MICROBIAL MAT COMMUNITIES IN HAWAIIAN LAVA CAVES

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Abstract

Microbial mats are a prominent feature in many Hawaiian lava caves, but little research has been done on these communities. Since 2008, we have sampled 16 lava caves on the Big Island of Hawai`i for microbial communities for scanning electron microscopy (SEM) analysis, cultivation, and DNA sequencing. These caves occurred in areas of Hawai`i that varied in rainfall from 47—401 cm per year. Sampled communities included microbial mats of various colors from white to tan, yellow, and orange; white mats floating on puddles in the floor; and butterscotch-colored organic ooze. We also sampled "microbes that masquerade as minerals" to determine whether mineral deposits contained substantial microorganisms. SEM studies revealed diverse morphologies across the lava caves, with coccoid and filamentous shapes predominating. Culture media inoculated with microbial mat or mineral deposits on site in Hawaiian lava caves revealed morphologies consistent with Actinobacteria and many cultures demonstrated the presence of fugitive dyes that were aqueously soluble. DNA analysis revealed that the white wall microbial mats differed from the yellow, pink, and orange mats, which were more similar to each other. Actinobacteria dominated the latter deposits. Overall, the type of sample (mat versus mineral versus surface soil) made the greatest composition difference.

Introduction

Hawaiian lava caves are rich in colorful microbial mats such as the yellow mats in Fig. 1. Yellow and white microbial mats predominate in most lava caves in areas with higher rainfall, with occasional pink, orange, and shades in between occurring in some mats. In more arid areas, such as those found in the vicinity of Ocean View, HI, white mats dominate the caves in the deeper, more humid regions. Microbial mats can consist of individual colonies (Fig. 2A), thick mats of microorganisms (Fig. 2C), organic ooze (Fig. 2B), or floating colonies on pools (Fig. 2D). In areas nearer the entrances of these semi-arid regions, mineral deposits that contain abundant microorganisms dominate, including some moonmilk deposits. More rare and unusual, are the copper containing deposits (Fig. 3).

Figure 1: Yellow microbial mats line walls of this Hawaiian lava cave. Photo by K. Ingham.

Studies by Hathaway et al. (2014) of lava cave microbial communities previously have revealed diverse communities using an older sequencing technique (Sanger sequencing). Our goal was to use next generation 454 sequencing to do a more comprehensive survey of the microbial communities in Hawaiian lava caves.

Methods

Beginning in 2008, we have sampled 16 lava caves for microbial communities for scanning electron microscopy (SEM) analysis, cultivation, or DNA sequencing. Rainfall on the surface above these caves varied from 47—401 cm per year, with the least amount being above the caves in the Kipuka Kanohina System and the greatest amount being in caves in the vicinity of Hilo, HI. Microbial mats varied in color from white to tan, yellow, orange and pink. Other samples included white mats floating on puddles in the floor and butterscotch-colored organic ooze. Samples were taken aseptically using a flame-sterilized cold chisel, and were immediately covered with sucrose lysis buffer to preserve the DNA (Giovannoni et al. 1990). Rock chips with microbial mats sampled for scanning electron microscopy (SEM) were mounted directly on SEM sample stubs in the field.

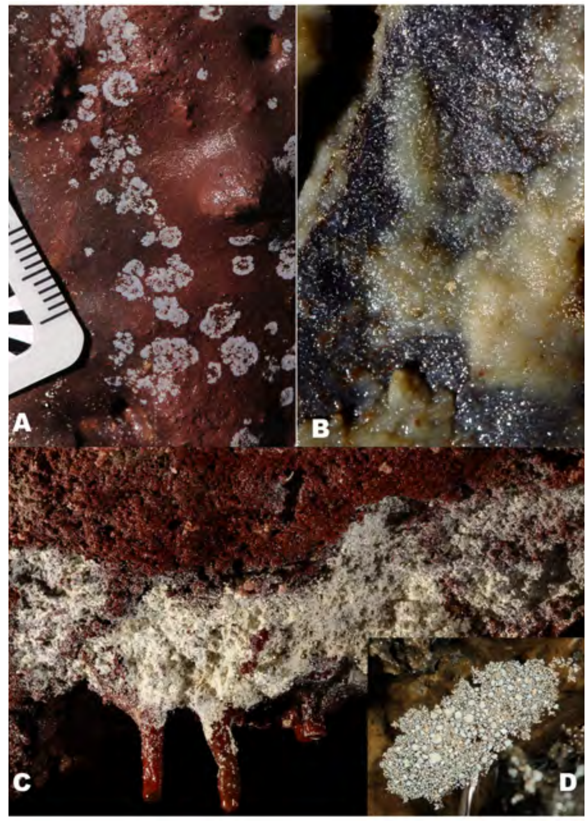

Figure 2: Macroscopic images of microbial colonies and deposits in Hawaiian lava caves. Photos by K. Ingham.

