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The transition to microbial photosynthesis in hot spring ecosystems

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ABSTRACT

Even casual observations of continental hot springs reveal that photosynthesis has its limits. In an effort to explore the transition to photosynthesis, field measurements of temperature and pH were made at 996 hot spring locations at Yellowstone National Park ranging from 14° to 94 °C and pH from 0.8 to 9.7. In addition, sulfide measurements were made in 426 of these locations showing concentrations up to 8820 μg L^{-1} total sulfide. These data indicate that the previously established upper temperature (73–75 °C) for the transition to photosynthesis is reached in many basic hot springs, but that the transition occurs at lower temperature with decreasing pH below ~6.5. As an example, no strong evidence for photosynthesis was found above 45 °C at pH ~2. In several locations, photosynthesis appears to be suppressed despite temperatures and pH values that permit photosynthesis elsewhere. Sulfide concentrations may be responsible for the suppression of photosynthesis at these sites. Total sulfide concentrations were observed to decrease downstream in hot spring outflow channels. Abiotic processes (degassing, oxidation, mineral precipitation, etc.) are too slow to account for these decreases, suggesting an explanation from microbial sulfide oxidation that is supported by field experiments. Microbial sulfide oxidation may determine the ultimate suitability of some hot springs for microbial photosynthesis.

1. Introduction

Through photosynthesis, energy from the sun is used by autotrophic organisms to fix carbon dioxide to form organic matter (symbolized here as CH_2O), and the process can be oxygenic or anoxygenic depending upon the electron donor. In oxygenic photosynthesis, water is the electron donor:

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2 \tag{1}$$

whereas in anoxygenic photosynthesis, another molecule such as hydrogen sulfide is the electron donor:

$$CO_2 + 2H_2S \rightarrow (CH_2O) + 2S + H_2O. \tag{2}$$

Both of these processes occur in hot spring environments; however, there appear to be conditions beyond which photosynthetic life does not occur.

Upper temperature limits for microbial life and microbial photosynthetic life have been noted by researchers in Yellowstone National Park for over 120 years. Weed (1889) found "white vegetation" at a temperature of 85 °C and "yellow green vegetation" at 68 °C. Setchell (1903) recorded "chlorophylless *Schizomycetes* (Bacteria)" enduring a temperature of 89 °C, whereas "chlorophyllose *Schizophycae* (Cyanophyceae)" only inhabited waters up to 75–77 °C. Kempner (1963) and Brock (1967a, 1978) noted the photosynthetic upper temperature limit as 73° and 75 °C, respectively. In contrast, the maximum growth temperature of the non-photosynthetic hyperthermophilic Archaea, *Pyrolobus fumarii*, isolated from a submarine black smoker, was found to be 113 °C (Blöchl et al., 1997), and the growth of other submarine hydrothermal microbial strains was documented at 121 °C (Kashefi and Lovley, 2003) and 122 °C (Takai et al., 2008). Life, then, does not appear to be limited by the temperatures reached in continental hot springs, but perhaps photosynthetic life is.

Temperature is not the only factor constraining microbial photosynthesis. It is likely that in certain situations additional factors might inhibit photosynthesis including light availability, pH, salinity, nutrient supply, and presence of toxins, among other factors. The purpose of this reconnaissance study was to measure temperature, pH, and sulfide concentration in the field, in order to locate where the transition to photosynthesis occurs. Hot springs provide an ideal study site because the transition is commonly distinct and can be bracketed by field measurements. The transition to photosynthesis can be inferred by observing that the pigments involved in photosynthesis occur in some places but not in others, and in many instances there is a sharp edge where photosynthesis begins. In this

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study, we visually located the transition to photosynthesis by the presence or absence of these pigments.

