bay. "V" followed by a number indicates the vent sampled while "Bulk" indi - cates a non-vent sample from the water column. Temperatures listed above each sample are in °C. No direct count data of bacteria in the bulk water sample were available.

magnitude less than (or ~1%) the total count. This is because using growth media of any type selects only for the organisms that can grow on that particular medium, and the vast majority of bacteria out there are unlikely to all grow on the same medium. This low ability to culture microorganisms extends to just about every habitat that has been studied and has inspired the use of molecular approaches for assessing microbial diversity and ecology. These molecular approaches allow the assessment of microbial diversity and identification of microorganisms without cultivation (e.g., Amann et al. 1995).

We have been using a combination of enrichment culture and molecular methods to assess the procaryotic microorganisms from both hydrothermal vent and bulk water samples (Figure 3). These include members of both the *Bacteria* and *Archaea*. On the enrichment side, we can focus on groups of microorganisms that grow under very specific conditions and utilize the chemistry of the hydrothermal vent emanations for growth or energy (e.g., Remsen et al. 1990). We can then isolate individual microorganisms and characterize and identify them. This was generally the methodology used by microbial ecologists everywhere before the advent of molecular techniques. Now, however, to identify and characterize a single type of bacterium not only are phenotypic attributes used (e.g., morphology, fine structure, growth substrates, conditions for growth, etc.), but so are genotypic characteristics determined through molecular techniques. These allow the investigator to get a clearer picture of the bacterium in question.

Molecular Analyses for Identification and Diversity

One of the genes most used to deduce the position of a bacterium phyloge-

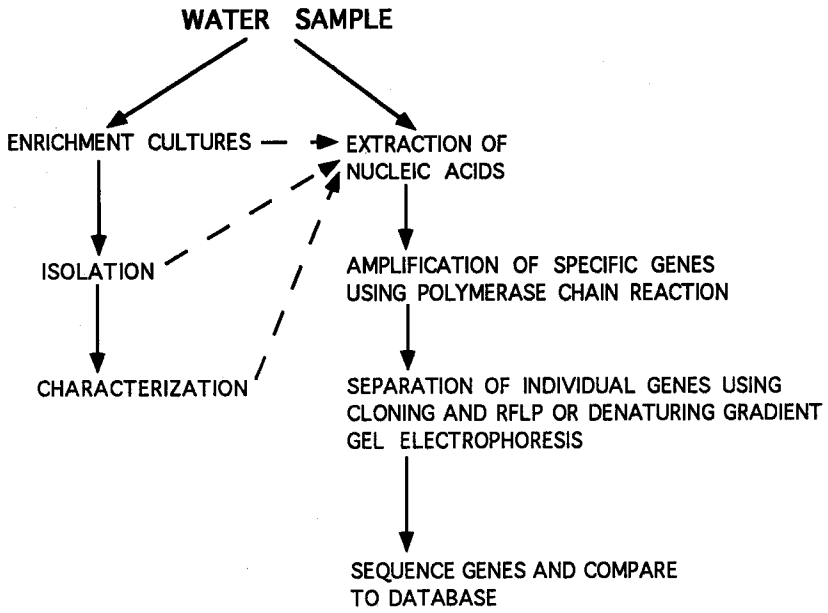

Figure 3. Flow chart showing combination of enrichment culture and molecular techniques used to examine hydrothermal vent and water column samples from Yellowstone Lake.

netically is the one that codes for a portion of the ribosome, a cellular structure where protein synthesis occurs that is found in all living organisms. In order to better study one gene, it is amplified using a process called the polymerase chain reaction (PCR). For a complete description of the process see the article by Mullis (1990). To amplify a certain gene, small pieces of DNA, called *primers*, are used. The primers are designed to be specific for the gene in question and are complementary to short sequences of the gene. They initiate making a copy of the gene of interest, which in the PCR is repeated many times. Amplification of the gene with the PCR results in billions of copies of the gene, making it easier to work with. After amplification, the sequence of bases that make up the gene is determined. So, if a bacterium has been isolated and we want to identify it using molecular tools, we determine the sequence of bases in the gene that codes for the subunit of the ribosome, called the 16S subunit, and compare this sequence to other known sequences that exist in databases. From this comparison we can examine the relatedness of one bacterium to another, or to a whole range of other bacteria, or even resolve its identity (Amann et al. 1995).

