

Role of Lanthanides in the Complex Interplay of Alternative Enzyme Systems Involved in Methanotrophy

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Project Goals: This project addresses the structure and function of microbial communities active in methane consumption, using lake sediment as a model. Through manipulations of synthetic communities and systems biology approaches, we are striving to understand the molecular mechanisms that form a basis for specific interspecies interactions in microbial oxidation of methane. In this phase of the project our goals were: 1) Evaluate behavior of model synthetic communities comprised of major functional guilds defined in prior research, and assess performance of these communities; 2) Evaluate the functions and the regulation of the alternative methanol dehydrogenases, previously identified as the cross-talk points among keystone species in methane-utilizing communities; 3) Obtain enzyme-level insights into lanthanide-dependent methanol oxidation systems in these keystone species.

We followed species dynamics and gene expression patterns in synthetic bacterial communities consisting of species that compete for methane, and species unable to consume methane, which could only succeed through cooperative interactions (a total of 50 species). We demonstrated that these communities mostly select for two functional guilds, methanotrophs of the family *Methylococcaceae* and non-methanotrophic methylotrophs of the family *Methylophilaceae*, these taxonomic guilds outcompeting all other species included in the synthetic mix. The metatranscriptomics analysis uncovered that in both *Methylococcaceae* and *Methylophilaceae*, some of the most highly transcribed genes were the ones encoding methanol dehydrogenases (MDH). Remarkably, expression of alternative MDH genes (*mxoA* versus *xoxF*), previously shown to be subjects to the 'rare Earth element switch', was found to depend on environmental factors such as nitrogen source and methane and O₂ partial pressures, while also being species-specific. Along with multiple *xoxF* genes, multiple genes encoding divergent cytochromes were highly expressed in both *Methylophilaceae* and *Methylococcaceae*, suggesting their function in methanol metabolism, likely as electron acceptors from XoxF enzymes. This experiment tested a synthetic community model that is much simplified compared to natural communities consuming methane, but more complex than the previously utilized two-species model. The performance of this model identified prominent species for future synthetic ecology experiments and highlighted both advantages of this approach and the challenges that it presents.

We analyzed the physiological effects of a mutation in *xoxG*, a gene encoding a novel cytochrome XoxG(4) identified through metatranscriptomics, and compared these to the effects of mutation in XoxF, a lanthanide-dependent methanol dehydrogenase, at the enzyme activity level and also at the community function level, using *Methylomonas* sp. LW13 as a model

organism. Through comparative phenotypic characterization, we establish XoxG as the second protein directly involved in lanthanide-dependent metabolism, likely as a dedicated electron acceptor from XoxF. However, mutation in XoxG caused a phenotype that was dramatically different from the phenotype of the mutant in XoxF, suggesting a secondary function for this cytochrome, in metabolism of methane. We further purified XoxG(4) and demonstrated that this protein is a true cytochrome *c*, based on the typical absorption spectra, and we demonstrated that XoxG can be directly reduced by a purified XoxF, supporting one of its proposed physiological functions. Overall, our data continued to suggest the complex nature of the interplay between the calcium-dependent and the lanthanide-dependent alcohol oxidation systems, while they also suggested that addressing the roles of these alternative systems is important at the enzyme as well as community function level, in addition to gene transcription level.

To evaluate the properties of XoxF enzymes in major community partners, we expressed, purified and biochemically characterized three enzymes, two belonging to the XoxF4 phylogenetic clade, from *Methylothermobacter mobilis* JLW8, and one belonging to the XoxF5 clade, from *Methylothermobacter* sp. LW13, and demonstrated that, while all dependent on lanthanides as cofactors, they possess different properties in terms of kinetics and substrate specificities.

Overall, our recent data provide new insights and expand our understanding of the role of metals in methanol oxidation, an essential step in methanotrophy, and further point to the complex nature of its biochemistry. Our data strongly suggest that, likely, a balance between the two types of MDH enzymes, calcium-dependent versus lanthanide-dependent, rather than the on/off switch, provide metabolic robustness to natural populations of methanotrophs and non-methanotrophic methylotrophs.

Publications

1. Zheng Y, Huang J, Zhao F, Chistoserdova L (2018) Physiological effect of XoxG(4) on lanthanide-dependent methanotrophy. *mBio* Submitted.
2. Chistoserdova L (2018) Applications of methylotrophs: can single carbon be harnessed for biotechnology? *Curr Opin Biotechnol* Submitted.
3. Chistoserdova L, Kalyuzhnaya MG (2018) Current trends in methylotrophy. *Trends Microbiol* Submitted.
4. Chistoserdova L (2018) Methanotrophy: an evolving field. In: Kalyuzhnaya M, Xing X-H Eds, *Methane biocatalysis: Paving the way to sustainability*. Springer, Springer-Verlag, Berlin Heidelberg, Germany In Press.
5. Yu Z, Beck DAC, Chistoserdova L (2017) Natural selection in synthetic communities highlights the roles of *Methylococcaceae* and *Methylophilaceae* and suggests differential roles for alternative methanol dehydrogenases in methane consumption. *Front Microbiol* 8:2392.
6. Yu Z, Chistoserdova L (2017) Communal metabolism of methane and the rare Earth element switch. *J Bacteriol* pii:JB.00328-17.
7. Chistoserdova L (2017) Omics approaches to studying methylotrophs and methylotroph communities. In: Marco D Ed, *Metagenomics: current advances and emerging concepts*. *Curr Issues Mol Biol* 24:119-142.

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