Systems Biology Meets Enzymology: Recent Insights into Communal Metabolism of Methane and the Role of Lanthanides

Zheng Yu[1], Yue Zheng[1,2,3], Jing Huang[1] and Ludmila Chistoserdova[1]*

[1]Department of Chemical Engineering, University of Washington, Seattle, WA, USA.
[2]CAS Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China.
[3]University of Chinese Academy of Sciences, Beijing, China.

*Correspondence: milachis@uw.edu

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Abstract

In this review article, we cover the recent developments in understanding the principles and the mechanisms by which microbial communities participating in methane consumption in natural environmental niches are assembled, and the physiological and biochemical mechanisms and regulators that allow efficient carbon transfer within the communities. We first give a brief overview of methanotrophy. We then describe the recent evidence on non-random assembly of bacterial communities that utilize carbon from methane, based on stable isotope probing experiments as well as on results from natural community manipulations followed by metagenomic analysis. We follow up by highlighting results from synthetic methanotrophic community manipulations identifying the importance of a lanthanide switch that regulates alternative methanol dehydrogenase enzymes in these communities. We further expand on the recently uncovered significance of lanthanides in methylotrophy and review data on the biochemical properties of representatives of two different clades of lanthanide-dependent enzymes. We also provide an overview of the occurrence and the distribution of the lanthanide-dependent alcohol dehydrogenases in the bacterial domain, these data strongly suggesting significance of these metals beyond methylotrophy.

Introduction

A historical outlook on methane oxidation

Methane is an abundant hydrocarbon on Earth. While braking the strong bond between carbon and hydrogen in this molecule is thermodynamically difficult, this reaction is readily catalysed by specialized bacteria, the methanotrophs, at ambient temperatures (Lawton and Rosenzweig, 2016). The ability of microbes to utilize methane as a substrate has been discovered over a century ago, giving rise to the field of science known as the Methylotrophy field (reviewed in Chistoserdova, 2018). It has been especially active since the early 1970s, after multiple methanotrophs have been cultivated in pure cultures (Whittenbury *et al.*, 1970). Those were the so-called aerobic methanotrophs, belonging to the Proteobacteria Phylum of Bacteria, of which originally two types were recognized, gammaproteobacterial (Type I) and alphaproteobacterial

(Type II) methanotrophs (Trotsenko and Murrell, 2008). Key enzymes involved in the primary oxidation of methane in these bacteria have been characterized as two alternative methane monooxygenases, particulate (pMMO) and soluble (sMMO), respectively, the former reliant on copper-mediated catalysis and the latter dependent of iron-mediated catalysis (Ross and Rosenzweig, 2017). The enzyme involved in oxidation of methanol, which is a product of methane oxidation, methanol dehydrogenase (MDH) of the MxaFI-type, has also been thoroughly studied over years, uncovering its calcium dependence and regulation by the presence of methanol (Anthony and Zatman, 1967a,b; Anthony, 2004). However, very recently, an alternative MDH has been identified (see below), suggesting metabolic flexibility for this oxidative step as well. Downstream of methanol, formaldehyde and, sequentially, formate can also be oxidized via alternative enzymes and pathways, further expanding the flexible metabolic scheme of methylotrophy (Chistoserdova, 2011a). Carbon assimilation by proteobacterial methanotrophs has been originally attributed to two specific biochemical pathways, the ribulose monophosphate cycle (in gammaproteobacterial methanotrophs) and the serine cycle (in alphaproteobacterial methanotrophs; Anthony 1982). From recent genomic studies, the gammaproteobacterial methanotrophs also encode at least parts of the serine cycle, but its role in carbon assimilation by these bacteria is still not well understood (But *et al.*, 2017). Some methanotrophs also encode and express genes for the Calvin-Benson-Bassham cycle (Baxter *et al.*, 2002; Kao *et al.*, 2004), this cycle potentially serving as an additional pathway for carbon assimilation. Carbon in the form of CO_2 is also assimilated through the ethylmalonyl-CoA pathway (Anthony, 2011) by the alphaproteobacterial methanotrophs (Chistoserdova *et al.*, 2009).

Two novel taxa within Bacteria capable of methanotrophy have been discovered relatively recently. These are the Verrucomicrobia (Sharp *et al.*, 2014) and the yet unnamed phylum NC10 (Ettwig *et al.*, 2010; Versantvoort *et al.*, 2018). In both of these novel methanotroph taxa, the traditional pathways for methane oxidation are recognized, while carbon assimilation takes place at the level of CO_2 (Khadem *et al.*, 2011; Rasigraf *et al.*, 2014). While the verrucomicrobial methanotrophs are aerobes, the NC10 methanotrophs are considered to be anaerobes (Ettwig *et al.*, 2010; Welte *et al.*, 2016). The latter, so far, have not been isolated in pure cultures, as they potentially require functional partners to carry out methane oxidation anaerobically.

Anaerobic oxidation of methane can also be carried out by the archaeal ANME-types (Hinrichs *et al.*, 1999; Boetius *et al.*, 2000; Orphan *et al.*, 2001). In these species, methanotrophy is carried out through a reverse methanogenesis pathway (McGlynn, 2017), and carbon from methane is assimilated via the Wood–Ljungdahl pathway (Meyerdierks *et al*, 2010; Haroon *et al.*, 2013). Thus, the metabolic scheme employed by methanotrophic archaea is radically different from the one employed by bacteria. These archaeal species are typically engaged in syntrophic co-metabolism involving bacterial species, and, like anaerobic NC10 bacteria, they have not yet been isolated as pure cultures (Knittel and Boetius, 2009; Trembath-Reichert *et al.*, 2016).

Lanthanides in methanotrophy

Over two decades ago, a homologue of the mxaF gene has been identified in several methylotroph genomes (Chistoserdova and Lidstrom, 1997), but it's true function, in encoding an alternative MDH, XoxF, has been revealed only recently, by uncovering its reliance on the so-called rare earth elements lanthanides (Ln; Chistoserdova, 2016; Chistoserdova and Kalyuzhnaya, 2018). The discovery of the role of the mysterious homologue of MxaF (Chistoserdova, 2011a), as an alternative MDH requiring Ln for both expression and activity has stirred-up the field of methylotrophy (Chistoserdova, 2016), raising numbers of questions fundamental not only to microbial physiology and biochemistry, but also to the broader fields of basic chemistry and geobiology: why Ln, and how? Since their discovery, Ln were deemed biologically inert due to their very low solubility (Lim and Franklin, 2004). However, the methylotrophs apparently possess mechanisms for solubilizing Ln and employing them in their central metabolism not only in environments in which Ln concentrations are relatively high (Pol *et al.*, 2014), but also in environments in which Ln concentrations are very low (Chistoserdova, 2016; Shiller *et al.*, 2017).

