Aquificales in Yellowstone National Park

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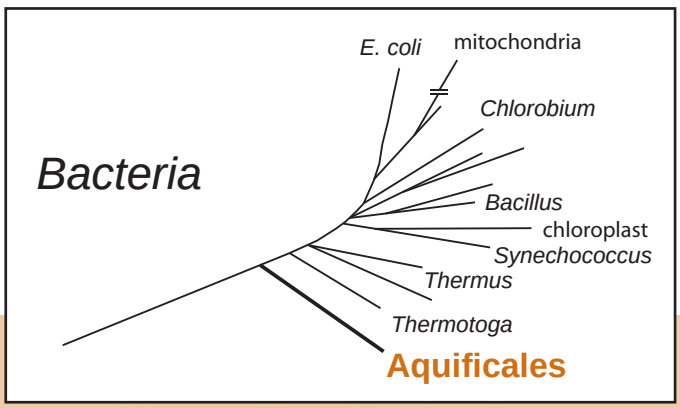

ABSTRACT

Aquificales are metabolically versatile chemolithoautotrophic thermophilic bacteria. This group is widespread in both deep-sea and terrestrial hydrothermal systems. In Yellowstone National Park, they were first described in early descriptions of the biology of the park, and later captured the attention of many microbiologists including Brock, Stahl, Pace, and others. There are four genera currently described from Yellowstone: *Thermocrinis*, *Sulfurihydrogenibium*, *Hydrogenobacter* and the only acidophilic genus, *Hydrogenobaculum*. Aquificales appear to fix CO_2 using the reductive TCA cycle, although several species can also obtain carbon from organic sources such as acetate and formate. Hydrogen and sulfur appear to be the preferred electron donors. Furthermore, many of these filamentous bacteria are associated with visible iron and sulfur mineral precipitation, which points to their overall importance in biogeochemical cycling in hot spring ecosystems.

Key Words

Aquificales
biomineralization
chemolithoautotrophy
thermophiles

1.0 INTRODUCTION

"In one pool, where the pink bacteria were present, …we carried out several detailed studies on these high temperature bacteria and our results are clear-cut. In springs of neutral to alkaline pH, bacteria live at temperatures right up to the boiling point…"

—T.D. Brock 1979, referring to publications of 1969/70.

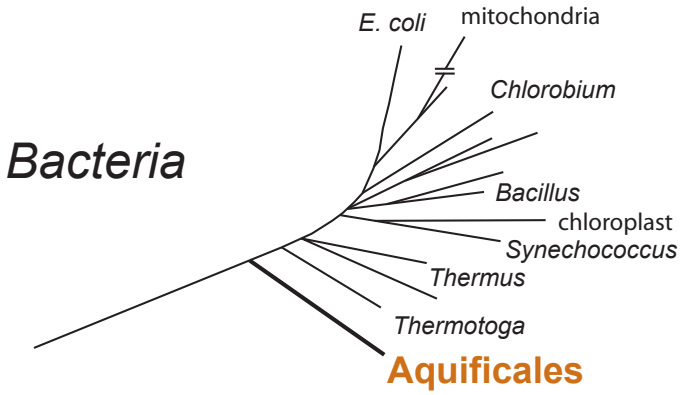

Figure 1A. Schematic 16S rRNA tree of the domain *Bacteria*, showing the position of the order Aquificales.

The order, Aquificales (**Figure 1A**), has captured the imagination of biologists visiting hot springs throughout the world, and has established upper temperature limits of life for more than a century. As early as the late 1800s, colorless filamentous life in Yellowstone's hot springs (up to 89°C) was noted by the California phycologist, W.A. Setchell. In Setchell's unpublished manuscript on Yellowstone algae, he includes the description of what were most likely Aquificales—"the filamentous types occurred in such masses and in connection with such high temperatures as to make them noticeable." He describes three different species *Chlamydothrix calidissima*, *Chlamydothrix penicillata*, and *Thiothrix carnea*, located in the Black Sand Basin, Firehole Pool in Firehole Basin, and Norris Geyser Basin (Brock 1978). The two *Chlamydothrix* species were located in springs up to 89°C and *T. carnea* at 82°C, forming "gelatinous tufts" that ranged from whitish to flesh- or salmon-colored. One can only surmise that these descriptions refer to members of Aquificales—most likely related to the genus *Thermocrinis*. (It is possible that *T. carnea*, if located in an acidic pool in Norris Geyser Basin, was a member of the genus *Hydrogenobaculum*; or if located in a near-neutral pH spring, was related to *Sulfurihydrogenibium*.)

It took many years to reveal the true identity of these conspicuous filamentous thermophiles. Many attempts to grow these organisms from the outflow channel at Octopus Springs were unsuccessful. Some researchers tried to extract enough nucleic acids (RNA) from the filaments to determine the 5S rRNA identity (Stahl et al. 1985), and these extractions were also unsuccessful. Not until molecular phylogenetic approaches based on polymerase chain reaction (PCR) were used to describe microbial diversity in the environment were the flesh-colored pink filaments in the outflow channel at Octopus Springs identified as members of Aquificales—the deeply diverging branch in the small subunit (16S) rRNA tree of life (Reysenbach et al. 1994). A few years later, Huber et al. (1998) were able to grow this organism under hydrogen-oxidizing conditions and named the filaments *Thermocrinis ruber*. Subsequently, a lower temperature but phylogenetically-distinct relative was described from Calcite Springs, Mammoth Hot Springs, and Obsidian Pool (Reysenbach et al. 2000a; Graber et al. 2001). This group appeared to be associated with more sulfidic systems and often appeared to be linked to biomineralization (Reysenbach et al. 1999; Skirnisdottir et al. 2000).