The strength of the molecular–noncultural methodology is that bacteria do not have to be grown or isolated before they can be studied. As illustrated in Figure 3, a sample can be directly analyzed starting with the extraction of nucleic acids followed by amplification of genes, most likely the 16S ribosomal DNA (rDNA) gene, with the PCR. The situation is somewhat different from that described

above for a single bacterium. Instead of just having the gene from a single species of bacteria, when amplifying the 16S rDNA gene from the nucleic acids extracted from a natural sample, one presumably ends up with this gene from the DNA of every bacterium in the sample. This is analogous to having a large bowl of spaghetti, when what is wanted are the sequences on the individual strands of spaghetti that are each from different cells. Somehow, the strands must be separated before their sequences can be effectively analyzed.

Basically, two types of methods are used to get the single strands out of the bowl. The first is cloning. This is the insertion of the single strands into a small circle of DNA, called a *plasmid,* in a bacterium, usually a strain of *Escherichia coli*. As it grows and divides, the bacterium produces many copies of the plasmid containing the strand of DNA of interest. The gene of interest is recovered and analyzed with a treatment called *restriction fragment length polymorphism* (RFLP). This process uses enzymes called *restriction enzymes* that cut strands of DNA in very specific locations. These locations are in separate places in genes from different bacteria. Therefore, after treating the recovered cloned 16S rDNA with restriction enzymes, the patterns between clones are compared by separation in an agarose gel by a process known as *electrophoresis* (Figure 4). Because the locations where the restriction enzymes cut the DNA are in separate places in different bacteria, each different type should be represented by a distinct pattern on the gel, while those with the same pattern should represent the same bacterium. Examination of the different RFLP patterns from two vent water samples suggests that the bacterial diversity in the vents is quite distinct (Figure 5). However, this information needs to be confirmed after the 16S rDNA clones are

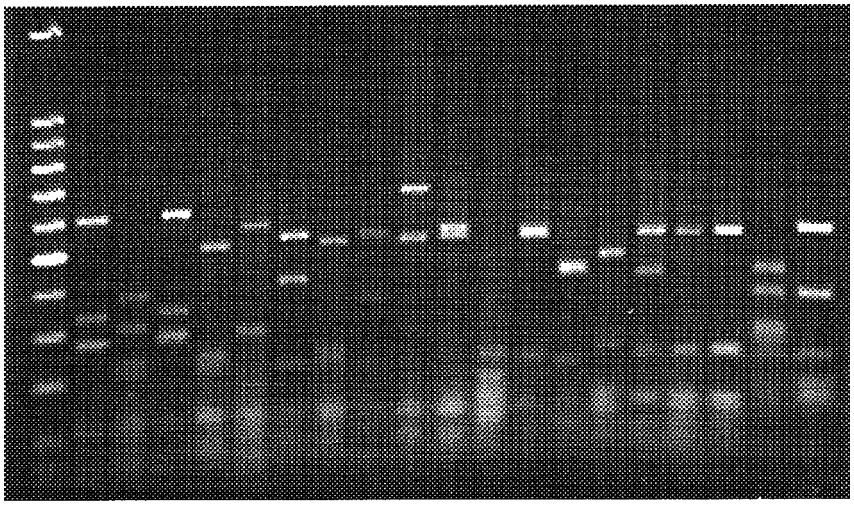

Figure 4. Example of a restriction fragment length polymorphism (RFLP) pattern from a clone library created after amplification using the polymerase chain reaction (PCR) of DNA extracted from a hydrothermal vent water sample. Of the 19 patterns generated, 15 appear to be distinct, indicating a diverse bacterial population.