Laboratory experiments with Ln have mostly employed unnaturally high concentrations of Ln, in the form easily dissolved salts (Fitryanto *et al.*,

2011; Farhan Ul Haque *et al.*, 2015; Gu *et al.*, 2016; Chu and Lidstrom, 2016; Chu *et al.*, 2016). Thus, one needs to consider these results while exercising caution that, in real life, similar conditions probably do not exist. Whenever tested, the addition of Ln ions to growth media has caused a dramatic, arresting effect on the expression of the genes for the classic, calcium-dependent MDH (MxaFI), while positively effecting transcription of *xoxF* (Gu *et al.*, 2016; Chu and Lidstrom, 2016; Chu *et al.*, 2016; Zheng *et al.*, 2018), the mechanism that became known as the Ln switch (Chu and Lidstrom, 2016; Chu *et al.*, 2016). In the presence of Ln, the MxaFI MDH could be removed without a significant effect on growth, while XoxF could be removed when Ln were not present (Farhan Ul Haque *et al.*, 2015; Chu and Lidstrom, 2016; Chu *et al.*, 2016; Zheng *et al.*, 2018). However, the Ln switch was proven to be very fragile. When XoxF mutants were grown in the presence of Ln, suppressor mutants appeared at high frequency, suggesting deregulation and suggesting that some type of selective pressure for such deregulation exists, at least under the conditions of high Ln concentrations (Chu *et al.*, 2016; Zheng *et al.*, 2018). On another hand, there are many examples of methylotrophs that only contain XoxF types of MDH, thus demonstrating that the MxaFI type is an auxiliary enzyme while the XoxF type is essential (Hou *et al.*, 2008; Vekeman *et al.*, 2016). Moreover, many genomes contain multiple, sometimes divergent copies of xoxF genes, suggesting further complexity in the alcohol oxidation potential (Mustakhimov *et al.*, 2013; Huang *et al.*, 2018).

When both the Ca and the Ln forms of MDH are encoded, it is possible that the Ln switch may be a subject to different settings, as opposed to being simply 'on' or 'off'. For example, when assessed in a species encoding both types, both were enzymatically active over a range of Ln concentrations, despite differences in the transcriptional responses (Zheng *et al.*, 2018). Simultaneous expression of both proteins has also been observed in natural settings such as the plant phyllosphere (Delmotte *et al.*, 2009). It is thus likely that each enzyme may play a specialized role in the methylotrophy metabolism, by exploiting not only different metal dependencies, but, potentially, different redox properties, and the presence of both types of enzymes likely confers enhanced environmental fitness.

Interspecies interactions in metabolism of methane

Community function, (re)discovered?

While the process of anaerobic methane oxidation appears to be a communal function (Trembath-Reichert *et al.*, 2016), likely due to the energetic or the metabolic constraints of such a metabolism (Thauer and Shima, 2008), the aerobic methanotrophs have been cultivated as pure cultures for decades, and this is how their metabolism and their physiology have been studied over years (Trotsenko and Murrell, 2008; Chistoserdova and Lidstrom, 2013). However, a possibility of community function in aerobic methane oxidation has also been suggested, based on the observations of the tight associations between the methanotrophs and the generalist heterotrophs during the attempts of obtaining methanotrophs in pure cultures (Dalton, 2005; Dedysh and Dunfield, 2017). The potential of methanotrophs in engaging in communal behaviour has been rediscovered more recently, and, unlike the early notions of methanotrophs supporting random communities of generalist heterotrophs, the recent observations suggest that the communal relationships may not be random after all. For example, stable isotope probing experiments with a lake sediment community responding to methane stimulus have produced interesting and not entirely expected results: the label from methane appeared to be split between a major primary methane consumer, a *Methylobacter* species, and a non-methanotrophic methylotroph, a *Methylotenera* species (Kalyuzhnaya *et al.*, 2008; Fig. 10.1). This result was puzzling because methylotroph communities have been known to be very diverse in the study site, and alternative methanotroph species such as *Methylomonas* and *Methylosinus,* have been readily cultivated from the site, while the *Methylobacter* species remained overlooked (Chistoserdova, 2011b). As to the partner species, many species have been identified in the site that showed robust methylotrophic or growth in laboratory (Beck *et al.*, 2015), but only *Mathylotenera* species were able to partner with *Methylobacter* species in consuming carbon from methane (Kalyuzhnaya *et al.*, 2008). Reconstruction of the metabolisms of the major species involved in methane consumption in these experiments has uncovered a potential for a link between methane oxidation and

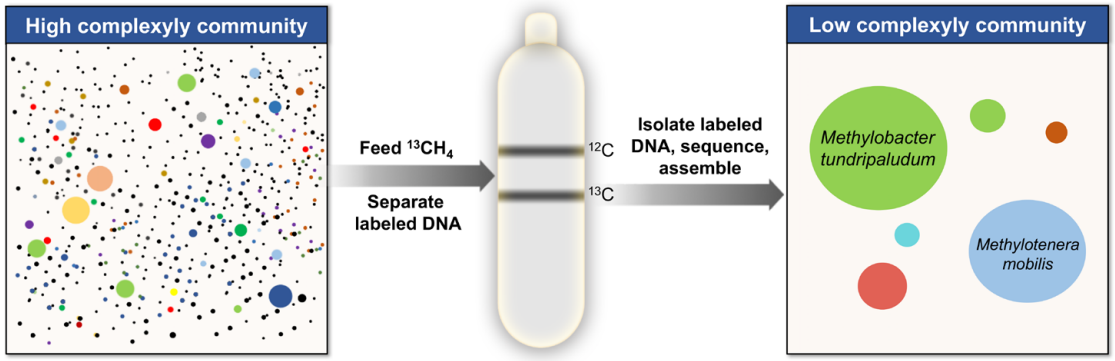

Figure 10.1 Summary of the stable isotope probing experiment followed by metagenomic sequencing. *Methylobacter* and *Methylotenera* were identified as the most competitive consumers of carbon from methane, based on sequence read abundance in the metagenome. See Kalyuzhnaya *et al*. (2008).

denitrification, and thus a potential for adaptation to the conditions of low molecular oxygen availability (Kalyuzhnaya *et al.*, 2008). This hypothesis was tested in additional experiments with labelled methane, under conditions of variable O_2 and nitrate concentrations (Beck *et al.*, 2013). In these experiments, once again, the *Methylobacter* types and the *Methylotenera* types were the species most actively consuming methane in both oxic and hypoxic conditions, and nitrate had a stimulatory effect on both species, further supporting the notion that the two species may be engaged in communal behaviour (Beck *et al.*, 2013). Co-occurrence of the *Methylococcaceae* types along with the *Methylophilaceae* types has been also noted in natural freshwater and soil environments (Martineau *et al.*, 2010; He *et al.*, 2012; Karwautz *et al.*, 2018; Biderre-Petit *et al.*, 2018), while in marine environments, the *Methylococcaceae* type primary methane oxidizers appear to cooperate with *Methylophaga* species as the major carbon sharing partners (Kessler *et al.*, 2011; Paul *et al.*, 2017).

The hypothesis of the non-random partner selection has been tested more directly by manipulating natural communities, as parts of microcosms stimulated with methane (Oshkin *et al.*, 2015). When natural sediment communities were fed methane, in the presence of nitrate, in conditions of either 'high' or 'low' dioxygen partial pressures, very rapid reduction in community complexity occurred, as judged by 16S rRNA gene marker surveys, selecting again for the *Methylococcaceae* and the *Methylophilaceae* types, along with select representatives of non-methylotrophic heterotrophs such as Burkholderiales and Bacteroidetes (Oshkin *et al.*, 2015). Further microcosm experiments that imposed a stricter control of dioxygen concentrations in the headspace allowed for distinguishing genus-specific selection for both major partners: the 'high' oxygen conditions selecting for the *Methylosarcina/Methylophilus* partnerships, and the 'low' oxygen conditions selecting for the *Methylobacter/Methylotenera* partnerships (Hernandez *et al.*, 2015). Thus, it is possible that discrete niche conditions, such as the different ratios of O_2/methane along their counter-gradients typically observed in methane-producing environments such as lake sediments, may be fine-tuning the specific methanotroph–non-methanotroph partnerships, selecting for organisms with optimized metabolic schemes for most successful partnerships.

Insights from synthetic community manipulations

Synthetic communities of organisms relevant to communal methane oxidation have been employed as a simplified and a better controlled model to test the findings from the natural community experiments. Some of the questions addressed via the synthetic community model were whether the dynamics of the natural communities were determined by the initial titres of species present in native conditions, or whether the species most successful in methane consumption were biochemically equipped to be more competitive, under specific conditions created as parts of a specific experiment.

