Goldschmidt 2025 Abstract
https://doi.org/10.7185/gold2025.28238

Biosensor-mediated isolation of anaerobic arsenic-methylating bacteria

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Arsenic (As) is a toxic metalloid that occurs naturally in the environment, where inorganic arsenic is mainly present as arsenate (As(V)) and arsenite (As(III)). Some microbes can methylate As(III) and produce mono-, di-, and trimethylated As compounds. Both inorganic and methylated As species can be taken up by rice plants and accumulate in rice grains thereby posing a threat to the safe consumption of rice, particularly dimethylated As. In addition, dimethylated As can also cause straighthead disease in rice plants, decreasing productivity.

Although both aerobic and anaerobic microbes may carry the gene responsible for As methylation (*arsM*), flooding of rice paddy fields leads to increased methylation and accumulation of methylarsenicals in rice, indicating a crucial role for anaerobic methylators in soil. However, very few anaerobic As-methylating organisms have been isolated to date, impeding studies to understand the biological role of anoxic As methylation and the environmental factors controlling it.

Traditional methods for isolating environmental anaerobes are demanding and low-throughput. Additionally, screening for As methylation relies on testing numerous individual isolates using analytical tools such as HPLC-ICP-MS, that are slow and cumbersome. We developed a high-throughput isolation approach that traps and cultivates single microbes in hydrogel capsules, sorts them using fluorescence-activated cell sorting (FACS), and distributes compartmentalized isolates into individual microwells for further growth and testing.

This approach generates many isolates that can be screened for As methylation. To do so, we created two *E. coli* based whole-cell biosensors that respond to methylated arsenic by expressing a reporter (GFP). We then combined the high-throughput isolation technique with biosensor-mediated screening to identify potential arsenic methylators. This targeted approach allowed us to successfully isolate two anaerobic As(III)-methylating bacteria from a flooded paddy soil. The novelty of this approach opens the door to many applications in the field of arsenic geomicrobiology, where high-throughput isolation techniques are rare and functional screening analytically challenging.