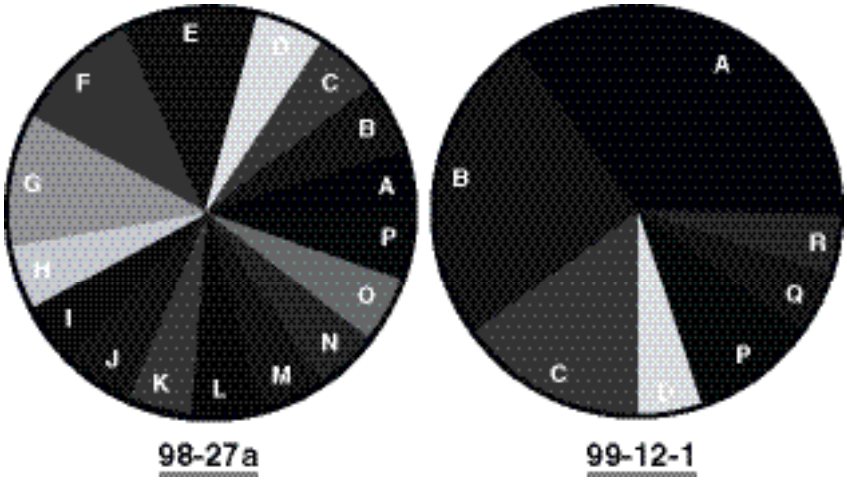

Figure 5. Comparison of RFLP patterns from two separate hydrothermal vents. The size of each pie piece indicates the proportion of the total number of clones examined with the same RFLP pattern. Clearly the diversity of bacteria in the two vents is different.

sequenced and the sequences compared. We have used, and are continuing to use, this approach to examine the diversity of *Bacteria* and *Archaea* both in hydrothermal vent waters and in the water column.

The second method involves separating the amplified DNA in gel electrophoresis. One way to do this is by what is called *denaturing gradient gel elec - trophoresis* (DGGE; e.g., Ferris et al. 1996). Each species of bacterium in a mixed microbial community will have a different sequence in its 16S rDNA gene. These can be separated into distinct bands in an acrylamide gel that contains an increasing gradient of a denaturant; due to their composition, each will denature and stop at a different concentration of denaturant in the gel. Each distinct band in the gel may represent a different type of bacterium. This can be confirmed by excising the bands and sequencing them. Currently, this technique is also being used on samples collected from Yellowstone Lake.

Molecular Analyses to Study Microbial Distribution

In addition to examining microbial diversity, molecular techniques can also be used to determine the presence and distribution of microorganisms with specific metabolic activities. One example is a gene for an enzyme that is involved in the oxidation of methane. The enzyme is called *methane monooxygenase* and is found in the bacteria that utilize methane as a source of both energy and carbon. These bacteria are called *methanotrophs* and may be important in parts of Yellowstone Lake because of the presence of methane in both water column and hydrothermal vent samples from some of the lake basins (Remsen et al. 1990). By taking the DNA extracted from a water sample (Figure 3) the genes for the methane monooxygenase can be amplified using specific primers (Cheng et al. 1999). By serially diluting the DNA before the PCR amplification, the number

of copies of the gene in a sample can be determined by most probable number (MPN) PCR based on the analysis of replicates diluted to extinction (e.g., Fode-Vaughan et al. 2001). In other words, the dilutions in which a signal is detected after amplification are representative of the concentration of the gene in the sample. An example using the primers for the methane monooxygenase on a water sample from Yellowstone Lake is presented in Figure 6. This methodology will allow the comparison of the distribution of the gene copies with the concentration of methane in water samples.