Scanning Electron Microscopy

Samples were air dried, and coated with Au-Pd metal for imaging in the laboratory. They were then examined on a JEOL 5800 SEM equipped with an energy dispersive X-ray analyzer (EDX), at high vacuum with an accelerating voltage of 15 keV with a beam current between 0.1 to 0.01 nA.

Cultivation

Microbial colonies were inoculated into a variety of culture media, optimized for lower nutrient, mineral-rich environments, on site in the caves and allowed to grow for months to years to encourage the growth of more slow-growing cave-adapted microorganisms. Cultures that revealed the presence of fugitive dyes had portions of the agar excised from the cultures, dissolved in pH 7 phosphate buffer and measured with a Nanodrop 1000 UV/Vis spectrophotometer. Long-term incubations of some Hawaiian cultures from 2008 collections were grown in calcite-containing media (10, 35, and 50 mg/L CaCO3 where saturation at 20oC is ~350 mg/L, Donor & Pratt, 1969). Cultures were also grown on silica containing media, using sodium silicate (a.k.a. waterglass) as silica source at concentrations of 10 and 60 mg/L, which are representative of natural waters (e.g. Miretzky et al 2001; Asano et al 2003)

DNA extraction and sequencing

DNA was extracted and purified using the MoBio PowerSoilTM DNA Isolation Kit using the manufacturer's protocol (MoBio, Carlsbad, CA), with exception of using beat beating instead of vortexing. Extracted DNA was sent to MR DNA (**http://mrdnalab.com/**) for next gen 454 sequencing. Returned sequences were processed and analyzed used Qiime (**qiime.org**).

Figure 3: Copper-containing stalactite from a Hawaiian lava cave near Hilo. Photo by K. Ingham.

Results and Discussion

Scanning Electron Microscopy

SEM studies revealed diverse morphologies including beads-on-a-string (Fig. 4A, white line with a solid white ball), filaments (Fig. 4B, C, D), reticulated filaments (Fig. 4E, F), rows of rods embedded in biofilm (Fig. 4C, leftmost black line with solid black square), and cocci (Fig. 4A with white line with open white circle, B), some with extensive hair-like or knobby extensions. Biofilm was extensive in many samples (Fig. 4C, black lines with solid squares on ends). Some of the mineral deposits contained more unusual minerals, such as copper silicates and vanadium oxides. Moonmilk deposits revealed calcite ("lublinite") crystals, microbial filaments, beads-on-a-string, and possible opal deposits (not shown). Reticulated filaments are relatively rare in lava cave microbial mats, with the exceptions of a white mat (Fig. 4E) and copper silicate speleothems from the Kipuka Kanohina System and in Blair Cave (Fig. 4F).

Cultivation

Culture media inoculated on site in Hawaiian lava caves revealed morphologies consistent with Actinobacteria and many cultures demonstrated the presence of fugitive pigments that were aqueously soluble and sensitive to light. Pigment colors range from pink and red to orange and disappear in cultures exposed to light after they are initially incubated in the dark. The spectra from portions of the agar excised and analyzed with a spectrophotometer, closely resemble index spectra of beta-carotene (Miller et al 1935 and many subsequent studies), with a number of additional peaks not yet identified that may be due to other pigment components or to other adsorptive materials in the agar.

Some cultures demonstrated significant ability to precipitate calcite and silica if provided in the media. Precipitate halos of microcrystalline calcite primarily on the growing margins were observed. This material coated the entire colony after several years of growth and the colony eventually slowed its growth to undetectable, which may be due to a combination of aging of the cells or to the growth kinetics of calcium carbonate crystallization (e.g. Nancollas & Reddy, 1971). Cultures grown in the presence of a small concentration of sodium silicate to mimic typical silica concentrations in natural waters (e.g. Correll et al 2000) have a different precipitation pattern and simply appear to coat themselves in a silica gel that then hardens. The cultures are being maintained to determine whether such amorphous silica will eventually crystallize.