Complex synthetic communities were mixed of 50 species representing bona fide methanotrophs, including the *Methylobacter* and *Methylosarcina* species closely related to the ones identified via stable isotope probing, as well as via microcosm manipulations (Kalyuzhnaya *et al.*, 2008; Oshkin *et al.*, 2015; Hernandez *et al.*, 2015), the *Methylotenera* and *Methylophilus* species identified in prior experiments, and of species that have been isolated from the native environment and grown methylotrophically in laboratory, but not detected in the microcosm experiments (Beck *et al.*, 2015). In addition, a handful of non-methylotrophic heterotrophs were employed to test whether they would persist in communities fed exclusively methane as a carbon source, at variable concentrations of methane and dioxygen, in the presence of alternative (nitrate versus ammonium) nitrogen sources. These synthetic communities were treated similarly to the natural communities (Oshkin *et al.*, 2015; Hernandez *et al.*, 2015), and their dynamics were followed through 16S rRNA gene profiling and through the analysis of select metatranscriptomes, matched to the respective 50 genomes (Yu *et al.*, 2017; Fig. 10.2). The synthetic communities, in general, followed the pattern of the natural communities in terms of the *Methylococcaceae* and the *Methylophilaceae* establishing themselves as the most abundant species. However, when considered at the genus level, the major species differed from the ones in natural community microcosms. The species known for most robust growth in laboratory, the *Methylomonas* species among the *Methylococcaceae* and the *Methylophilus* species among the *Methylophilaceae* gradually outcompeted other species in most microcosms, suggesting that some of the controls present in natural communities, such as, for example, any predatory species, have not been recaptured in the synthetic communities (Yu *et al.*, 2017). Thus, while providing more straightforward and more easily controlled models, synthetic communities function somewhat differently, favouring fast-growing species. This conclusion has been further tested in a simplified model that competed thee major *Methylococcaceae* species against each other under a variety of conditions, confirming that growth rate played a role in the outcomes of the competition experiments (Yu *et al.*, 2016).

One advantage of the synthetic communities is the resolution they offer for the analysis of the metatranscriptomic data, allowing for matching transcript reads to the well assembled and annotated genomic scaffolds (Yu *et al.*, 2017). The strain-resolved transcript abundance analyses produced interesting results: in each of the actively expressing methylotrophs, some of the genes most responsive to the specific experimental conditions were the genes encoding alternative MDH

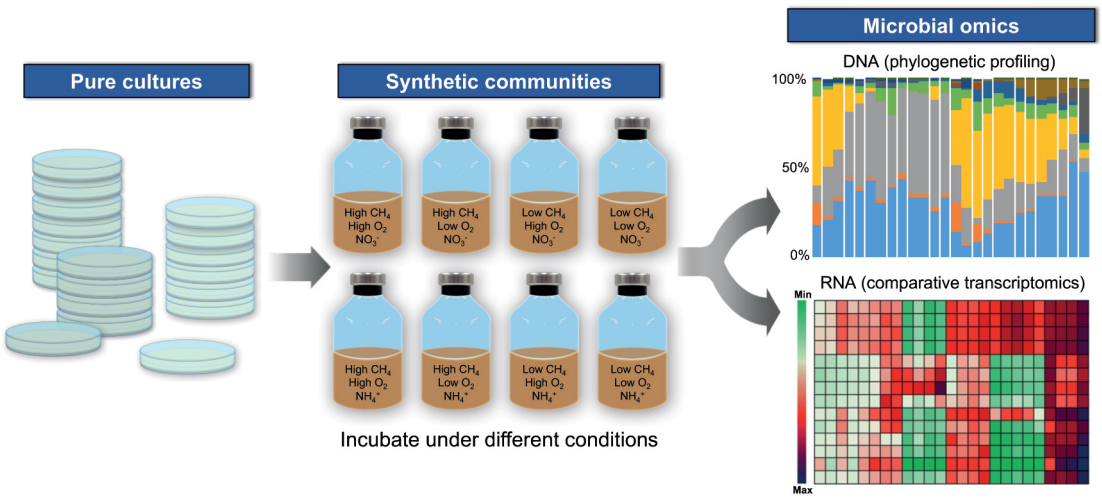

Figure 10.2 Schematic of the synthetic community experiment. Pure cultures were mixed and incubated under different conditions with methane as the only carbon source. Competitive species were identified via iTag analysis, and metabolic features were identified via comparative transcriptomics. See Yu *et al*. (2016, 2017).

enzymes, MxaFI and XoxF. The expression patterns were found very distinct dependent on whether nitrate or ammonium were supplied, or whether 'low' or 'high' concentrations of O_2 and methane were present. Moreover, expression patterns differed among different species and among different MDH enzymes. While the *Methylococcaceae, Methylocystaceae, Rhodocyclaceae,* and the *Methylophilus* species expressed *mxaFI* genes at high levels when 'high' O_2, 'high' methane, and nitrate were supplied, they expressed *xoxF* genes at higher levels when ammonium was supplied instead, or when methane supply was limited. Under the 'high' methane, 'low' O_2, NO_3^- regimen, genes for both enzymes were expressed at approximately equal levels in these species. In contrast, *Methylophilaceae* other than *Methylophilus* (mostly represented by unclassified *Methylophilaceae*) displayed higher expression of *xoxF* genes under all regimens (Yu *et al.*, 2017). It is important to note that the *xoxF* genes that were highly and differentially expressed by major community partners belonged to two main phylogenetic clades (Chistoserdova, 2011a), as follows. While the *Methylococcaceae, Methylocystaceae,* and *Rhodocyclaceae* possessed and express the *xoxF5* clade genes, the *Methylophilaceae* possessed and expressed the *xoxF4* clade genes. Proteins classified as XoxF4 and XoxF5 share approximately 50% amino acid identity, similarly to the identity divergencies between MxaF and XoxF4 and XoxF5 proteins, respectively (Huang *et al.*, 2018, 2019).

The complexity of the lanthanide-dependent methanol oxidation system

The Ln switch mechanism has been proposed as the result of the experiments with pure cultures of methanotrophs, using typical laboratory conditions, i.e. high methane and high dioxygen concentrations and nitrate as the source of nitrogen (Farhan Ul Haque *et al.*, 2015; Gu *et al.*, 2016; Chu and Lidstrom; 2016; Chu *et al.*, 2016; Zheng *et al.*, 2018). Even in these conditions, in some studies, the switch was shown to act in this manner only in the absence of copper, suggesting that, at least in some organisms, the Cu switch may override the Ln switch (Gu *et al.*, 2016). Sampling of the communal transcript response, under a variety of experimental conditions, produced a much more complex picture of how the Ln switch regulates transcription of alternative MDH genes. It appeared that the Ln switch is not only responsive to the presence of Ln, but also to the partial pressures of both methane and dioxygen, as well to the nitrogen source. In addition, a response has been recorded to both the phylogenetic positioning of an organism, and to the phylogenetic affiliation of a XoxF enzyme, providing a very complex picture of how different XoxF enzymes may contribute to the communal function in methane oxidation and, potentially, to interspecies communications (Yu *et al.*, 2017; Fig. 10.3).