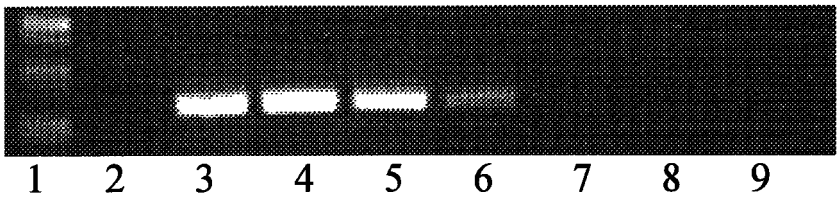

Figure 6. Example of a serial dilution of DNA extracted from a Yellowstone Lake water sample followed by amplification using the PCR of genes specific for the particulate methane monooxygenase enzyme, which is found in the vast majority of bacteria that uti - lize methane for both a source of energy and carbon. The last dilution (Lane 7, 1:10,000 dilution) in which a signal is amplified is representative of the concentration of the gene in the extracted DNA. Lane 1, DNA size markers; Lane 2, no DNA control; Lane 3, undi - luted DNA from sample; Lane 4, 1:10 dilution; Lane 5, 1:100 dilution; Lane 6, 1:1000 dilution; Lane 7, 1:10,000 dilution; Lane 8, 1:100,000 dilution; Lane 9, 1:1,000,000 dilu - tion.

A New Genus from Yellowstone Lake: Thermodesulfovibrio

Are there new microorganisms in Yellowstone Lake? In this case, the word "new" merely implies that they have not been previously isolated and characterized by humans. Any "new" microorganisms have probably been around for a very long time. The terrestrial thermal features of Yellowstone National Park have long been the source of a variety of novel microorganisms (e.g., Brock 1994). This should also be true for the hydrothermal features of Yellowstone Lake. An example of a new microorganism isolated from a hydrothermal vent in Sedge Bay is the obligate anaerobic thermophilic bacterium *Thermodesulfovibrio yellowstonii* (Henry et al. 1994; Maki 2001). This bacterium (Figure 7) has an optimum growth temperature of 65˚C, reduces sulfate to sulfide, and oxidizes some organic carbon sources (Henry et al. 1994; Maki 2001). Analysis of its 16S rDNA sequence reveals it to be a member of the phylum *Nitrospirae*, a deeply branching group of the *Bacteria* domain (Maki 2001). Since its isolation and characterization (Henry et al. 1994), the 16S rDNA sequence for the genus *Thermodesulfovibrio* has been reported from a terrestrial hot spring in Yellowstone National Park (Hugenholtz et al. 1998) and thermophilic granular sludges (Sekiguchi et al. 1998). In addition, a second species, *Thermodesulfovibrio islandicus*, has been isolated from a microbial mat in a thermal spring in Iceland (Sonne-Hansen and Ahring 1999). It's clear that this bac-

terium, originally isolated from Yellowstone Lake, represents a new genus that has a worldwide distribution.