DNA sequencing

Hawaiian lava caves contain a wealth of microbial colony morphologies and bacterial phyla that vary by color of microbial mat, by cave, and by type of

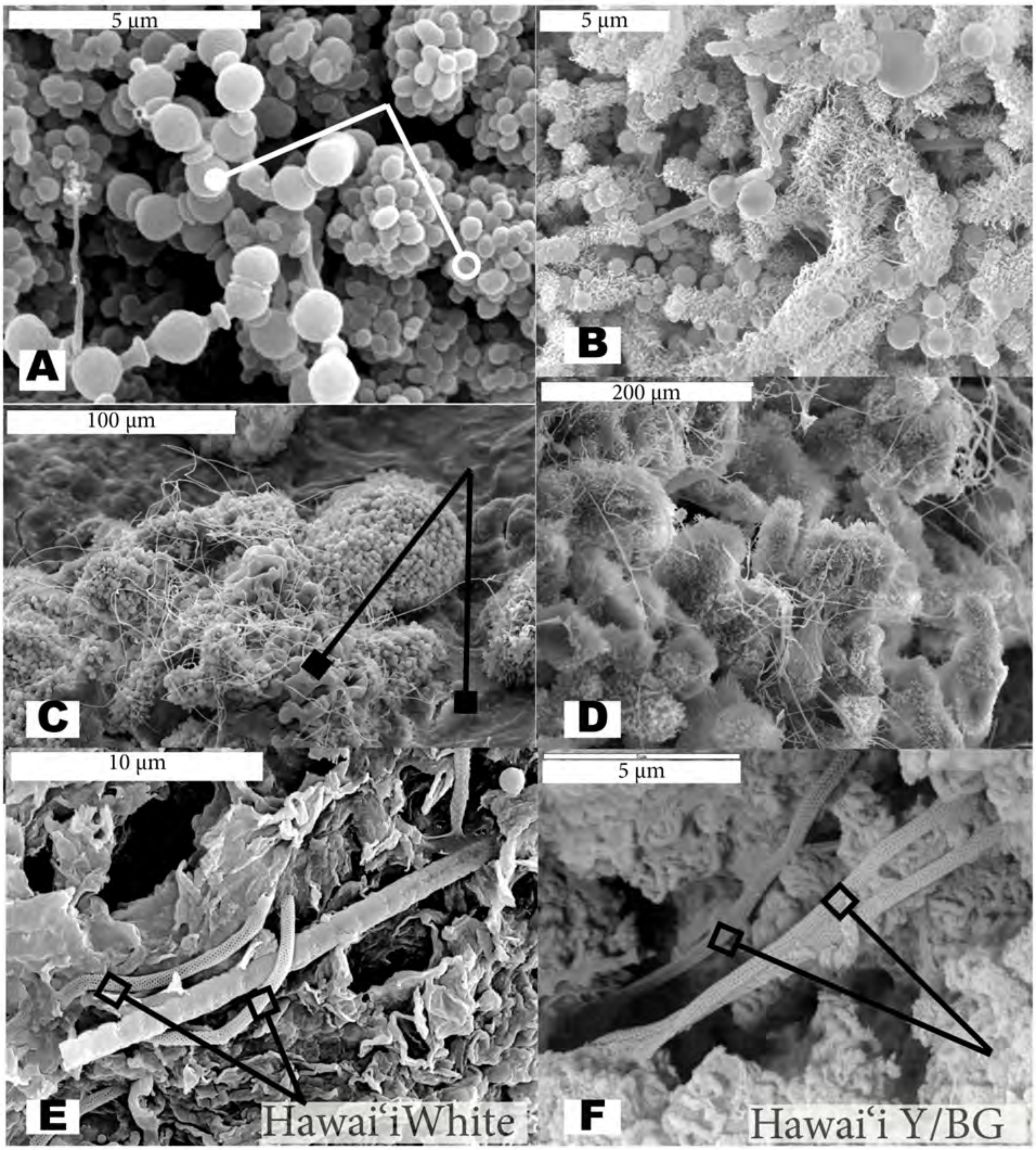

Figure 4: Scanning electron micrographs of white (A, C, E) and yellow (B, D) microbial mats, and a blue-green speleothem (F).

microbial deposit. Common bacterial phyla present in the communities included Alpha-, Beta-, and Gammaproteobacteria, Actinobacteria, Acidobacteria, and Nitrospirae (Fig. 5).