Since these initial studies on Aquificales' taxonomy, ecology, and physiology, many additional insights into the diversity of this group have emerged. Numerous lineages are now in cultivation establishing the global distribution of Aquificales in terrestrial hot springs and shallow marine and deep-sea environments (e.g., Yamamoto et al. 1998; Stöhr et al. 2001; Eder and Huber 2002; Reysenbach et al. 2002; Takai et al. 2001, 2003; Nakagawa et al. 2003; Aguiar et al. 2004; for reviews, Reysenbach et al. 2001; Reysenbach and Shock. 2002). Furthermore, with the genome sequence of *Aquifex aeolicus* (Deckert et al. 1998),

interest in this group has increased significantly as illustrated by the exponential increase in related publications (**Figure 1B**). The release of the genome sequences (**www.tigr.org**) of three other members of this order, *Persephonella marina*, *Sulfurihydrogenibium azorense*, and "*Sulfurihydrogenibium yellowstonense*" (pending), will no doubt continue to fuel research on Aquificales (see below).

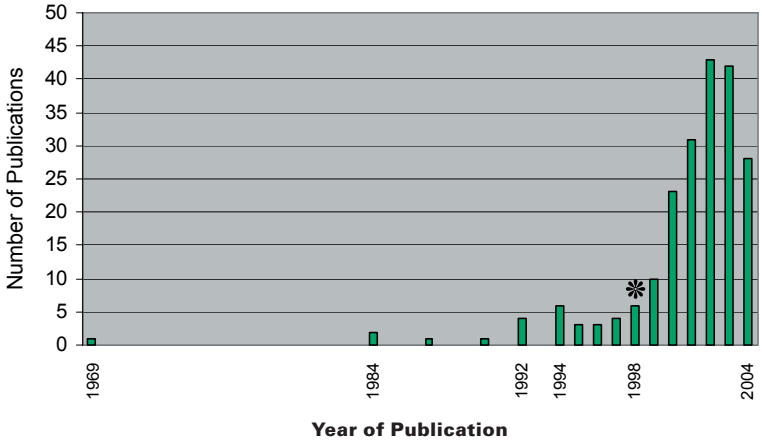

Figure 1B. The impact of genome sequencing. This graph illustrates the research impact of the genome sequence of *Aquifex aeolicus* as measured by an increased number of publications dealing with aspects of Aquificales' biology, ecology and genetics. The entire genome of *A. aeolicus* was published in 1998 (Deckert et al.), as indicated by the asterisk. Partial count for 2004.

2.0 TAXONOMY OF AQUIFICALES

Based on phylogenetic analysis of the 16S rRNA gene sequences, Aquificales is the deepest lineage within the domain *Bacteria* (Burggraf et al. 1992; Pitulle et al. 1994). However, the *A. aeolicus* genome sequence (Deckert et al. 1998; Wolf et al. 2001) and RNA polymerase sequence comparisons do not fully support the deeply rooted position of this order.

Members of Aquificales have been isolated from several systems. *A. aeolicus*, *Aquifex pyrophilus*, *Hydrogenobacter halophilus*, and *Hydrogenothermus marinus* have been isolated from shallow marine systems (Huber et al. 1992; Nishihara et al. 1990; Stöhr et al. 2001). *Hydrogenobacter [Calderobacterium] hydrogenophilum*, *Hydrogenobaculum [Hydrogenobacter] acidophilum*, *Hydrogenobacter thermophilus*, *T. ruber*, and *S. azorense* are present in terrestrial hydrothermal systems (Kryukov et al. 1983; Kawasumi et al. 1984; Shima and Suzuki 1993; Huber et al. 1998; Kristjansson et al. 1985; Aguiar et al. 2004). Additionally, evidence of Aquificales has been found in heated compost (Beffa et al. 1996), deep gold mines (Takai et al. 2001), and from deep-sea hydrothermal vents—namely *P. marina* (Reysenbach et al. 2000a), *Persephonella guaymasensis* (Gotz et al. 2002), and *Persephonella hydrogenophila* (Nakagawa et al. 2003). Within Aquificales, the *Aquifex-Hydrogenobacter-Thermocrinis* lineage is a separate clade from the genera *Persephonella*, *Hydrogenothermus*, and *Sulfurihydrogenibium*; forming Aquificaceae and Hydrogenothermaceae, respectively (**Figure 2**).

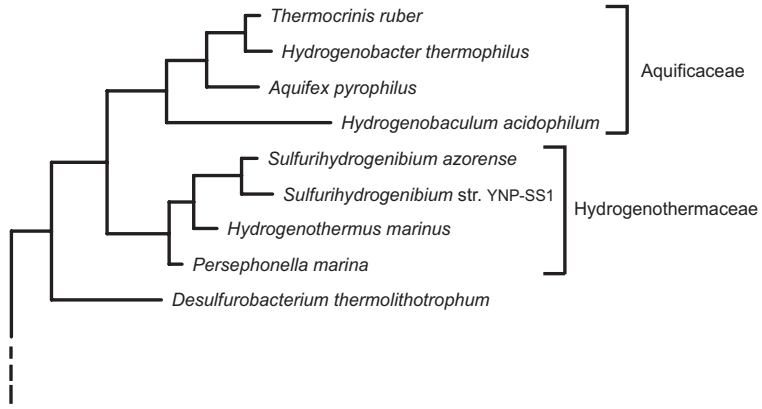

Figure 2. Maximum likelihood phylogenetic tree based on the small subunit rRNA molecule showing representatives of Aquificaceae and Hydrogenothermaceae.