Further insights into the Ln switch were gained by manipulating synthetic bacterial communities in their simplest, two-species form, combining a *Methylobacter* type methanotroph

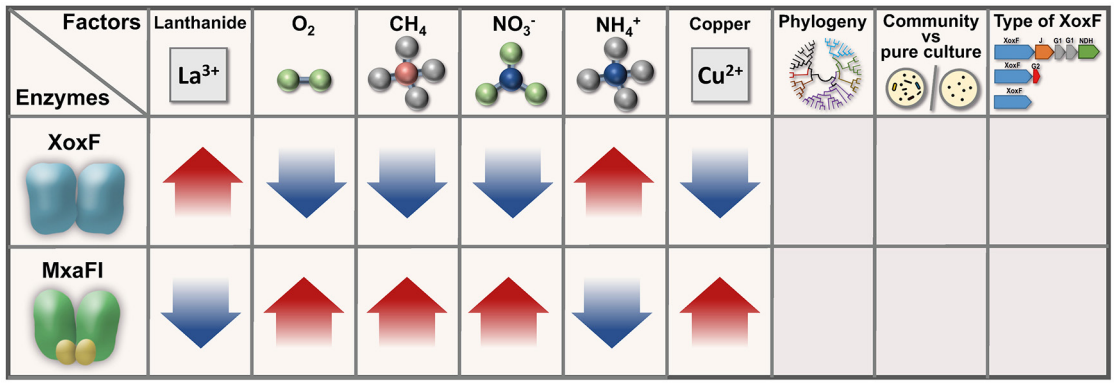

Figure 10.3 Cartoon depicting factors regulating expression of genes encoding XoxF and MxaFI methanol dehydrogenases. Red arrow, up-regulated; blue arrow, down-regulated. See Krause *et al*. (2017), Yu *et al*. (2017) and Zheng *et al*. (2018).

with two different *Methylotenera* partners, into functional methane-oxidizing communities, in separate two-species community experiments (Krause *et al.*, 2017). These were grown in the presence of Ln, either as communities or as axenic cultures, and their transcriptomes were compared (Krause *et al.*, 2017). Pure cultures of the *Methylobacter* species demonstrated the predicted pattern for MDH gene expression: the *xoxF* gene being induced and the *mxa* genes being repressed. Remarkably, in cocultures, this pattern was reversed, and *Methylobacter* cultures expressed the *mxa* genes (Krause *et al.*, 2017). In this case, it appeared that community living has overruled the Ln switch, even in the presence of Ln (Fig. 10.3). It has been proposed that the reversal of the Ln switch was a result of interspecies interactions, the *Methylotenera* species potentially releasing a signal to upregulate the *mxa* machinery in *Methylobacter*, resulting in methanol release (Krause *et al.*, 2017). However, this conclusion on the requirement of the *mxa* system for methanol release needs further investigation. In a more recent study, knockout mutants of a *Methylomonas* species separately expressing either MxaFI or XoxF both appeared to maintain populations of the satellite *Methylophilaceae* of similar size, while the sizes of the satellite populations were correlated with the concentrations of the methanol released (Zheng *et al.*, 2018).

When multiple *xoxF* genes are present in the genomes, these genes are also subjects to differential regulation. For example, the *Methylotenera* species employed in the two-species communities showed reversed transcriptional regulation of the alternative *xoxF* genes, in response to communal living (Krause *et al.*, 2017).

Insights into lanthanide-dependent catalysis

The complexity of the Ln switch has highlighted the necessity of a better understanding of the biochemical properties of XoxF enzymes. It needs to be noted that the diversity of enzymes referred to XoxF has been appreciated for a long time, and, as early as 2011, they have been classified into five divergent phylogenetic clades (Chistoserdova, 2011a). More recent phylogenetic inferences suggested that Ln dependencies may extend beyond these five clusters, and, perhaps, even beyond primary alcohol/aldehyde dehydrogenase enzymes (Keltjens *et al.*, 2014; Chistoserdova and Kalyuzhnaya, 2018). A second aspartate has been proposed to be essential in the active site of XoxF-type enzymes (Pol *et al.*, 2014; Jahn *et al.*, 2018; Lumpe *et al.*, 2018), and this aspartate appears to be conserved in many divergent clades of XoxF and XoxF-like enzymes (Keltjens *et al.*, 2014; Chistoserdova and Kalyuzhnaya, 2018). Two clades, XoxF4 and XoxF5 appear to be especially widely spread among the proteobacterial methylotrophs, and intriguingly, these two types appear to be mutually exclusive: while a variety of Alpha-, Beta- and Gammaproteobacteria encode, sometimes multiple, XoxF5 types, the XoxF4 types are only encoded by the members of *Methylophilaceae* (Betaproteobacteria; Huang *et al.*, 2018). Thus, among the main partners in methane oxidation, as described above, the *Methylococcaceae* encode the XoxF5 type and the *Methylophilaceae* encode the XoxF4 type. The uncompetitive methanol consumers, such as *Rhodocyclaceae, Methylobacterium, Hyphomicrobium, Methylopila* types, etc. based on the synthetic community experiments, (Yu *et al.*, 2017) also only encode and express XoxF5 types, raising a question whether the XoxF4 type, possessed by the *Methylophilaceae* gives them advantage in competing for methanol spilled by the *Methylococcaceae*? This hypothesis was tested by purifying and evaluating the catalytic properties of XoxF5 and XoxF4 enzymes from select organisms (Fitriyanto *et al.*, 2011; Huang *et al.*, 2018, 2019). Both types revealed similarities in terms of Ln dependences and in terms of a broad range of substrates. However, the catalytic efficiencies of the XoxF5 enzymes appeared to be generally higher than the catalytic efficiencies of the XoxF4 enzymes (Fitriyanto *et al.*, 2011; Huang *et al.*, 2018, 2019; Fig. 10.4), thus not supporting the original hypothesis. What does this result mean for understanding the relationships among partner species sharing carbon from methane (Krause *et al.*, 2017; Yu and Chistoserdova, 2017)? The discrepancies between the measured kinetic constants and the observed communal behaviour further point to the limitations of our understanding of the metabolic step mediated by alternative MDH enzymes. One obvious problem is the reliance on the artificial dye assay, practised since the 1960s, for measuring the activity of MDH enzymes (Anthony and Zatman, 1967a,b). However, more likely, enzyme

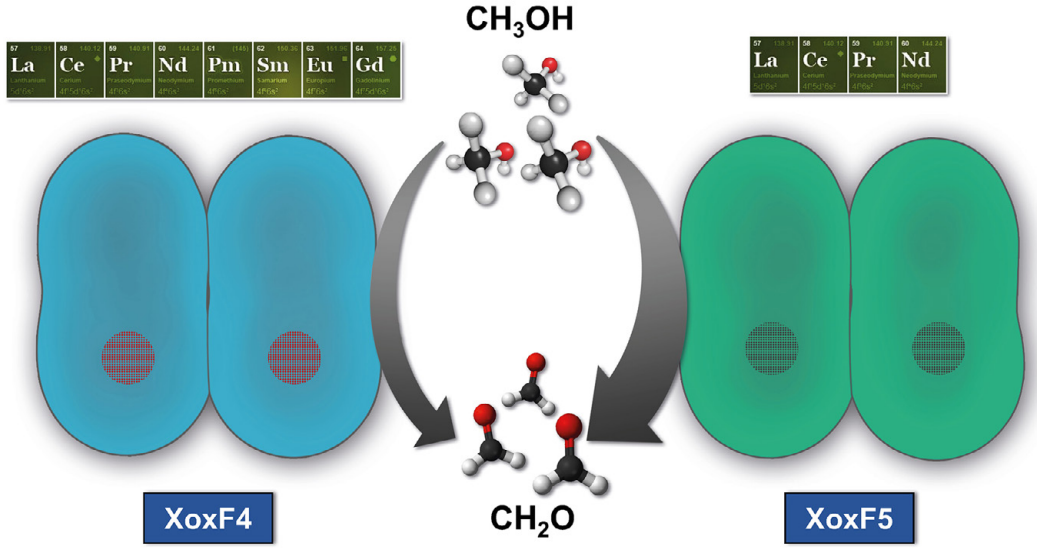

Figure 10.4 Cartoon depicting catalytic properties of XoxF4 versus XoxF5. While XoxF4 enzymes reveal activity with a broader range of lanthanides, XoxF5 enzymes reveal higher catalytic efficiency (size of arrow). The range of metals supporting activity is shown for each enzyme. See Fitriyanto *et al*. (2011) and Huang *et al*. (2018, 2019).