The Actinobacteria, which give caves their musty odor, were more abundant in microbial mats. Looking at the overall patterns observed in the phyla plot, it's apparent that the yellow and pink-orange microbial mats are very similar, while the white microbial mats are more varied and substantially different from the other colors. White floating mats were much less diverse than wall microbial mats and were very different in composition from microbial mats. Organic ooze varied from sample to sample, but usually contained a moderate number of Nitrospirae sequences and was very different in composition from wall microbial mats. The organic ooze from Kula Kai Caverns in the Kipuka Kanohina System had abundant Firmicutes and Gammproteobacteria (Fig. 5).

Common genera present included *Bacillus, Nitrospira, Crossiella,* and *Euzebya* (Fig. 6). The latter two genera are recently described Actinobacteria genera that have been found in several cave culture-independent studies. *Crossiella* sequences were present in large numbers in some of the cave microbial mats. *Nitrospira* is a genus of bacteria that is known for its oxidation of nitrite to nitrate. Recently a new member of the *Nitrospira* genus has been shown to be able to execute the entire nitrification cycle, taking ammonia all the way to nitrate (Daims et al. 2015). The genus *Chloracidobacterium,* first identified in 2007 (Bryant et al. 2007), is currently only known for its photosynthetic lifestyle. The identification of this genus in all of the caves studied, and in particular in Kazumura and Textbook Tunnel Caves, raises the possibility that other, non-photosynthetic species exist in this genus. Alternatively, the *Chloracidobacterium* in the cave could employ a mixotrophic metabolism in which they use heterotrophy when in the dark. Or, their presence could be evidence of re-inoculation of the lava caves from surface microorganisms entering the cave by being lofted on air masses or fluid-borne by infiltration of waters from the surface.

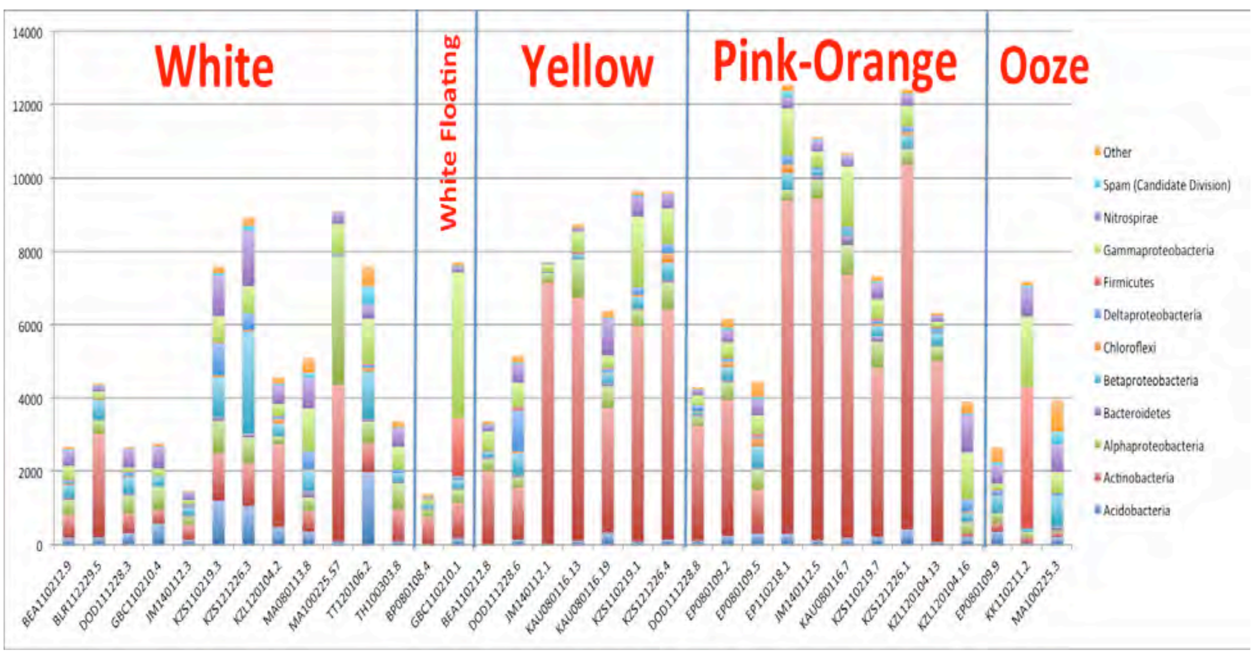

Figure 5: Phyla of bacteria present in white wall microbial mats, white floating colonies, yellow and pink to orange wall microbial mats, and organic ooze deposits.

