Arsenic methylation across microbial phyla

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Arsenic (As) undergoes extensive microbial cycling in the environment, and, because of the toxicity of this metalloid, many bacteria and archaea harbour genes that confer resistance to it [1]. As methylation is one of the many microbially-mediated process, it consists in the addition of one or several methyl groups to inorganic arsenic generating volatile and non-volatile arsenicals. The reaction is catalysed by the enzyme arsenite methyltransferase (ArsM). It has been proposed as a detoxification mechanism but also as an activation pathway or a precursor reaction for the synthesis of more complex arseno-organic molecules. As methylation has been subject of study due to the presence of methylarsenicals in rice, a major source of arsenic in the human diet. The methylarsenicals are derived from microorganisms in paddy soils given that rice plants lack the ability to catalyse the reaction [2].

In paddy soils, *arsM*-harbouring microorganisms are phylogetically diverse and abundant [3] but it is still unclear whether all synthesize functional proteins and active methylate As once present in the soil. Previous studies have identified a few active As methylators, most of them aerobic microbial species, including the Bacteroidete *Arsenicibacter rosenii* SM-1 [4]. In contrast, only two anaerobic species were identified: the Firmicute *Clostridium* sp. BXM [5] and the archaeon *Methanorsarcina acetivorans* C2A [6]. Thus, the goal of this study is twofold: first, to test the As-methylation activity of *arsM*-harbouring microbial species belonging to various phyla and representing diverse metabolisms present in paddy soils and, second, to systematically probe the functionality and the *in vivo* activity of the enzymes encoded in their *arsM* genes.

As(III) methylation and volatilization was tested across five anaerobic microbial strains: two archaea (*Methanosarcina mazei* Gö1, *Methanosarcina acetivorans* C2A); two Firmicutes (*Anaeromusa acidaminophila* DSM 3853, *Clostridium pasteurianum* DSM 525); a Deltaproteobacterium (*Geobacter metallireducens* GS-15); and two aerobic strains: a Streptomycete (*Streptomyces vietnamensis* DSM 41927); and a Bacteroidete (*Arsenicibacter rosenii* SM-1). All *arsM* genes were cloned into the arsenic-sensitive *Escherichia coli* AW3110(DE3) and As methylation quantified.

The results show that most of the strains were not able to methylate As despite harbouring *arsM* genes that encode functional ArsM proteins. Our first conclusion is that the presence of *arsM* does not equate As methylation even in the presence of the metalloid. Further, we hypothesize that more efficient As detoxification pathways might be prevalent, precluding methylation, therefore more work is warranted to deconvolute *arsM* regulation.

REFERENCES

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