Using the small subunit rRNA sequence information obtained from Aquificales' isolates, oligonucleotide probes have been developed that are specific for this order (Harm-

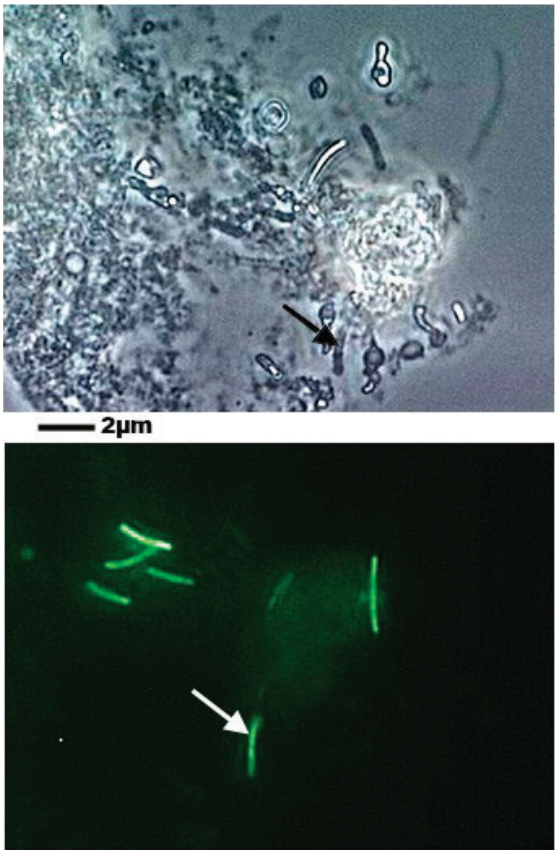

Figure 3. ***In situ*** **hybridization of black filamentous biomass from Calcite Springs, Yellowstone National Park, with a fluorescein-labeled oligonucleotide probe specific for Hydrogenthermaceae 16S rRNA.** Top panel is a micrograph using phase contrast microscopy of the same field as that of the fluorescent image (bottom panel), 100X. Arrow shows the same rod in both panels.

sen et al. 1997). We have modified this probe and used it as a primer to explore the global distribution of Aquificales (primer sequence aqfx540R-5'-TCGCGCAACGTTCGGGACC-3'; **Table 1, next page**). Additionally, a probe was designed that appears to be specific for the Hydrogenothermaceae (Hydroth840R 5'- GTAGCCCACATCTAGCAT-3'; **Figure 3**). Single mismatches occur in *Persephonella* and *S. azorense*. Although the conditions for probe hybridization have not been optimized, this probe and the general Aquificales probe are useful tools for monitoring enrichment cultures and for detecting relative abundances in the environment. Rusch and Amend (2004) also reported an Aquificales probe (Aqui1197) that has been used successfully.

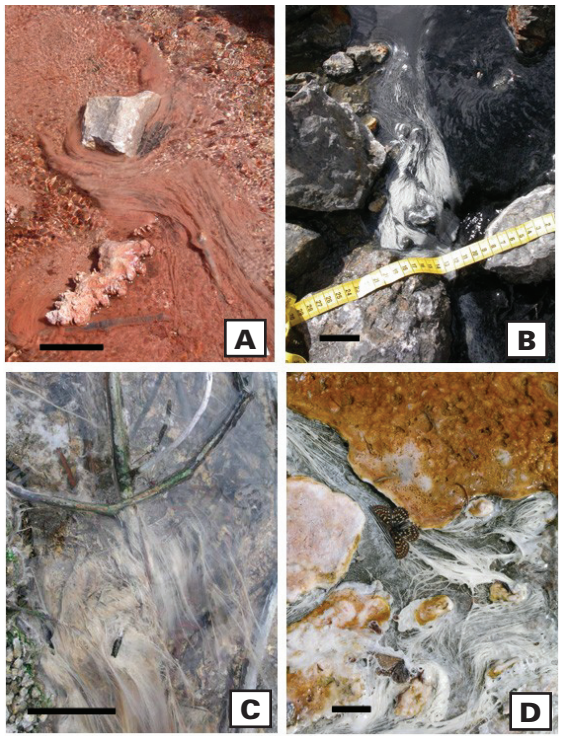

Figure 4. Filamentous communities in Yellowstone of predominantly Aquificales members. A. *Thermocrinis* sp. from Artist Paintpots; **B.** *Sulfurihydrogenibium* sp. from Calcite Springs; **C.** *Hydrogenobaculum* sp. from Nymph Creek; and **D.** *Sulfurihydrogenibium* from Narrow Gauge. Bar is approximately 3 cm.