Interactions of microorganisms with their environment are observed but not always understood. Consider temperature, for instance. Documented upper temperature limits to photosynthetic life have afforded speculation about the cause—including arguments about protein stability, the stability of enzyme–substrate complexes (Brock and Brock, 1966); the destruction of proteins, DNA, or RNA (Kempner, 1963); the limitations of the photosynthetic apparatus itself (Meeks and Castenholz, 1971; Castenholz, 1969); the alteration of energy flow from phycocyanin to chlorophyll at high temperatures (Meeks and Castenholz, 1978a) and the thermolability of the CO_2 assimilation mechanism (Meeks and Castenholz, 1978b)—but no real answers as to why these limits exist. Our efforts to explore the relations among the occurrence of visible microbial photosynthesis, temperature, pH, and sulfide concentration in the field were done to provide additional quantitative geochemical constraints on the problem. Answering *why* there is no microbial photosynthesis observed above 75 °C is left to biochemical research beyond the scope of this study.

In hot spring outflow channels, the transition to photosynthesis was located by the presence of biofilms that showed green, orange, brown, yellow (in association with green) and/or purple pigmentation. In addition, warm springs cloudy with green pigmentation were assumed to host photosynthesis. This method is unlikely to generate a false positive, but at a low abundance of phototrophs could produce a false negative. Color variations are likely to reflect variations in the combinations of pigments present. Chlorophylls have a characteristic green to blue-green color due to their two major absorption bands in the red or near-infrared and blue or near-ultraviolet regions of the electromagnetic spectrum (Blankenship, 2002). Carotenoids and phycobiliproteins are accessory pigments found in cyanobacteria that transfer energy to chlorophylls. Carotenoids absorb in the 400–500 nm range, making them orange and yellow. Carotenoids are also known to provide indirect protection from high solar irradiance by quenching various forms of reactive oxygen or inhibiting their production (Norris et al., 2002). Phycobiliproteins absorb from 550–650 nm. Phycoerythrin and phycocyanin are red and blue phycobiliproteins, with absorptions around 550 and 620–640 nm respectively. A purple extract from the acidophile *Zygogonium* absorbs in the 500–550 nm range (Alston, 1958). In contrast, the light-harvesting pigment complex of *Thermochromatium tepidum* has a maximum in its absorbance at 920 nm (Madigan, 2003).

In some cases, certain colors are suggestive of the presence of specific microbes. Cyanobacteria, the only oxygenic photosynthetic bacteria, are typically green, using chlorophyll *a* and phycobiliproteins. The other four groups of anoxygenic photosynthetic bacteria are the green sulfur (bacteriochlorophylls *a* and *c*, *d*, or *e*), green nonsulfur (bacteriochlorophylls *a* and *c*), purple sulfur (bacteriochlorophyll *a* or *b*), and purple nonsulfur (bacteriochlorophyll *a* or *b*). *Chloroflexus*, among the green nonsulfur bacteria, is widely distributed in Yellowstone (Ruff-Roberts et al., 1994; Pierson et al., 1999; Pierson and Parenteau, 2000; Nübel et al., 2002; van der Meer et al., 2003) and is known to be green under anaerobic conditions and orange under aerobic conditions (Pierson and Castenholz, 1971, 1974). *Roseiflexus*, a red to reddish-brown, chlorosome-less anoxygenic filamentous photoheterotrophic bacterium, first isolated from a bacterial mat in a Japanese hot spring (Hanada et al., 2002), as well as closely-related filamentous anoxygenic phototrophic bacteria, can also be found in Yellowstone hot springs (Boomer et al., 2000, 2002; Klappenbach and Pierson, 2004). Among the photosynthetic, eurkaryotic microorganisms, the bluish-green *Cyanidium caldarium* is a common inhabitant of acidic thermal waters (Brock, 1978; Lehr et al., 2007), a strain of the related *Galdieria sulphuraria* is reported from Yellowstone (Cozzolino et al., 2000; Toplin et al., 2008), as well as strains of the red algaes *Chlorella*, *Paradoxia*, and *Cyanidioschyzon* (Ferris et al., 2005). Filamentous green algae among the *Zygnemataceae* also inhabit cooler acidic regions of Yellowstone hot spring ecosystems (Lynn and Brock, 1969; Rothschild, 2001).