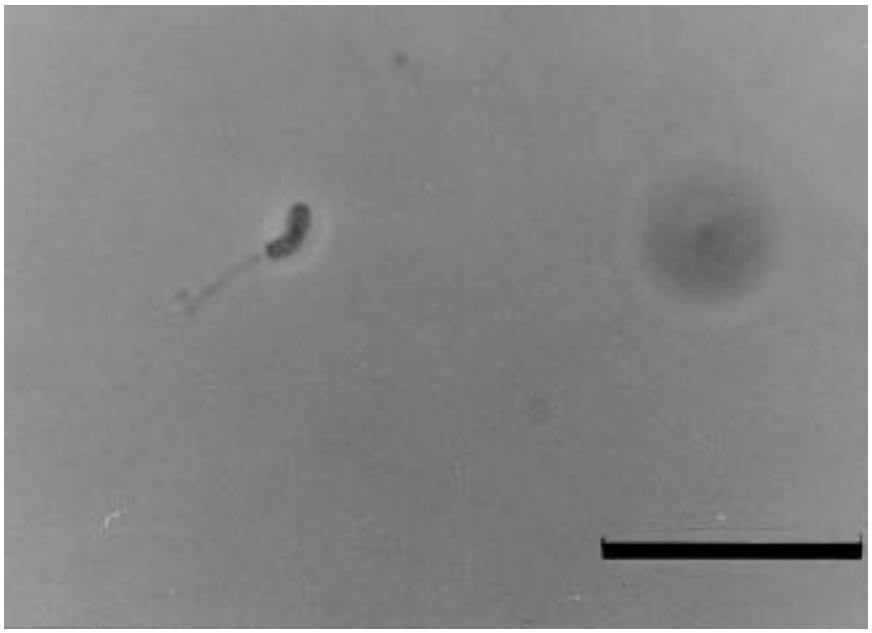

Figure 7. Photomicrograph of Thermodesulfovibrio yellowstonii taken with a phase contrast microscope after using a flagella stain. Bar = 10 µm.

Conclusions

For a microbial ecologist, Yellowstone Lake represents both a challenge and an opportunity. The challenge comes in effectively collecting samples from some of the difficult locations the vents are found in. The opportunity is in the potential of finding some unusual new microorganisms. The chemical variety of geothermal features on the bottom of the lake suggest that they will be as important to microbial ecology, and microbiology in general, as the terrestrial hot springs, geysers, fumaroles, and mudpots in the rest of Yellowstone National Park have been. The molecular approaches we have taken, although many of the studies are still preliminary in nature, have indicated a wide diversity of both *Archaea* and *Bacteria* associated with the vents. Although getting all of these bacteria into pure culture is highly unlikely, through enrichment cultures and isolations there is a strong possibility in finding some bacteria that have not been previously described. The lake and its hydrothermal features should be a source of fascinating results for some time to come.

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References

Amann, R.I., W. Ludwig, and K-H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* 59, 143–169.

Brock, T.D. 1966. *Principles of Microbial Ecology*. Englewood Cliffs, N.J.: Prentice-Hall.

———. 1994. *Life at High Temperatures.* Yellowstone National Park, Wyo.: Yellowstone Association.

Buchholz, L.A., P.D. Anderson, R.L. Cuhel, J.V. Klump, J.E. Kostka, R.W. Paddock, C.C. Remsen, J.S. Maki, and D. Lovalvo. 1995. Employment of ROV techniques and SCUBA in Yellowstone Lake. In *Diving for Science 1995: Proceedings of the American Academy of Underwater Sciences Fifteenth Annual Scientific Diving Symposium*. D.E. Harper, Jr., ed. Nahant, Mass.: American Academy of Underwater Sciences, 1–7.

Castenholz, R.W. 1969. Thermophilic blue-green algae and the thermal environment. *Bacteriological Reviews* 33, 476–504.

Cheng, Y.S., J.L. Halsey, K.A. Fode, C.C. Remsen, and M.L.P. Collins. 1999. Detection of methanotrophs in groundwater by PCR. *Applied and Environmental Microbiology* 65, 648–651.

Ferris, M.J., G. Muyzer, and D.M. Ward. 1996. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Applied and Environmental Microbiology* 62, 340–346.

Fode-Vaughan, K.A., C.F. Wimpee, C.C. Remsen, and M.L.P. Collins. 2001. Detection of bacteria in environmental samples by direct PCR without DNA extraction. *BioTechniques* 31, 598–607.

Gresswell, R.E., W.J. Liss, and G.L. Larson. 1994. Life-history organization of Yellowstone cutthroat trout *(Oncorhynchus clarki bouvieri)* in Yellowstone Lake. *Canadian Journal of Fisheries and Aquatic Sciences* 51(Supplement 1), 298–309.

Henry, E.A., R. Devereux, J.S. Maki, C.C. Gilmour, C.R. Woese, L. Mandelco, R. Schauder, C.C. Remsen, and R. Mitchell. 1994. Characterization of a new thermophilic sulfate-reducing bacterium, *Thermodesulfovibrio yellowstonii*, gen. nov. and sp. nov.: Its phylogenetic relationship to *Thermodesulfobacterium commune* and their origins deep within the bacterial domain. *Archives of Microbiology* 161, 62–69.