3.0 DISTRIBUTION OF AQUIFICALES IN YNP

Members of both Aquificaceae and Hydrogenothermaceae are present in Yellowstone (**Figure 4**), where Aquificaceae generally occupy the higher temperature regimes and are hyperthermophiles, growing best above 80°C. This group is represented by *Thermocrinis* spp. (75-92°C, ~pH 8) and has not been reported to be associated with biomineralization; however, its role in siliceous sinter formation has been proposed (Blank et al. 2002; Reysenbach and Cady 2001). At lower temperatures (60-80°C), Aquificaceae are represented by *Hydrogenobacter* and *Hydrogenobaculum*. The latter are limited to low pH environments (e.g., pH~3.0)

Table 1. **A preliminary survey of Aquificales in Yellowstone National Park***

Site	*Hydrogeno-baculum*	*Thermocrinis*	*Sulfuri-hydrogenibium*	Latitude	Longitude	Reference
LGB River group (002L)+		+		44°31.49′	110°47.31′	‡
LGB Celestine Pool (008L) +		+		44°32.59′	110°48.24′	‡
LGB River (021L)			+	44°33.26′	110°49.59′	
LGB River (024L)			+	44°33.25′	110°49.56′	
LGB River(027L)		+		44°33.23′	110°49.56′	‡
LGB River (029L)		+		44°33.22′	110°49.56′	
LGB River (033L)			+	44°33.23′	110°49.52′	‡
LGB River (034L)			+	44°33.25′	110°49.54′	‡
LGB River (035L)			+	44°33.25′	110°49.55′	‡
LGB River (041L)		+		44°33.47′	110°50.4′	‡
LGB River (044L)		+	+	44°33.41′	110°49.59′	‡
NGB	+					Donahoe-Christiansen et al, 2004
NGB (054N)	+			44°43.59′	110°49.54′	
NGB (060N)		+		44°43.53′	110°42.40′	
NGB (063N)	+			44°43.27′	110°42.19′	
NGB (068N)	+			44°43.42′	110°42.9′	
Ledge Geyser (069N)		+	+	44°43.40′	110°42.11′	‡
Porcelin Basin (073N)	+			44°43.28′	110°42.10′	‡
Crater Hills (078CH)	+			44°39.9′	110°28.58′	
Crater Hills (080CH)	+			44°39.7′	110°28.59′	
LGB Porcupine (083L)			+	44°34.14′	110°48.38′	‡
LGB Sentinel Meadows (090L)		+		44°33.59′	110°51.48′	‡
MM Highland Spring (116MM) +		+		44°36.51′	110°35.15′	‡
MM Glen Africa Basin (117MM) +		+		44°36.51′	110°35.15′	‡
LSB (130LS)			+	44°24.1′	110°49.32′	‡
LSB (149LS) +	+	+		44°24.55′	110°48.41′	‡
LSB (155LS)		+		44°24.58′	110°48.40′	‡
SGB (170S) +		+		44°21.80′	110°48.12′	‡
SGB (174S)			+	44°21.50′	110°47.45′	‡
SGB (180S)			+	44°21.40′	110°47.45′	‡
SGB (196S)		+		44°21.13′	110°47.56′	‡
SGB (207S)		+		44°21.21′	110°47.52′	‡

and are abundant at Nymph Creek (Ferris et al. 2003; Reysenbach, unpublished observations; **Table 1**; **Figure 4**); Norris Geyser Basin (Langner et al. 2001; Jackson et al. 2001); Artist Paintpots (**Table 1**); and in acidic streams in the Obsidian Pool area (Reysenbach, unpublished data). *Sulfurihydrogenibium*, a member of the Hydrogenothermaceae, is prevalent in many springs with pH 6-8 and temperatures between ~60°C and 75°C such as Mammoth Hot Springs (Fouke et al. 2000); Calcite Springs and Obsidian Pool (Reysenbach et al. 2000a; Graber et al. 2001; Hugenholtz et al. 1998); and at Sylvan, Rainbow, and Washburn Hot Springs (**Table 1**).

Hydrogenobacter is often isolated from springs that contain *Sulfurihydrogenibium* as the dominant member, but is rarely detected in environmental 16S rDNA clone libraries or in denaturing gradient gel electrophoresis (DGGE) analyses. Both genera are often associated with hot springs that have higher total dissolved sulfide concentrations (up to 12 mg/L). Although these two organisms appear

Table 1. Continued

Site	*Hydrogeno-baculum*	*Thermocrinis*	*Sulfuri-hydrogenibium*	Latitude	Longitude	Reference
SGB (2000-15SL)			+	44°21.4′	110°47.44′	
Josephs' Coat (264JC)			+	44°44.21′	110°20.10′	‡‡
Josephs' Coat (271JC)			+	44°44.41′	110°19.18′	‡‡
HSB (282HSB)	+			44°44.59′	110°15.19′	‡‡
Rainbow Springs (287RNB)			+	44°46.70′	110°16.14′	‡
Smoke Jumper HS (2000-33SJHS)	+			44°24.40′	110°57.5′	
Smoke Jumper HS (2000-38SJHS)	+			44°24.36′	110°57.28′	
Smoke Jumper HS (2000-41SJHS)			+	44°24.57′	110°57.28′	
Smoke Jumper HS (2000-42SJHS)		+	+	44°24.57′	110°57.20′	
Sylvan springs (SV1)	+					‡‡
Sylvan springs (SOS)			+			‡‡
Artist Paint Pots (AP1-3)	+					‡‡
Artist Paint Pots (AP3-1)	+					‡‡
Calcite Springs			+	44°54.292	110°24.242′	Reysenbach et al., 2000
Washburn Hot springs			+			Spear et al., this volume
Octopus Spring		+				Blank et al., 2002
Queen's Laundry		+				Blank et al., 2002
Black Pool		+				Blank et al., 2002
Abyss Pool		+				Blank et al., 2002
Boulder Spring		+				Blank et al., 2002
Eclipse Geyser		+				Blank et al., 2002
Mammoth Hot Springs			+			Bonheyo, et al, submitted.
Obsidian Pool area			+			Graber et al. 2001
Obsidian Pool+		+	+			Hugenholtz, et al 1998
Nymph Creek	+					Ferris, et al 2003