Sample locations for the present study were selected to cover wide ranges of temperature, pH, and chemical composition. We sampled several basic springs in the Lower Geyser Basin including sites in Sentinel Meadow (Mound Spring, Flat Cone, Steep Cone, Queen's Laundry and other unnamed springs), Boulder Spring, Imperial Meadow, White Creek (Octopus Spring, among others), Rabbit Creek in the Midway Geyser Basin, Heart Lake and Lone Star. Several near neutral and acidic springs were sampled in the general vicinity of Obsidian Pool in the Mud Volcano area. Additional acidic springs were sampled at Crater Hills north of Mud Volcano, and Sylvan Springs in the Gibbon Geyser Basin. Finally, we sampled several geochemically distinct springs and mudpots at Washburn Springs on the southeastern flank of Mt. Washburn, and Calcite Springs along the Yellowstone River. Related geochemical information about many of these sites, maps, and additional information is provided by Shock et al. (2010).

2. Methods

The temperature, pH, and conductivity measurements were taken in the summers of 2003 through 2009 with a pH meter (WTW Model pH330i), and a hand-held salinity, conductivity, and temperature system (YSI Model 30). Following the manufacturer protocol, the WTW electrode was kept in a solution of potassium chloride to prevent ion leakage through the reference junction, maximizing electrode life and minimizing response time. WTW specifications report an accuracy of ±0.01 for pH and ±0.1 °C for temperature. YSI specifications indicate an accuracy of ±0.5% for the specific conductance and ±0.1 °C for the temperature. The temperature was also recorded at some locations using a Taylor professional digital pocket thermometer (Model 9841) with an accuracy of ±0.1 °C. Observing the fluctuations inherent to the hot spring environment on the hand-held meters, the errors in all measurements are taken to be five times the manufacturer specifications (pH ±0.05, conductivity ±2.5%, and temperature ±0.5 °C).

The concentration of the total dissolved sulfide was measured in the field at 37 sites using a Hach sulfide kit and a Hach DR/2010 Portable Datalogging Spectrophotometer (test #690, wavelength of 665 nm, range of 0 to 600 $\mu g\ L^{-1}$ total sulfide). An additional 389 measurements were made using a Hach DR/2400 or DR/2800 Portable Datalogging Spectrophotometer. The method relies upon the reaction of hydrogen sulfide and acid-soluble metal sulfides with N,N-dimethyl-*p*-phenylenediamine oxalate to form methylene blue (adapted from Standard Methods for the Examination of Water and Wastewater, after Cline, 1969). Care was taken to perform this test immediately after collecting the sample, and to avoid agitating the sample more than necessary in order to minimize the loss of sulfide. The protocol was similar at each site, but the dilution factor was adjusted to accommodate high and low concentrations of sulfide. Some sulfide loss probably occurs when a sample is diluted, making these measurements lower limits. Field accuracy and precision were not tested, although the precision as reported by the manufacturer, using standard solutions of 250 $\mu g\ L^{-1}$ total sulfide, is ±3 $\mu g\ L^{-1}$ total sulfide. The estimated detection limit is 10 $\mu g\ L^{-1}$ total sulfide.

Our analytical strategies varied subtly depending on hot spring discharge and known sulfide concentrations. At sites surrounding Obsidian Pool, as well as some of the Sentinel Meadow and White Creek locations, the dilution was one part sample to one part deionized water. Each sample was collected in a polyethylene beaker that was rinsed three times. The 25-mL spectrophotometric vials were rinsed three times with several mL of deionized water. Using 10-mL pipettes, 12.5 mL of sample was added to the sample vial, and 12.5 mL of deionized water was added to the blank. To each vial, 1 mL of Hach Reagent 1 (sulfuric acid) was added followed by 1 mL of Hach Reagent 2 (potassium dichromate). Both the sample and the blank were diluted with 12.5 mL of deionized water. Both vials were agitated