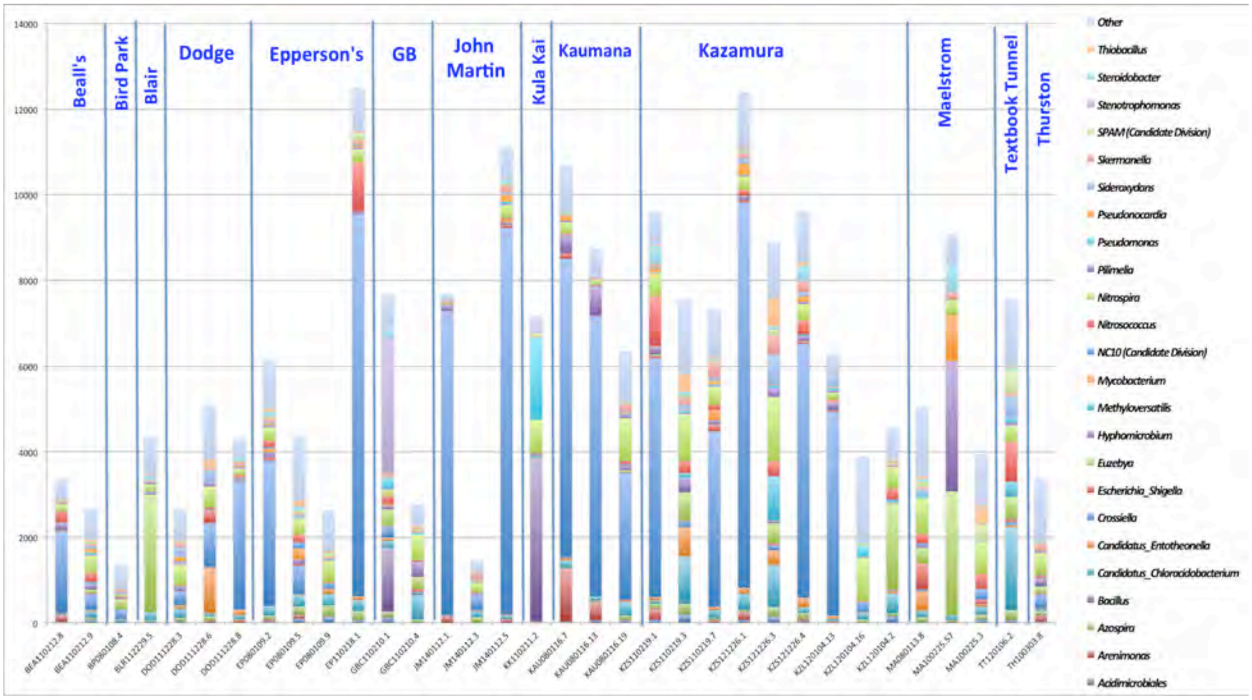

Figure 6: Bacterial genera present in Hawaiian lava caves by cave sampled.

Conclusions

Hawaiian lava cave microbial mats, floating microbial colonies, and organic ooze deposits contain a wealth of microbial diversity with eight major bacterial phyla and many less abundant bacterial phyla present. Scanning electron microscopy has provided us with an intimate look at the microbial morphology diversity present. Most notable are the extensive rods, cocci, and filaments with hair-like and knobby extensions from their cells. Also of interest is the presence of reticulated filaments found in many carbonate caves worldwide. Yellow, pink and orange microbial mats are much more similar in composition to each other and differ substantially from white microbial mats, which are more diverse in their composition. Organic ooze deposits differ from each other and from microbial mats. Cultivation of microorganisms from these mats is shedding light on some of their properties, such as the ability to precipitate calcium carbonate and silica. This study sheds light on the nature of these diverse microbial mats and suggests there is much left to study.

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