*If a phylotype is not detected, it may still be present in the hot spring, but just not detected in the sample analyzed.
LGB Lower Geyser Basin NGB Norris Geyser Basin
SGB Shoshone Geyser Basin LSB Lone Star Geyser Basin
HSB Hot Springs Basin MM Mary Mountain
+ Phylotype O1aA90 was from Harris et al. 2004 was also detected
‡This study using Aquifex-specific‡ primers or bacterial primers‡‡

to share overlapping niches, *Sulfurihydrogenibium* is often the only organism detected in clone libraries and by fluorescent *in situ* hybridization (e.g., **Figure 3**), yet *Hydrogenobacter* is often isolated in cultures from these samples. For example, hydrogen-oxidizing enrichment cultures from Calcite Springs' samples dominated by *Sulfurihydrogenibium* resulted in the isolation of *Hydrogenobacter* (**Figure 5, next page**). However, we were only able to isolate *Sulfurihydrogenibium* (the dominant band in the environmental sample) in S^0-oxidizing enrichments, and the new species is *S. yellowstonense* (Nakagawa et al. submitted). Additionally, of 203 clones screened from a bacterial 16S rDNA clone library generated from a Calcite Springs' sample, 14 different restriction fragment length polymorphism (RFLP) patterns were detected, of which 13 were most closely related to *Sulfurihydrogenibium* (Takacs-Vesbach and Reysenbach, unpublished results). The other clone type was more closely related to *Geothermobacterium ferrireducens*, an organism that has previously been isolated from this environment (Slobodkin et al. 1997). Similar observations were

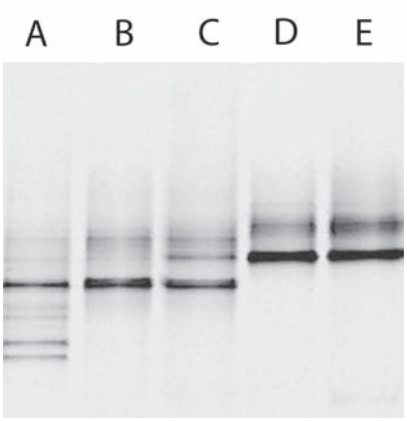

Figure 5. DGGE gel showing PCR products (16S rDNA 338FGC/aqfx540R) amplified from cultures and environmental samples. Note how Hydrogenobacter is eventually selected for. **A.** Y03ASS1-5 black filaments environmental sample; **B.** Original H_2-oxidizing enrichment; **C.** H_2-oxidizing culture after 2 transfers; **D.** H_2-oxidizing culture after 7 transfers; **E.** Hydrogenobacter str. SS1. Multiple bands on a DGGE gel does not necessarily indicate that a culture is not pure. Some Aquificales have at least 2 16S rRNA genes, that migrate differently on DGGE gels (data not shown), or different bands are the same sequence but migrate differently due to different melting profiles of the DNA. Both these features are disadvantages for the use of DGGE analysis for diversity assessments. DGGE conditions were as follows: 6% acrylamide (37.5:1 acrylamide:bis-acrylamide), 30-70% Urea-Formamide gradient, 60°C, 3.5 hr run time at 200V.

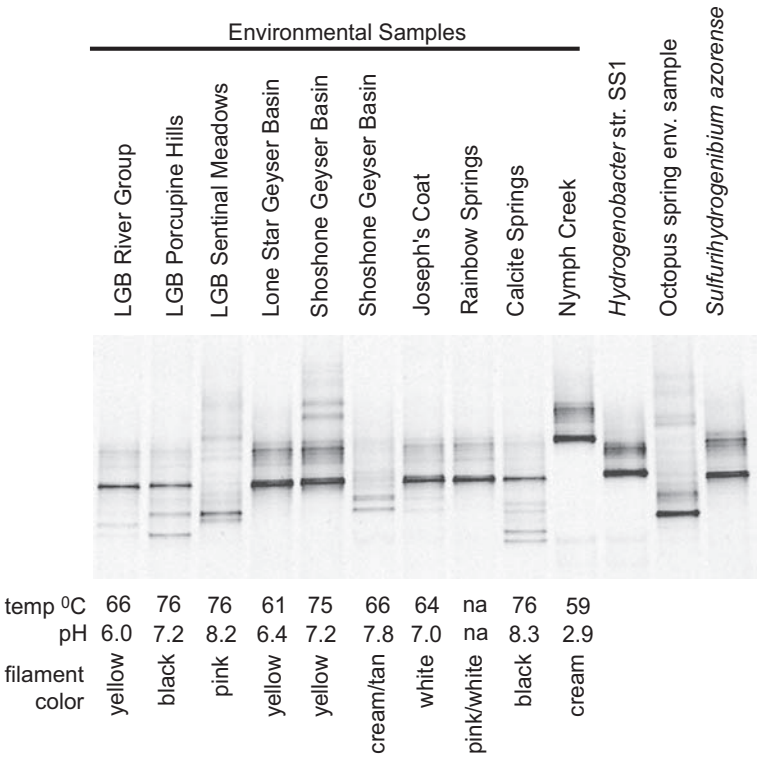

Figure 6. DGGE gel showing PCR products (16S rDNA 338FGC/aqfx540R) amplified from Yellowstone environmental samples and Aquificales cultures. DGGE conditions were as follows: 6% acrylamide (37.5:1 acrylamide:bis-acrylamide), 30–70% Urea-Formamide gradient, 60°C, 3.5 hr run time at 200V. This gel does not represent the Aquificales diversity per se, as sequencing of DGGE bands reveals that less than 10% of the bands are non-Aquificales sequences (OPB14- a candidate division OP, Hugenholtz et al. 1998; or clone O1aA90of candidate division OP11, Harris et al. 2004).

obtained from a spring feeding into Obsidian Pool (Graber et al. 2001). Likewise, DGGE analyses throughout the park have not revealed *Hydrogenobacter* sequences, yet *Thermocrinis*, *Sulfurihydrogenibium*, and *Hydrogenobaculum* are found as predicted (**Table 1**; **Figure 6**).

