31. High Throughput Isolation and Environmental Isolate Characterization in ENIGMA

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Project Goal: The goal of ENIGMA's Environmental Isolation and Characterization Campaign is to answer outstanding questions in microbial ecology that are best addressed with a combination of microbial isolation and downstream functional characterization of those isolates. The specific goal of the Isolation project within this campaign is to develop high throughput isolation platform for cultivating and subsequently investigating microbes performing metabolic processes of interest at the ENIGMA field site, Oakridge Field Research Center.

The constraints of obtaining isolates from key environments have undermined testing and validating hypotheses that emerge from powerful gene-based technologies. Moreover, how individual microbes function, predictions about how they might function, and the identification of genes and pathways critical for fitness under a given condition can be best answered by lab- based controlled experiments with the representative microbial isolates from the field. Towards this direction, we are developing and testing different approaches to obtain isolates from Oakridge FRC, which contains high plumes of uranium, technetium, nitrate, volatile organic compounds and has a pH gradient from 3-10.

Our first approach uses microtiter plates for high throughput, and aims to recover a large number of diverse culturable aerobic and anaerobic strains across multiplexed conditions of soluble electron acceptors, pH, salinity and metals among others. As a result, several hundred strains have been isolated from selected groundwater wells across geochemical gradients, including representatives from those genera identified to be most abundant by the ENIGMA 100 Well 16S- survey Campaign. These were cultivated using a combined approach of both minimal and rich complex media. Select isolates are being characterized in details as part of the ENIGMA Printable Worlds and Environmental Characterization campaigns.

Our second approach is directed towards specific enrichments and isolation of strains that metabolize unique substrates and/or catalyze novel metabolisms. As part of this, we have identified strains capable of oxidizing dissolved organic matter (humics), and metals such as Fe coupled to nitrate reduction. Some of these isolates are denitrifiers, while others reduce nitrate to ammonia. We have also focused on isolating specific phylogenetic groups of interest. Several *Pseudomonas spp* have been isolated from wells with different nitrate content, and although closely related by 16S rRNA sequence, they vary greatly in their ability to reduce nitrate ranging from 10mM to 300mM. We are investigating the role of metals (such as Mo, Cu, Fe) as limiting factors on their ability to reduce nitrate in monocultures and co-cultures, and performing pan- genome analysis as part of ENIGMA Metals and Environmental Characterization Campaigns.

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