METAGENOMIC SEQUENING OF THE RIO TINTO. S. S. Johnson[1], L. Amaral-Zettler[2], B. Haas[3], R. Amils[4], C. Carr[5], G. Ruvkun[6]

[1]Harvard University, (78 Mount Auburn St., Cambridge, MA 02138, sjohnson@fas.harvard.edu), [2]Marine Biological Laboratory, (7 MBL St., Woods Hole, MA 02543, amaral@mbl.edu), [3]Broad Institute, (320 Charles St., Cambridge, MA 02139, bhaas@broadinstitute.org), [4]Centro de Astrobiología, (28850 Torrejón de Ardoz, Madrid, Spain, ramils@cbm.uam.es), [5]Massachusetts Institute of Technology, (77 Massachusetts Avenue, 54-418, Cambridge, MA 02139, chrisc@mit.edu), [6]Massachusetts General Hospital, (Department of Molecular Biology, ruvkun@molbio.mgh.harvard.edu).

Introduction: Microbial life is continually being discovered in Earth environments in exceedingly harsh conditions, demonstrating the surprising adaptability of microbes. The Rio Tinto in Southwest Spain is one such site. The waters of the Rio Tinto precipitate a diverse suite of iron sulfates and oxides, several of which resemble those found at the landing site of the Opportunity Rover on Mars. Highly acidic conditions also dominate the Rio Tinto system; the average pH is only 2.3 [1]. At its headwaters, acid mine drainage mixes with natural sources of acidity. Although this area has been mined for ores for several thousands of years [2], there is isotopic evidence (in the form of ancient iron oxide terraces, that suggests an age of 2-6 My [1, 3, 4], and paleosols that predate the Pliocene) that the Rio Tinto system is also driven strongly by chemolithotrophic metabolism [5].

Here we present initial results from the first metagenomic sequencing effort in the Rio Tinto. We focus on samples from the middle section of the Rio Tinto, from the Berrocal sampling region. The microbial diversity at this station is well-characterized for bacteria, archaea and eukaryotes. It is thought to be representative of the approximately 70 km-long acidic river.

Materials and Methods: Sediment and water samples were collected in 50 ml Falcon tubes at the Berrocal site. Sampling conditions and DNA extraction were as described in [6]. Sequencing was completed on the Broad Institute's Titanium 454, and preliminary sequence identifications were made using the SEED database in MG-RAST with a maximum e-value of 0.01 [7].

Results: A total of 130,141 sequences were recovered, approximately 102,616 from sediment (of which 39,728 could be classified phylogenetically) and 27,525 from the water column (of which 7,820 could be classified phylogenetically). The average sequence length was 397 bp and 329 bp, respectively.

The most frequent sequences among the metagenomic data matched organisms detected using more classic approaches, like cloning or in situ hybridization, for example, *Acidiphilum* and *Acidithiobacillus ferrooxidans*. We also detected several organisms that have not been seen before in the Rio Tinto, most likely due to methodological limitations. Among those, two stand out.

We found low levels of many types of sulfate-reducing bacteria within our our sediment and water samples, including multiple species of *Desulfobacterales* and *Desulfovibrionales*. These bacterial activities are responsible of closing the sulfur cycle in the Tinto ecosystem.

We also identified several sequences from *Magnetococcus*, *Magnetospirillum magneticum*, *Magnetospirillum gryphiswaldense,* and *Magnetospirillum magnetotacticum* within the sediment and water samples. This represents the first time magnetotactic bacteria have been detected in the Rio Tinto. While magentotactic bacteria are not well characterized, it is known that they most often thrive in areas with low to no oxygen. In the Northern Hemisphere, where geomagnetic north also points down, magnetotactic bacteria aligned to the geomagnetic field move down through the water column into areas with less oxygen. *Magnetospirillum magnetotacticum* are often found just above the anoxic zone occupied by methanogens and sulfate-reducging bacteria, which lessens the competitive pressure for nutrients [8]. It is also known that *Magnetospirillum magneticum* contribute to the iron cycle by transforming Fe to Fe_3O_4 or Fe_3S_4 [9].

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