ARPHA Conference Abstracts 6: e108172
doi: 10.3897/aca.6.e108172

Conference Abstract

Genomic Approaches and Analyses of Slow-Growing Obligate Iron-Metabolizing Microbes

David Hsu[‡,§], Abhiney Jain[‡,§], Halle R Kruchoski[‡,§], Daniel R Bond[‡,§], Jeffrey A. Gralnick[‡,§]

‡ BioTechnology Institute, University of Minnesota, St Paul, United States of America
§ Department of Plant and Microbial Biology, University of Minnesota, St Paul, United States of America

Corresponding author: Jeffrey A. Gralnick (gralnick@umn.edu)

Received: 17 Jun 2023 | Published: 17 Oct 2023

Citation: Hsu D, Jain A, Kruchoski HR, Bond DR, Gralnick JA (2023) Genomic Approaches and Analyses of Slow-Growing Obligate Iron-Metabolizing Microbes. ARPHA Conference Abstracts 6: e108172. https://doi.org/10.3897/aca.6.e108172

Abstract

The biogeochemical cycling of iron is a vastly important process that has been a major factor defining life on Earth both before and after the Great Oxidation Event. While both abiotic and biotic factors contributing to the iron cycle have been studied for many years, the bulk of studies on iron metabolizing organisms has focused on a select few, easily manipulated model organisms. Recent discoveries have identified several unique and difficult to work with organisms from iron rich environments that survive solely on iron as either an electron acceptor or donor. The Fe(III)-reducing, Gram-positive Firmicute *Metallumcola ferriviriculae* MK1 was recently isolated from the Soudan Underground Mine in northern Minnesota from brine waters that intersect 2.7 Ga banded iron formations within the Canadian Shield. *M. ferriviriculae* MK1, which grows anaerobically using Fe(III)-citrate as its sole electron acceptor, is also mesophilic, spore-forming, culturable, and rich in multiheme cytochromes. Multiheme cytochromes are a well-established mechanism for Fe(III) reduction among the model Gram-negative microbes, such as *Shewanella oneidensis* and *Geobacter sulfurreducens*, but is poorly studied in Gram-positives. While the slow growth times of *M. ferriviriculae* MK1 make it difficult to study in the laboratory, the genome encodes homologs to multiheme cytochromes utilized by *G. sulfurreducens* for Fe(III) reduction. Specifically, two gene clusters (MK1_2258-2259 and MK1_2264-2265) each encode proteins homologous to the *b*-type cytochrome domain (60% and 61% sequence similarity, respectively) and *c*-type cytochrome domain (44.9% and 47.14%,

respectively) of *cbcL*, which is used for reduction of mid-range redox potential acceptors in *G*. *sulfurreducens* and MK1_1670 is homologous to *imcH* (54.3% sequence similarity), which is used for higher redox potential acceptors. Additionally, the MK1 genome contains genes associated with sporulation, including genes encoding the master sporulation regulator *spo0A*, the peptidoglycan remodeling enzymes *spoIID*, *spoIIP*, and *spoIIM*, the spore morphogenesis protein *spoIVA*, and the small, acid-soluble spore proteins *sspA*, *sspB*, *sspC*, *sspD*, and *sspF*. Extraction and isolation of MK1 spores will facilitate evaluation of sporulation and germination conditions to shed light on a crucial preservation mechanism from an organism found in an environment with limited nutrients. Further evaluation into this novel organism can also give us insights into the microbial impacts on the iron cycle in the deep terrestrial biosphere. Another microbe of interest that was isolated from an iron-rich environment is the obligate Fe(II)-oxidizing *Mariprofundus ferrooxydans* PV-1, which was enriched from iron-rich mats associated with hydrothermal vents at the Kama'ehuakanaloa Seamount (previously Lo'ihi) in Hawaii. *M. ferrooxydans* PV-1 is a stalk-forming Fe(II)-oxidizing microbe and the first *Zetaproteobacterium* characterized. While *M. ferrooxydans* PV-1 can reliably be grown (doubling time of ~12 hours) in liquid medium traditional genetic methods are challenging because it does not make colonies on agar plates. To identify essential and non-essential genes, we developed a conjugation method using *E. coli* and successfully generated a transposon library in *M. ferrooxydans*. Libraries were grown under kanamycin selection with Fe(II)-chloride as the sole electron donor and samples isolated for analysis at two different timepoints. Deep sequencing of the mutant population at these timepoints was performed and the reads were mapped back to the *M. ferrooxydans* PV-1 genome to identify transposon insertion sites. Initial evaluation of the reads has identified 31 transposon insertion sites found within both the first- and second-generation populations, with 21 hits representing stable insertions in non-essential genes, including a predicted malate dehydrogenase (SPV1_772) and a predicted phospholipase (SPV1_8286), that may be useful future targets for gene insertion sites. Further evaluation comparing the read depth between the generations should identify genes that were selected for or against during the subculturing. These studies and approaches are providing insights into the roles these challenging, non-model organisms are performing in iron-rich environments, which provides a better context for the biogeochemical cycling of iron in relevant biospheres.

Keywords

biogeochemical cycling of iron, non-model micoroorganisms, transposon mutagenesis

Presenting author

David Hsu

Presented at

Abstract submission for poster presentation at ISEB-ISSM 2023 under the Emerging Tools & Areas of Scientific Inquiry Theme, with a focus on Microbial diversity and ecology and Microbial geochemistry and geomicrobiology Theme area

Conflicts of interest

The authors have declared that no competing interests exist.