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Cultivation and isolation of anaerobic soil microorganisms using semi-permeable capsules

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Arsenic (As) is a toxic contaminant that is prevalent in the environment. In soils, it is mostly found in its inorganic forms, either as As(V) in oxic environments or as As(III) in anoxic environments. In rice paddy fields, the practice of flooding mobilizes arsenic in the soil solution as As(III) which is then enzymatically transformed by soil microorganisms. In particular, some microbial species methylate As(III) to form organic products that they release extracellularly and that the plant takes up inadvertently. The methylated products harm the crops (straighthead disease) and pose a threat to the safe consumption of rice, since they also translocate from the root to the grain.

However, the microbial species responsible for arsenic methylation in flooded soils are unknown, and the mechanisms (abiotic drivers, ecological function) underlying this process remain to be determined. At present, only a few anaerobic isolates were found to methylate arsenic, although the gene encoding the responsible enzyme is widespread in microbial genomes. Yet the reported isolates poorly methylate arsenic as compared to soil microbial communities. Further understanding has been limited by the difficulty of isolating anaerobes with this phenotype.

To facilitate this task, we explore a novel cultivation and isolation approach leveraging picoliter-sized hydrogel capsules. Microbial cells extracted from soil are individually trapped and cultivated in these compartments (semi-permeable capsules) which can further be sorted by fluorescence-activated cell sorting (FACS). In order to specifically screen for arsenic-methylating microorganisms, we recently developed a whole-cell biosensor that fluoresces upon sensing the methylated products. We aim to enclose this biosensor in capsules along with individual soil microbes and grow the cells in a medium amended with As(III), hoping to see an increase in fluorescence in capsules containing methylators. Ultimately, we seek to use this fluorescence signal as a sorting criterion to specifically collect capsules containing methylating species.

At this stage, semi-permeable capsules were shown to support the growth of microbial species with various anaerobic metabolisms (fermentation, Fe(III) reduction, sulfate reduction, methanogenesis, acetogenesis), and were shown to enrich for obligate anaerobes. Finally, this approach was used to isolate obligate anaerobes from a paddy soil.