

Pyrotag Sequencing Across Three Domains and Two Seasons Unveils the Río Tinto's 'Rare Biosphere'. L. A. Amaral-Zettler¹, E. R. Zettler², and R. Amils^{3,4} ¹The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution (Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543 USA, amaral@mbi.edu), ²Sea Education Association (PO Box 6, Woods Hole, MA 02543, USA, ezettler@sea.edu) ³Centro de Biología Molecular (Universidad Autónoma de Madrid, Madrid, 28049 Spain) ⁴Centro de Astrobiología (INTA-CSIC, Torrejón de Ardoz, 28850 Spain, ramils@cbm.uam.es)

Introduction: The Río Tinto in Southwestern Spain is a terrestrial analog for Mars that offers an opportunity to explore microbial community structure across all three domains of life – Bacteria, Archaea and Eukarya. Despite extremely acidic and high heavy metal conditions, a variety of traditional and molecular ecology approaches have revealed abundant microbial life in the Río Tinto [1-3]. High-throughput molecular approaches such as Serial Analysis of Ribosomal Sequence Tags of the V6 hypervariable region of the small subunit (SSU) ribosomal RNA (rRNA) gene (SARST-V6) [4] and high-throughput capillary sequencing of the V4-V8 hypervariable region of the SSU rRNA gene [5] have shown that microbial diversity in the Río Tinto is not limited to only dominant phylotypes, but also consists of moderate and low abundance microbial populations. Next generation sequencing platforms such as 454 pyrosequencing provide a window into the membership of these low abundance populations and invite us to contemplate the role these rare taxa play in the ecosystem functioning of extreme environments like the Río Tinto.

Methods: Short sequence tags from hypervariable regions in rRNA genes (pyrotags) provide us with measures of diversity (species or OTU richness) and relative abundance (evenness) of OTUs in microbial communities. When combined with the massively parallel capacity of next generation DNA sequencing technology they allow for the simultaneous sequencing of hundreds of thousands of templates overshadowing conventional sequencing methods by orders of magnitude. By applying next generation amplicon sequencing of V6 hypervariable SSU rRNA gene regions for bacteria and archaea and V9 hypervariable regions of the SSU rRNA gene for eukaryotes, we have for the first time sequenced at a depth an order of magnitude greater than ever before. This deep sequencing has offered a closer look at the Río Tinto's 'Rare Biosphere' [6-7] across all three domains of life.

Summary: We recovered more than 420,000 pyrotags from river water samples taken during the dry and rainy seasons in three geochemically distinct sampling stations in the Río Tinto. As predicted for harsh or impacted environments, bacterial, archaeal, and eukaryotic populations were all dominated by a few Operational Taxonomic Units (OTUs) with many low

abundance OTUs. Some of the pyrotags we recovered were from taxa that have been detected but never sequenced directly from the Río Tinto before. Examples included rotifers from the eukaryotic domain, methanogens in the archaeal domain and sulfate reducers among the bacterial domain. Some pyrotags were rare in the dry season but abundant in the rainy season. Examples included *Galionella*-like tags that were found in higher abundance at the Berrocal sites during the rainy season but were absent in the dry season. Likewise, *Acidiphilium*-related tags were found at a higher relative abundance during the dry season than the rainy season. Other rare tags appeared to be consistently rare. At least a fraction of these were related to bacterial symbionts living in association with heterotrophic protists in the river. Another source of rare taxa in the Río Tinto appeared to be coming from fecal contamination in one of our sites during the dry season. The extent to which allochthonous sources contribute to the Río Tinto's 'Rare Biosphere' requires further investigation.

References:

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