efficiencies and their differential functions could only be understood when considered in the context of natural redox metabolic complexes, which would include the primary alcohol-oxidizing enzyme, their natural electron acceptors (dedicated or promiscuous cytochromes), and the respective cytochrome oxidases, and these additional electron transfer chain components remain poorly understood for XoxF type MDH enzymes. Recently, complex relationships for XoxF enzymes with the downstream redox enzymes have been suggested, with different types of cytochromes potentially metabolically coupling with different types of XoxF enzymes (Yu *et al*., 2017; Zheng *et al*., 2018), and these metabolic connections and their metabolic efficiencies need to be further characterized, to advance our understanding of Ln-linked versus Ca-linked methanol oxidation in methanotrophs and non-methane-oxidizing methylotrophs, as well as their potential involvement in the community function.

The inferred diversity and distribution of lanthanide-dependent alcohol dehydrogenases

Besides XoxF-type MDH enzymes, two enzymes have been recently biochemically characterized, named ExaF/PedH, which also depend on Ln for activity, revealing highest affinities towards ethanol, thus these enzymes were assumed to be functional ethanol dehydrogenases (Good *et al*., 2016; Wehrmann *et al*., 2017). In *Pseudomonas putida*, which also encodes a Ca version of this enzyme (PedE/QedH), expression of the two enzymes is regulated by a Ln switch (Wehrmann *et al*., 2018), a mechanism that is similar to the one described for XoxF (Chu and Lidstrom, 2016; Chu *et al*., 2016). How frequently do Ln-dependent enzymes occur in nature? Based on the conclusion that the Ln MDH enzymes are more widely spread among the methylotrophs, and based on their ancestral positioning in phylogenetic trees (Chistoserdova, 2011a; Keltjens *et al*., 2014; Chistoserdova and Kalyuzhnaya, 2018), they should be expected to be abundant beyond organisms with a demonstrated methylotrophic life style. Indeed, close homologues of XoxF5 are found among a variety of Proteobacteria, belonging, so far, to 12 orders, of which only one, the *Methylococcales*, is represented exclusively by the methylotrophs (Huang *et al*., 2019). Five of the remaining orders are represented by taxa that include methylotroph species but also species not known for methylotrophic life style, and the remaining six orders are only represented by non-methylotrophs, including such important orders as

Vibrionales and *Pseudomonadales* (Fig. 10.5; Huang *et al.*, 2019). While XoxF4 type Ln-MDH enzymes have only been identified so far in members of *Methylophilales*, these represent abundant environmental populations that play important functions in global carbon cycling (Sowell *et al.*, 2011; Salcher *et al.*, 2015; Gifford *et al.*, 2016). ExaF/PedH enzymes appear to be even more broadly spread among the Proteobacteria Phylum microbes, and these are found in bacteria belonging to 14 different orders (Fig. 10.5; Huang *et al.*, 2019), compared with 16 orders possessing the Ca-dependent homologues of ExaF/PedH, named ExaA/PedE (Fig. 10.5). The two counterparts co-occur in 14 orders

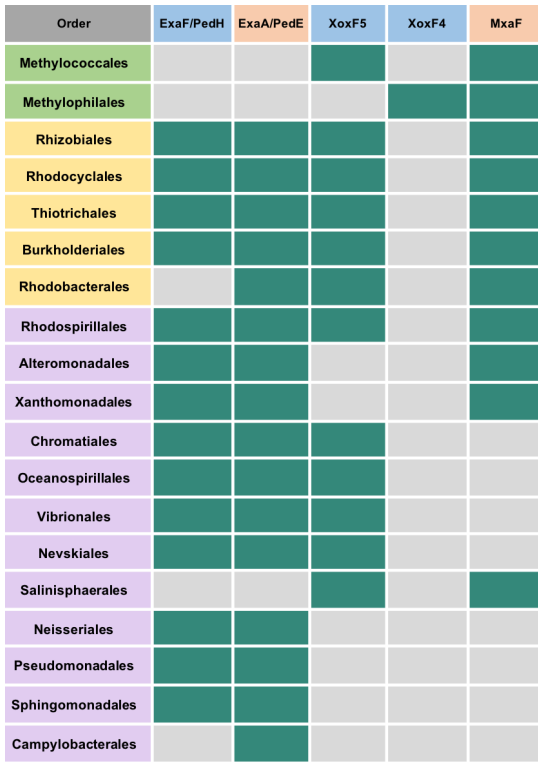

Figure 10.5 Occurrence and distribution of lanthanide-dependent alcohol dehydrogenases among Proteobacteria, compared with the occurrence and distribution of their calcium-dependent counterparts. Taxa are shown at the Order level. In green, taxa that only contain methylotroph species. In yellow, taxa that contain both methylotroph and non-methylotroph species. In purple, taxa that are not known to contain any methylotroph species. Enzymes highlighted in blue, Ln-dependent; in pink, Ca-dependent. Green, gene present, grey, gene absent. See Huang *et al*. (2019).

(Huang *et al.*, 2019). Note that Ln enzymes are also common in Verrucomicrobia and NC10 phylum microbes. However, the databases of the relevant genomes remain limited, while most of the proteins possessing the Ln-binding motif (Keltjens *et al.*, 2014) have not yet been biochemically characterized. However, the currently available genomic and biochemical data strongly suggest that Ln should be considered as true life metals, to join other metals recognized for their importance in biological functions, such as iron, calcium, copper, magnesium, zinc, cobalt, manganese, potassium. Based on the wide distribution of Ln-dependent counterparts of the Ca-dependent alcohol dehydrogenases, and based on existence of special cellular mechanisms of Ln acquisition and the Ln-dependent regulatory mechanisms, it is reasonable to expect that enzymatic reactions beyond alcohol oxidation, along with specific transport and regulation mechanisms, and potentially community interactions involving these reactions, may also be reliant on Ln. Thus further discoveries of Ln-dependent functions are expected.

Conclusions and future perspectives

The communal metabolism of methane, while not an entirely new concept, has regained interest, as the recent meta-omics data suggest that environmental co-occurrences of the methanotroph species along with non-methanotrophic methylotroph species may not be random, and, instead, these partnerships may be specific. While data are available on methanol being the one carbon source shared among the community members, it remains unknown why would the methanotrophs release methanol, and whether this carbon sharing is accidental or is specifically designed to feed populations of the satellite species. The feedbacks that these methanol-consuming populations may be providing to the methanotrophs remain unknown. However, evidence is also available that alternative enzymes involved in methanol oxidation in both the methane-oxidizing species and in the methanol-consuming species are some of the major metabolic sites for regulation, which follows a rather complex pattern, involving, as the major controlling factors, the availability of Ln, the partial pressures of O_2 and methane, the