As part of a National Park Service grant and a National Science Foundation Biodiversity Surveys grant, we have analyzed the microbial diversity of more than 200 park-wide samples using PCR-based approaches with bacterial and archaeal 16S rRNA gene primers. In these studies, we have detected a wide range of novel microbial lineages; however, Aquificales have not always been detected in springs that would likely harbor this group. We therefore used a different set of primers that specifically amplified Aquificales in many of these same samples (e.g., **Figure 6**). **Table 1** is a sampling of some of the results obtained. Unfortunately, only pH, temperature, and conductivity measurements accompany these samples, and in order to make correlations between microbial distributions and geochemistry,

Table 2. A selection of geochemical characteristics of Calcite Springs, Yellowstone National Park.

	Measure range*
pH	7.5-8.4
Conductivity	1.57-2.46 µS
H_2 (at 90°C)	22 nM
H_2(at 65°C)	2.3 nM
CH_4 (at 90°C)	7 nM
DOC	230 µM
DO_2	62.5 µM
NH_4	1756 µM
PO_4	1-5 µM
SO_4	6.25-31.7 mM
NO_3	0.203-0.525 µM
Fe	25-46 µM up to 615 µM in the sediments
As	~ 30.3 µM
Sr	19.7-31.1 µM
Cl	5.9-14.1 mM
δ-13$_C$	-15.07 to -17.37

*Range represents measurements accumulated over 5 years of sampling

more detailed aqueous chemistry is needed. However, it is interesting to note that although our primer set principally amplifies Aquificales, it also detected an uncultured phylotype that was originally detected using OP11-specific primers (Harris et al. 2004) and OP1 (a candidate division proposed by Hugenholtz et al. 1998). The co-occurrence of the OP11-like phylotype with *Thermocrinis* is notable, and points to the possibility of isolating this group under similar conditions, around pH 8 and 85°C.

4.0 AQUIFICALES AT CALCITE SPRINGS

The Calcite Springs area sits at the base of a high temperature hydrocarbon seep (Love and Good 1982; Kvenvolden et al. 1989; Clifton et al. 1990) along the Yellowstone River. Over the past 10 years, the geothermal landscape near Calcite Springs has changed dramatically. Springs present on the south side in the mid-1990s and visible from the Calcite Springs Overlook went dormant in the late '90s, but returned in 2004. Cave-ins are often evident in this dynamic area.

In general, the area's thermal springs are slightly alkaline (pH 7.4-8.4), although a few acidic streams (pH 3.0-4.0) have been detected. The alkaline streams are dominated by black mineral precipitation and black, yellow, and white filamentous biomass. The aqueous chemistry varies some with temperature—the most notable being gas concentrations (**Table 2**). Sulfate, ammonia, arsenic, and iron are also enriched in these streams.

Aquificales (*Sulfurihydrogenibium*) that occur in Calcite Springs are the community's dominant (>95% of the cell biomass) members (Reysenbach et al. 2000a) and are found between about 84°C and 50°C. Approximately six separate phylotypes have been detected that vary in 16S rRNA sequence by ~<1%. Interestingly, two different cultures have been isolated—one that grows best at 60°C and is 0.9% different in 16S rRNA sequence from the original dominant clone described from this area (pBB, Reysenbach et al. 2000a), and a second (*S. yellowstonense*) that grows best at 70°C and is 1.3% different from this clone (Nakagawa et al. submitted). Both were isolated under S^0-oxidizing autotrophic conditions. The different isolates and multiple phylotypes may represent different ecotypes. In a separate study in which we sampled an 84°C site repeatedly over a week-long period, although the Aquificales were dominant throughout, the less dominant members varied considerably over the time period as did the oxygen concentration in the geothermal fluid (Takacs-Vesbach and Reysenbach, unpublished data).

In addition, 16S rRNA gene sequences belonging to the beta-Proteobacteria have been detected (Mason 2003; **Figures 7 and 8, next page**) and are similar to the Obsidian Pool sequence obtained by Hugenholtz et al. (1998). This is noteworthy because the white filamentous biomass detected in Mammoth Hot Springs' pools was first named *Thermothrix*, a beta-proteobacterium (Caldwell et al. 1976), based on a S^0-oxidizing isolate from this area. However, from culture independent analyses, the bulk of the filaments are not Proteobacteria, but rather *Sulfurihydrogenibium*. In slower flowing streams at Calcite Springs, or where water pools, the communities are often more complex, characterized by more anaerobic conditions below the upper filamentous mat. Archaea have been detected belonging to the Korarchaeota, and Crenarchaeota related to *Thermofilum*, *Pyrobaculum*, and *Desulfurococcus*, and several uncultured phylotypes (Reysenbach et al. 2000b; Mason 2003).

