Goldschmidt 2021 Abstract
https://doi.org/10.7185/gold2021.7580

Identification of active arsenic-methylating organisms in anaerobic soil enrichment cultures using meta-omics

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Arsenic (As) is a naturally occurring toxic element that is ubiquitous in deltaic environment and that is of particular concern in South-East Asia. Microbial transformations of As include oxidation (trivalent As(III) to pentavalent As(V)), reduction (As(V) to As(III)), and methylation (inorganic As to methylated As). Here, we investigate enrichment cultures from a rice paddy soil in Vietnam, an environment in which As methylation is documented. The advent of As methylation in paddy soils has important implications for rice cultivation as dimethylarsinic acid is taken up by the rice plant, causing straight-head disease, which negatively impacts the crop's yield.

The soil enrichment exhibits methylation under anoxic conditions, providing the opportunity to address the gap in knowledge about which microorganisms are responsible for this process. To date, only sulfate-reducing bacteria (SRB) are thought to carry out anaerobic As methylation. Thus, a combination of metagenomic, metatranscriptomic and metaproteomic approaches were brought to bear on whether there is greater functional diversity within As methylators. The metagenomic approach included assembly (using MEGAHIT), binning (using a combination of binning approaches, CONCOCT, MetaBAT2, MaxBIN2 and the bin refinement algorithm, MetaWRAP), and identification of metagenome-assembled genomes (MAGs). MAGs harboring *arsM*, the gene encoding the arsenite S-adenosylmethionine methyltransferase enzyme responsible for As methylation, were considered as having the potential to be active As methylators. Each enrichment included 6 phylogenetically distinct MAGs harboring at least one *arsM* gene. A few of these MAGs (2 and 1, respectively) showed evidence for *arsM* or ArsM expression. Interestingly, while the *arsM*-harboring MAGs included SRB (Deltaproteobacteria), only the MAGs belonging to the family Clostridiales evidenced expression of *arsM*or ArsM. Interrogation of the metabolic activity (through expression) revealed that these MAGs are all active fermentative organisms in the soil enrichment.

We conclude that, in this system, Clostridiales species, operating as fermenters, are responsible for As methylation. Based on the metagenomic information obtained from the MAGs, targeted isolation was attempted. This finding expands the current understanding of the microorganisms capable of active As methylation and presents an example of the systematic use of meta-omic tools to identify a functional group of microorganisms.