phylogenetic makeups of the communities, and the types of MDH enzymes encoded in the genomes. Deciphering the details of this complex regulation presents a significant challenge for furthering our understanding of the communal methanotrophy under aerobic conditions. The potentially discrete functions of the MDH enzymes with alternative metal specificities, and, further, potentially discrete functions of the multiple Ln-dependent MDH enzymes encoded in many methylotroph genomes present another challenge. While a collection of the Ln-dependent alcohol dehydrogenases have been already characterized, more data are needed in order to decipher the differences among the distinct clades of Ln enzymes, at higher confidence. With this respect, the emergent problem is the paucity of the data on the classic, Ca-dependent MDH enzymes, despite the long history of these enzymes and the intense efforts on their investigation over decades. Next, the functions of the non-methylotrophic heterotrophs in the communities, while they typically are present at minor populations, also need to be addressed, and most parsimonious interspecies interaction scenarios elucidated: whether such heterotrophs are directly fed by the methanotrophs, and whether alternative carbon sources are consumed by them, such as acetate (Kalyuzhnaya *et al*., 2013), or whether the carbon flow also involves the major methylotroph partners. Further sampling of the potentially Ln-dependent enzymes is necessary, across the phylogenetic trees of the homologues, and their functions within their hosts or potentially within communities need to be revealed. Perhaps, deployment of Ln-binding motifs could identify Ln-dependent enzymes beyond alcohol dehydrogenases, furthering insights into Ln-dependent biological functions. Likely, the insights into the communal function in the aerobic methanotrophy, as a momentum of the ongoing effort, will identify and highlight similar patterns in communal behaviours relevant to other important microbially driven biogeochemical processes.

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References

Anthony, C. (1982). The Biochemistry of Methylotrophs (Academic Press, London, UK).

Anthony, C. (2004). The quinoprotein dehydrogenases for methanol and glucose. Arch. Biochem. Biophys. *428*, 2–9. https://doi.org/10.1016/j.abb.2004.03.038

Anthony, C. (2011). How half a century of research was required to understand bacterial growth on C1 and C2 compounds; the story of the serine cycle and the ethylmalonyl-CoA pathway. Sci. Prog. *94*, 109–137.

Anthony, C., and Zatman, L.J. (1967a). The microbial oxidation of methanol. Purification and properties of the alcohol dehydrogenase of *Pseudomonas* sp. M27. Biochem. J. *104*, 953–959.

Anthony, C., and Zatman, L.J. (1967b). The microbial oxidation of methanol. The prosthetic group of the alcohol dehydrogenase of *Pseudomonas* sp. M27: a new oxidoreductase prosthetic group. Biochem. J. *104*, 960–969.

Baxter, N.J., Hirt, R.P., Bodrossy, L., Kovacs, K.L., Embley, T.M., Prosser, J.I., and Murrell, J.C. (2002). The ribulose-1,5-bisphosphate carboxylase/oxygenase gene cluster of *Methylococcus capsulatus* (Bath). Arch. Microbiol. *177*, 279–289. https://doi.org/10.1007/s00203-001-0387-x

Beck, D.A., Kalyuzhnaya, M.G., Malfatti, S., Tringe, S.G., Glavina Del Rio, T., Ivanova, N., Lidstrom, M.E., and Chistoserdova, L. (2013). A metagenomic insight into freshwater methane-utilizing communities and evidence for cooperation between the *Methylococcaceae* and the *Methylophilaceae*. PeerJ *1*, e23. https://doi.org/10.7717/peerj.23

Beck, D.A., McTaggart, T.L., Setboonsarng, U., Vorobev, A., Goodwin, L., Shapiro, N., Woyke, T., Kalyuzhnaya, M.G., Lidstrom, M.E., and Chistoserdova, L. (2015). Multiphyletic origins of methylotrophy in Alphaproteobacteria, exemplified by comparative genomics of Lake Washington isolates. Environ. Microbiol. *17*, 547–554. https://doi.org/10.1111/1462-2920.12736

Biderre-Petit, C. Taib, N., Gardon, H., Hochart, C., and Debroas, D. (2018). New insights into the pelagic microorganisms involved in the methane cycle in the meromictic Lake Pavin through metagenomics. FEMS Microbiol. Ecol. https://doi.org/10.1093/femsec/fiy183 [Epub ahead of print].

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B.B., Witte, U., and Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature *407*, 623–626. https://doi.org/10.1038/35036572

But, S.Y., Egorova, S.V., Khmelenina, V.N., and Trotsenko, Y.A. (2017). Biochemical properties and phylogeny of hydroxypyruvate reductases from methanotrophic bacteria with different C1-assimilation pathways. Biochemistry Mosc. *82*, 1295–1303. https://doi.org/10.1134/S0006297917110074

Chistoserdova, L. (2011a). Modularity of methylotrophy, revisited. Environ. Microbiol. *13*, 2603–2622. https://doi.org/10.1111/j.1462-2920.2011.02464.x

Chistoserdova, L. (2011b). Methylotrophy in a lake: from metagenomics to single-organism physiology. Appl.

Environ. Microbiol. *77*, 4705–4711. https://doi.org/10.1128/AEM.00314-11

Chistoserdova, L. (2016). Lanthanides: New life metals? World J. Microbiol. Biotechnol. *32*, 138. https://doi.org/10.1007/s11274-016-2088-2

Chistoserdova, L. (2018). Methanotrophy: an evolving field. In Methane Biocatalysis: Paving the Way to Sustainability. Kalyuzhnaya, M., Xing, X.-H., eds (Springer, Springer-Verlag, Berlin Heidelberg), pp. 1–15.

Chistoserdova, L., and Lidstrom, M.E. (1997). Molecular and mutational analysis of a DNA region separating two methylotrophy gene clusters in *Methylobacterium extorquens* AM1. Microbiology *143*, 1729–1736. https://doi.org/10.1099/00221287-143-5-1729

Chistoserdova, L., and Lidstrom, M.E. (2013). Aerobic methylotrophic prokaryotes. In The Prokaryotes, Fourth Edition. Rosenberg, E., DeLong, E.F., Thompson, F., Lory, S., Stackebrandt, E., eds. (Springer, Berlin, Heidelberg), pp. 267–285.

Chistoserdova, L., and Kalyuzhnaya, M.G. (2018). Current trends in methylotrophy. Trends Microbiol. *26*, 703–714.

Chistoserdova, L., Kalyuzhnaya, M.G., and Lidstrom, M.E. (2009). The expanding world of methylotrophic metabolism. Annu. Rev. Microbiol. *63*, 477–499. https://doi.org/10.1146/annurev.micro.091208.073600

Chu, F., and Lidstrom, M.E. (2016). XoxF acts as the predominant methanol dehydrogenase in the Type I methanotroph *Methylomicrobium buryatense*. J. Bacteriol. *198*, 1317–1325. https://doi.org/10.1128/JB.00959-15

Chu, F., Beck, D.A., and Lidstrom, M.E. (2016). MxaY regulates the lanthanide-mediated methanol dehydrogenase switch in *Methylomicrobium buryatense*. PeerJ *4*, e2435. https://doi.org/10.7717/peerj.2435

Dalton, H. (2005). The Leeuwenhoek Lecture 2000 the natural and unnatural history of methane-oxidizing bacteria. Philos. Trans. R. Soc. Lond., B, Biol. Sci. *360*, 1207–1222.

Dedysh, S.N., and Dunfield, P.F. (2017). Cultivation of methanotrophs. In Hydrocarbon and Lipid Microbiology Protocols, McGenity, T.J., Timmis, K.N., Nogales Fernandez, B.N., eds (Springer-Ferlag, Berlin, Heidelberg), pp. 231–247.