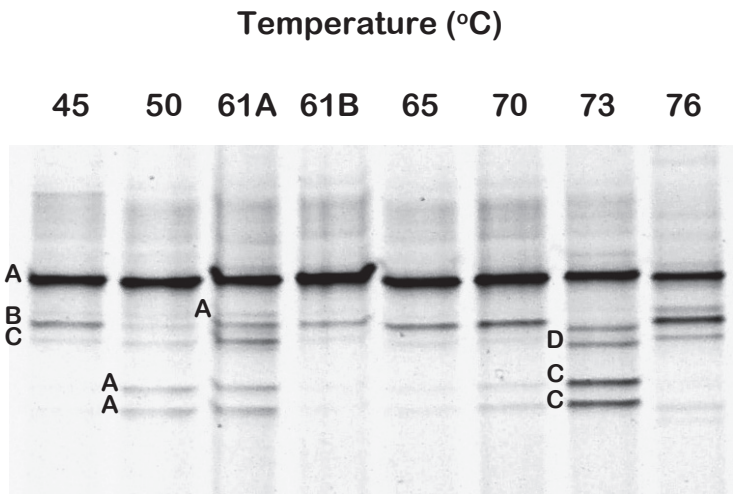

Figure 7. DGGE of 16S rDNA fragments (primers 338FGC/519R) from samples collected from Calcite Springs, 45–76°C. A sterile coring device with a diameter of 1 cm was used to standardize sample collection, and replicate samples (n=4) were taken to determine the spatial heterogeneity of the community. All the samples were immediately frozen at the site in liquid nitrogen until returned to the laboratory where they were stored at –80°C until DNA was extracted. The DGGE gradient was a 20-60% Urea-Formamide gradient. The closest match of partial 16S rDNA sequences: **A.** pBB, (Reysenbach et al. 2000); *Sulfurihydrogenibium*-Aquificales; **B.** β-Proteobacteria OPB30 (Hugenholtz et al. 1998); **C.** *Thermocrinis ruber* (Huber et al. 1998); **D.** Thermotogales OPB45 (Hugenholtz et al. 1998).

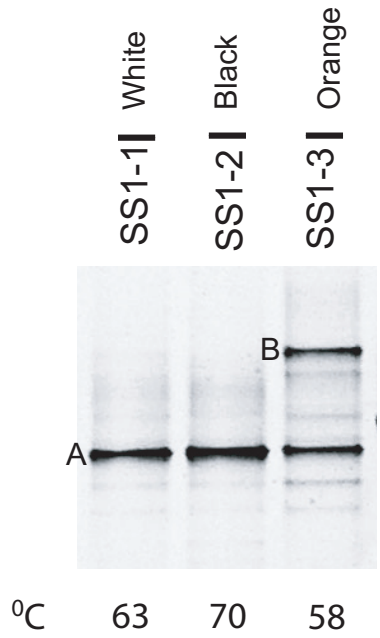

Figure 8. DGGE gel showing PCR products (16S rDNA 338FGC/519R) amplified from Calcite Springs, Yellowstone National Park environmental samples. Color (as reflected by the mineral precipitates) and temperature of the filamentous samples are depicted. The sequence of 'A' most closely matches *Sulfurihydrogenibium* and band 'B' most closely matches environmental clone OPB30 (Hugenholtz et al., 1998) which groups with the β-Proteobacteria. DGGE conditions were as follows: 6% acrylamide (37.5:1 acrylamide: bis-acrylamide), 30-70% Urea-Formamide gradient, 60°C, 3h run time at 200V.

5.0 BIOMINERALIZATION AT CALCITE SPRINGS

Although no extensive mineralogical analyses have been done at Calcite Springs, X-ray diffraction (XRD) analysis of mineral precipitates associated with the filaments revealed a mixture of calcite, pyrite, albite, quartz, pyrrohotite, and greigite (Reysenbach et al. 1999). Furthermore, many of the cells in the black filamentous mats have iron precipitates in their periplasmic space (Konhauser et al. 2003). The oxidation state of this iron is unknown and is under investigation using higher resolution techniques. The filaments are often coated with solid phases of different colors including black, grey, white-yellow, and orange precipitates likely corresponding to the formation of different minerals such as pyrrohotite, siderite, elemental sulfur, and Fe^{III} phases. Mineral differences generally follow temperature gradients (**Figure 7**) and probably reflect the changes in the chemistry (and oxygen concentrations) along the temperature gradient of the stream. Although the community structure does change some, it is clear that the dominant organism (phylotype and through fluorescent *in situ* hybridization; Reysenbach et al. 2000b) is *Sulfurihydrogenibium*.

Earlier studies at Calcite Springs reported the iron mineral precipitates associated with this hot spring (Reysenbach et al. 1999). Additionally, it was noted that layered black-to-grey sedimentary-type rock, primarily composed of calcite and some pyrite, was observed in the area, often overlaid by gypsum rubble. It was assumed that this represented the mineralized remains of the filamentous biomass that occurred in close proximity to these structures. Stages of this mineralization can be observed at