Hugenholtz, P., C. Pitulle, K.L. Hershberger, and N.R. Pace. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. *Journal of Bacteriology* 180, 366–376.

Klump, J.V., C.C. Remsen, and J.L. Kaster. 1988. The presence and potential impact of geothermal activity on the chemistry and biology of Yellowstone Lake, Wyoming. In

Global Venting, Midwater and Benthic Ecological Processes. M. DeLuca and I. Babb, eds. National Undersea Research Program Research Reports no. 88-4. Rockville, Md.: National Oceanic and Atmospheric Administration, National Undersea Research Program, 81–98.

Klump, J.V., R. Paddock, and D. Lovalvo. 1992. A multiple-loop , ROV controlled, in-situ water sampler. *Journal of Great Lakes Research* 18, 309–315.

Klump, V., T. Remsen, D. Lovalvo, P. Anderson, R. Cuhel, M. Kaplinski, J. Kaster, J. Maki, and R. Paddock. 1995. 20,000 leagues under Yellowstone Lake. *Yellowstone Science* 3:4, 10–14.

Maki, J.S. 2001. Genus IV. *Thermodesulfovibio*. In *Bergey's Manual of Systematic Bacteriology. Volume 1: The Archaea and the Deeply Branching and Phototrophic Bacteria*. 2nd ed. D.R. Boone and R.W. Castenholz, eds. New York: Springer-Verlag, 460–464.

Maki, J.S., and M. Hermansson. 1994. The dynamics of surface microlayers in aquatic environments. In *The Biology of Particles in Aquatic Systems*. 2nd ed. R.S. Wotton, ed. Boca Raton, Fla.: CRC Press, 161–182.

Marocchi, S., T. Remsen, and J. V. Klump. 2001. *Yellowstone Lake: Join the Expedition!* Whitefish Bay, Wisc.: Hammockswing Publishing.

Mullis, K.B. 1990. The unusual origin of the polymerase chain reaction. *Scientific American* 262:4, 56–61, 64–65.

Pierce, C. 1987. *The Lakes of Yellowstone: A Guide for Hiking, Fishing & Exploring*. Seattle: The Mountaineers.

Remsen, C.C., J.V. Klump, J. Kaster, R. Paddock, P. Anderson, and J.S. Maki. 1990. Hydrothermal springs and gas fumaroles in Yellowstone Lake, Yellowstone National Park, Wyoming. *National Geographic Research* 6, 509–515.

Sekiguchi, Y., Y. Kamagata, K. Syotsubo, A. Ohashi, H. Harada, and K. Nakamura. 1998. Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA analysis. *Microbiology* 144, 2655–2665.

Sherr, E.B., and B.F. Sherr. 1983. Double-staining epifluorescence technique to assess frequency of dividing cells and bacteriovory in natural populations of heterotrophic microprotozoa. *Applied and Environmental Microbiology* 46, 1388–1393.

Sonne-Hansen, J., and B. Ahring. 1999. *Thermodesulfobacterium hveragerdense* sp. nov., and *Thermodesulfovibrio islandicus* sp. nov., two thermophilic sulfate reducing bacteria isolated from a Icelandic hot spring. *Systematic and Applied Microbiology* 22, 559–564.

Theriot, E.C., S.C. Fritz, and R.E. Gresswell. 1997. Long-term limnological data from the larger lakes of Yellowstone National Park, Wyoming. *Arctic and Alpine Research* 29, 304–314.

Zinder, S.H., and A.A. Salyers. 2001. Microbial ecology—new directions, new importance. In *Bergey's Manual of Systematic Bacteriology. Volume 1: The Archaea and the Deeply Branching and Phototrophic Bacteria*. 2nd ed. D.R. Boone and R.W. Castenholz, eds. New York: Springer-Verlag, 101–109.

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