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von Mering, C., and Vorholt, J.A. (2009). Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc. Natl. Acad. Sci. U.S.A. *106*, 16428–16433. https://doi.org/10.1073/pnas.0905240106

Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M.M., Schreiber, F., Dutilh, B.E., Zedelius, J., de Beer, D., *et al.* (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature *464*, 543–548. https://doi.org/10.1038/nature08883

Farhan Ul Haque, M., Gu, W., DiSpirito, A.A., and Semrau, J.D. (2015). Marker exchange mutagenesis of *mxaF*, encoding the large subunit of the Mxa methanol dehydrogenase, in *Methylosinus trichosporium* OB3b. Appl. Environ. Microbiol. *82*, 1549–1555. https://doi.org/10.1128/AEM.03615-15

Fitriyanto, N.A., Fushimi, M., Matsunaga, M., Pertiwiningrum, A., Iwama, T., and Kawai, K. (2011). Molecular structure and gene analysis of Ce3+ -induced methanol dehydrogenase of *Bradyrhizobium* sp. MAFF211645. J. Biosci. Bioeng. *111*, 613–617. https://doi.org/10.1016/j.jbiosc.2011.01.015

Gifford, S.M., Becker, J.W., Sosa, O.A., Repeta, D.J., and DeLong, E.F. (2016). Quantitative transcriptomics reveals the growth- and nutrient-dependent response of a streamlined marine methylotroph to methanol and naturally occurring dissolved organic matter. MBio *7*, e01279–16.

Good, N.M., Vu, H.N., Suriano, C.J., Subuyuj, G.A., Skovran, E., and Martinez-Gomez, N.C. (2016). Pyrroloquinoline quinone ethanol dehydrogenase in *Methylobacterium extorquens* AM1 extends lanthanide-dependent metabolism to multicarbon substrates. J. Bacteriol. *198*, 3109–3118.

Gu, W., Farhan Ul Haque, M., DiSpirito, A.A., and Semrau, J.D. (2016). Uptake and effect of rare earth elements on gene expression in *Methylosinus trichosporium* OB3b. FEMS Microbiol. Lett. *363*, fnw129. https://doi.org/10.1093/femsle/fnw129

Haroon, M.F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., Yuan, Z., and Tyson, G.W. (2013). Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature *500*, 567–570. https://doi.org/10.1038/nature12375

He, R., Wooller, M.J., Pohlman, J.W., Catranis, C., Quensen, J., Tiedje, J.M., and Leigh, M.B. (2012). Identification of functionally active aerobic methanotrophs in sediments from an arctic lake using stable isotope probing. Environ. Microbiol. *14*, 1403–1419. https://doi.org/10.1111/j.1462-2920.2012.02725.x

Hernandez, M.E., Beck, D.A., Lidstrom, M.E., and Chistoserdova, L. (2015). Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation. PeerJ *3*, e801. https://doi.org/10.7717/peerj.801

Hou, S., Makarova, K.S., Saw, J.H., Senin, P., Ly, B.V., Zhou, Z., Ren, Y., Wang, J., Galperin, M.Y., Omelchenko, M.V., *et al.* (2008). Complete genome sequence of the extremely acidophilic methanotroph isolate V4, *Methylacidiphilum infernorum*, a representative of the bacterial phylum Verrucomicrobia. Biol. Direct *3*, 26. https://doi.org/10.1186/1745-6150-3-26

Huang, J., Yu, Z., and Chistoserdova, L. (2018). Lanthanide-dependent methanol dehydrogenases of XoxF4 and XoxF5 clades are differentially distributed among methylotrophic bacteria and they reveal different biochemical properties. Front. Microbiol. *9*, 1366. https://doi.org/10.3389/fmicb.2018.01366

Huang, J., Yu, Z., Groom, J., Cheng, J.-F., Tarver, A., Yoshikunin, Y., and Chistoserdova, L. (2019). Rare earth element alcohol dehydrogenases widely occur among globally distributed, numerically abundant and environmentally important microbes. ISME J. In Press.

Jahn, B., Pol, A., Lumpe, H., Barends, T.R.M., Dietl, A., Hogendoorn, C., Op den Camp, H.J.M., and Daumann, L.J. (2018). Similar but not the same: first kinetic and structural analyses of a methanol dehydrogenase containing a europium Ion in the

active site. Chembiochem. 2018 Mar 11. doi: 10.1002/cbic.201800130.

Kalyuzhnaya, M.G., Lapidus, A., Ivanova, N., Copeland, A.C., McHardy, A.C., Szeto, E., Salamov, A., Grigoriev, I.V., Suciu, D., Levine, S.R., *et al*. (2008). High-resolution metagenomics targets specific functional types in complex microbial communities. Nat. Biotechnol. *26*, 1029–1034. https://doi.org/10.1038/nbt.1488

Kalyuzhnaya, M.G., Yang, S., Rozova, O.N., Smalley, N.E., Clubb, J., Lamb, A., Gowda, G.A., Raftery, D., Fu, Y., Bringel, F., *et al*. (2013). Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. Nat. Commun. *4*, 2785. https://doi.org/10.1038/ncomms3785

Kao, W.C., Chen, Y.R., Yi, E.C., Lee, H., Tian, Q., Wu, K.M., Tsai, S.F., Yu, S.S., Chen, Y.J., Aebersold, R., *et al*. (2004). Quantitative proteomic analysis of metabolic regulation by copper ions in *Methylococcus capsulatus* (Bath). J. Biol. Chem. *279*, 51554–51560. https://doi.org/10.1074/jbc.M408013200

Karwautz, C., Kus, G., Stöckl, M., Neu, T.R., and Lueders, T. (2018). Microbial megacities fueled by methane oxidation in a mineral spring cave. ISME J. *12*, 87–100. https://doi.org/10.1038/ismej.2017.146

Keltjens, J.T., Pol, A., Reimann, J., and Op den Camp, H.J. (2014). PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. Appl. Microbiol. Biotechnol. *98*, 6163–6183. https://doi.org/10.1007/s00253-014-5766-8

Kessler, J.D., Valentine, D.L., Redmond, M.C., Du, M., Chan, E.W., Mendes, S.D., Quiroz, E.W., Villanueva, C.J., Shusta, S.S., Werra, L.M., *et al*. (2011). A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. Science *331*, 312–315. https://doi.org/10.1126/science.1199697

Khadem, A.F., Pol, A., Wieczorek, A., Mohammadi, S.S., Francoijs, K.J., Stunnenberg, H.G., Jetten, M.S., and Op den Camp, H.J. (2011). Autotrophic methanotrophy in verrucomicrobia: *Methylacidiphilum fumariolicum* SolV uses the Calvin-Benson-Bassham cycle for carbon dioxide fixation. J. Bacteriol. *193*, 4438–4446. https://doi.org/10.1128/JB.00407-11

Knittel, K., and Boetius, A. (2009). Anaerobic oxidation of methane: progress with an unknown process. Annu. Rev. Microbiol. *63*, 311–334. https://doi.org/10.1146/annurev.micro.61.080706.093130

Krause, S.M., Johnson, T., Samadhi Karunaratne, Y., Fu, Y., Beck, D.A., Chistoserdova, L., and Lidstrom, M.E. (2017). Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. Proc. Natl. Acad. Sci. U.S.A. *114*, 358–363. https://doi.org/10.1073/pnas.1619871114

Lawton, T.J., and Rosenzweig, A.C. (2016). Biocatalysts for methane conversion: big progress on breaking a small substrate. Curr. Opin. Chem. Biol. *35*, 142–149.