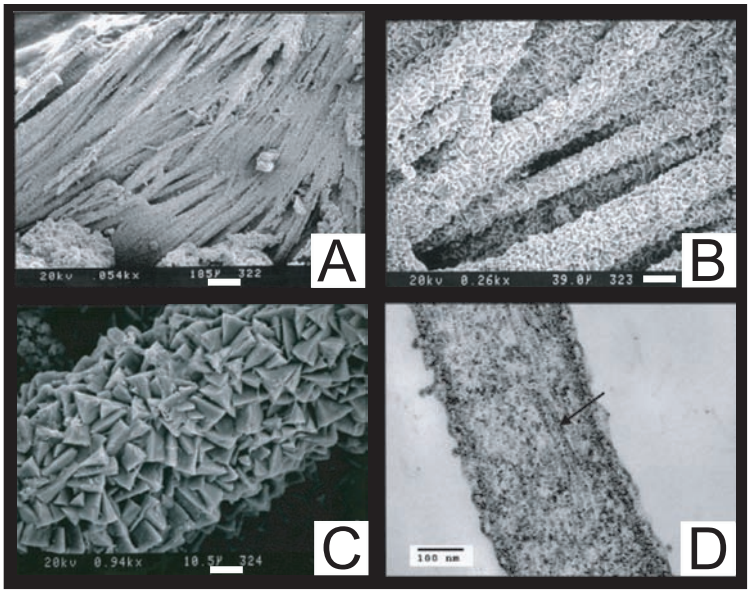

Figure 9. Electron micrographs of mineralized and living filaments from Calcite Springs, Yellowstone National Park. Scanning electron micrographs of mineralized filaments: **A.** Bar is 185 μm; **B.** Crystals are likely calcite, Bar is 39 μm; **C.** Bar is 10.5 μm; **D.** Transmission electron micrograph of a stained filament from communities at Calcite Springs. Arrow points to the internal structures that have been reported in the cultures of *Persephonella marina* (Gotz et al. 2002) and *Sulfurihydrogenibium azorense* (Aguiar et al. 2004).

Calcite Springs as streams dry up or are deviated. Scanning electron microscopy (SEM) images (**Figure 9**) reveal that mineralized filaments do not lose their structural integrity immediately. Preliminary lipid analysis of these structures revealed that although some of the fatty acids were retained, a degradation or shift in lipid profiles did occur with the shift from live to mineralized filaments (Simoneit and Reysenbach, unpublished results). Although cursory examination of thin sections of the rock did not show any possible microbial fossils, the early mineralized mats still showed some intact cells; in many the cytoplasm was already replaced by a mineral precipitate (**Figure 10**). Unfortunately, as the transmission electron microscopy (TEM) of the presented photomicrographs was not fitted with energy dispersive spectrometry (EDS), elemental analysis was not possible. Additionally, samples are being analyzed for stable isotopes of carbon.

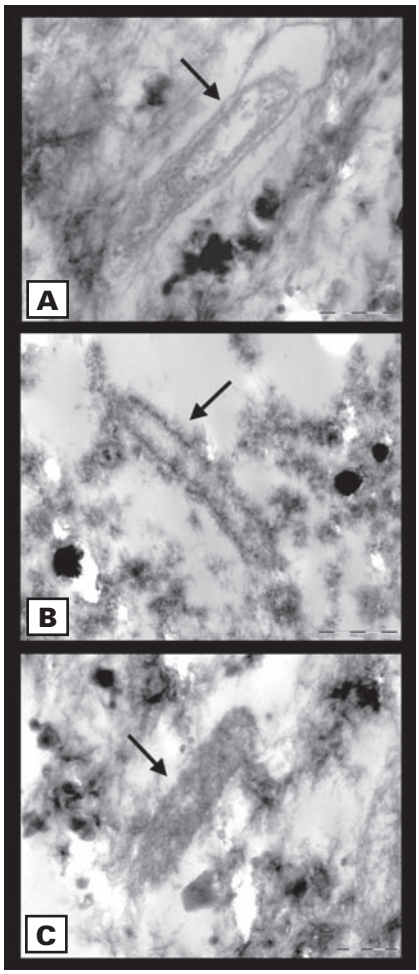

Figure 10. Transmission electron micrographs of mineralized filaments (as Figure 9, A-C) showing different stages of mineralization. This sample was collected from rocks in areas where the water was no longer flowing. Adjacent thermal springs (few centimeters to meters) had actively growing black filaments. **A.** Bar is 500 nm, cell is relatively intact with very little mineralization (arrow); **B.** Bar is 1000 nm, cell wall is mineralized and cytoplasm is partially mineralized (arrow); **C.** Bar is 500 nm, arrow indicates the mineralized cell.

6.0 CONCLUSIONS

Like the colorful photosynthetic microbial mats observed in lower temperature geothermal springs, the filamentous streamers have long caught the attention of Yellowstone's researchers. Consistently, the visible filamentous microbes occurring in both near-neutral (~pH 6-8) and acidic (~pH 2.5-4.5) hot springs belong to the order Aquificales—metabolically versatile chemolithoautotrophic thermophiles using an array of electron donors including H_2, sulfide, S^0, thiosulfate, and Fe^{II}. The widespread occurrence of Aquificales in Yellowstone's hot springs and throughout the world's terrestrial and deep-sea hot spring environments points to the importance of this group in the productivity of high temperature ecosystems.

ACKNOWLEDGEMENTS

ALR thanks Phil Hugenholtz for suggesting good regions for the Hydrothermaceae probe design, and Christy Callaghan for assisting with the manuscript preparation. Thanks to Carrie Guiles for assistance in the field. Research was supported by an NSF (DEB-0328326) grant to A.-L. Reysenbach, a National Park Service grant to A. Rodman, and a Biodiversity Inventory and Surveys grant to C. Takcas-Vesbach and subcontract to ALR (DEB-0206773). The TEM laboratory at Purchase College is supported by grant #0116457 from the Major Research Instrumentation Program of the National Science Foundation.

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