Lim, S., and Franklin, S.J. (2004). Lanthanide-binding peptides and the enzymes that Might Have Been. Cell. Mol. Life Sci. *61*, 2184–2188. https://doi.org/10.1007/s00018-004-4156-2

Lumpe, H., Pol, A., Op den Camp, H.J.M., and Daumann, L.J. (2018). Impact of the lanthanide contraction on the activity of a lanthanide-dependent methanol dehydrogenase - a kinetic and DFT study. Dalton Trans. *47*, 10463–10472. https://doi.org/10.1039/c8dt01238e

Martineau, C., Whyte, L.G., and Greer, C.W. (2010). Stable isotope probing analysis of the diversity and activity of methanotrophic bacteria in soils from the Canadian high Arctic. Appl. Environ. Microbiol. *76*, 5773–5784. https://doi.org/10.1128/AEM.03094-09

McGlynn, S.E. (2017). Energy metabolism during anaerobic methane oxidation in ANME Archaea. Microbes Environ. *32*, 5–13. https://doi.org/10.1264/jsme2.ME16166

Meyerdierks, A., Kube, M., Kostadinov, I., Teeling, H., Glöckner, F.O., Reinhardt, R., and Amann, R. (2010). Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. Environ. Microbiol. *12*, 422–439. https://doi.org/10.1111/j.1462-2920.2009.02083.x

Mustakhimov, I., Kalyuzhnaya, M.G., Lidstrom, M.E., and Chistoserdova, L. (2013). Insights into denitrification in *Methylotenera mobilis* from denitrification pathway and methanol metabolism mutants. J. Bacteriol. *195*, 2207–2211. https://doi.org/10.1128/JB.00069-13

Orphan, V.J., House, C.H., Hinrichs, K.U., McKeegan, K.D., and DeLong, E.F. (2001). Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science *293*, 484–487. https://doi.org/10.1126/science.1061338

Oshkin, I.Y., Beck, D.A., Lamb, A.E., Tchesnokova, V., Benuska, G., McTaggart, T.L., Kalyuzhnaya, M.G., Dedysh, S.N., Lidstrom, M.E., and Chistoserdova, L. (2015). Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. ISME J. *9*, 1119–1129. https://doi.org/10.1038/ismej.2014.203

Paul, B.G., Ding, H., Bagby, S.C., Kellermann, M.Y., Redmond, M.C., Andersen, G.L., and Valentine, D.L. (2017). Methane-oxidizing bacteria shunt carbon to microbial mats at a marine hydrocarbon seep. Front. Microbiol. *8*, 186. https://doi.org/10.3389/fmicb.2017.00186

Pol, A., Barends, T.R., Dietl, A., Khadem, A.F., Eygensteyn, J., Jetten, M.S., and Op den Camp, H.J. (2014). Rare earth metals are essential for methanotrophic life in volcanic mudpots. Environ. Microbiol. *16*, 255–264. https://doi.org/10.1111/1462-2920.12249

Rasigraf, O., Kool, D.M., Jetten, M.S., Sinninghe Damsté, J.S., and Ettwig, K.F. (2014). Autotrophic carbon dioxide fixation via the Calvin-Benson-Bassham cycle by the denitrifying methanotroph '*Candidatus* Methylomirabilis oxyfera'. Appl. Environ. Microbiol. *80*, 2451–2460. https://doi.org/10.1128/AEM.04199-13

Ross, M.O., and Rosenzweig, A.C. (2017). A tale of two methane monooxygenases. J. Biol. Inorg. Chem. *22*, 307–319. https://doi.org/10.1007/s00775-016-1419-y

Salcher, M.M., Neuenschwander, S.M., Posch, T., and Pernthaler, J. (2015). The ecology of pelagic freshwater methylotrophs assessed by a high-resolution monitoring and isolation campaign. ISME J. *9*, 2442–2453. https://doi.org/10.1038/ismej.2015.55

Sharp, C.E., Smirnova, A.V., Graham, J.M., Stott, M.B., Khadka, R., Moore, T.R., Grasby, S.E., Strack, M., and Dunfield, P.F. (2014). Distribution and diversity of

Verrucomicrobia methanotrophs in geothermal and acidic environments. Environ. Microbiol. *16*, 1867–1878. https://doi.org/10.1111/1462-2920.12454

Shiller, A.M., Chan, E.W., Joung, D.J., Redmond, M.C., and Kessler, J.D. (2017). Light rare earth element depletion during Deepwater Horizon blowout methanotrophy. Sci. Rep. *7*, 10389. https://doi.org/10.1038/s41598-017-11060-z

Sowell, S.M., Abraham, P.E., Shah, M., Verberkmoes, N.C., Smith, D.P., Barofsky, D.F., and Giovannoni, S.J. (2011). Environmental proteomics of microbial plankton in a highly productive coastal upwelling system. ISME J. *5*, 856–865. https://doi.org/10.1038/ismej.2010.168

Thauer, R.K., and Shima, S. (2008). Methane as fuel for anaerobic microorganisms. Ann. N. Y. Acad. Sci. *1125*, 158–170.

Trembath-Reichert, E., Case, D.H., and Orphan, V.J. (2016). Characterization of microbial associations with methanotrophic archaea and sulfate-reducing bacteria through statistical comparison of nested Magneto-FISH enrichments. PeerJ *4*, e1913. https://doi.org/10.7717/peerj.1913

Trotsenko, Y.A., and Murrell, J.C. (2008). Metabolic aspects of aerobic obligate methanotrophy. Adv. Appl. Microbiol. *63*, 183–229. https://doi.org/10.1016/S0065-2164(07)00005-6

Vekeman, B., Speth, D., Wille, J., Cremers, G., De Vos, P., Op den Camp, H.J., and Heylen, K. (2016). Genome characteristics of two novel Type I methanotrophs enriched from north sea sediments containing exclusively a lanthanide-dependent xoxf5-Type Methanol Dehydrogenase. Microb. Ecol. *72*, 503–509. https://doi.org/10.1007/s00248-016-0808-7

Versantvoort, W., Guerrero-Cruz, S., Speth, D.R., Frank, J., Gambelli, L., Cremers, G., van Alen, T., Jetten, M.S.M., Kartal, B., Op den Camp, H.J.M., *et al.* (2018). Comparative genomics of *Candidatus Methylomirabilis* Species and description of *Ca. Methylomirabilis Lanthanidiphila*. Front. Microbiol. *9*, 1672. https://doi.org/10.3389/fmicb.2018.01672

Vu, H.N., Subuyuj, G.A., Vijayakumar, S., Good, N.M., Martinez-Gomez, N.C., and Skovran, E. (2016). Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. J. Bacteriol. *198*, 1250–1259. https://doi.org/10.1128/JB.00937-15

Wehrmann, M., Billard, P., Martin-Meriadec, A., Zegeye, A., and Klebensberger, J. (2017). Functional Role of lanthanides in enzymatic activity and transcriptional regulation of pyrroloquinoline quinone-dependent alcohol dehydrogenases in *Pseudomonas putida* KT2440. MBio *8*, e00570–17.

Wehrmann, M., Berthelot, C., Billard, P., and Klebensberger, J. (2018). The PedS2/PedR2 two-component system is crucial for the rare earth element switch in *Pseudomonas putida* KT2440. mSphere *3*, e00376–18.

Welte, C.U., Rasigraf, O., Vaksmaa, A., Versantvoort, W., Arshad, A., Op den Camp, H.J., Jetten, M.S., Lüke, C., and Reimann, J. (2016). Nitrate- and nitrite-dependent anaerobic oxidation of methane. Environ. Microbiol. Rep. *8*, 941–955. https://doi.org/10.1111/1758-2229.12487

Whittenbury, R., Phillips, K.C., and Wilkinson, J.F. (1970). Enrichment, isolation and some properties of methane-utilizing bacteria. J. Gen. Microbiol. *61*, 205–218. https://doi.org/10.1099/00221287-61-2-205

Yu, Z., and Chistoserdova, L. (2017). Communal metabolism of methane and the rare Earth element switch. J. Bacteriol. *19*, pii JB.00323-17. https://doi.org/10.1128/JB.00328-17

Yu, Z., Krause, S.M., Beck, D.A., and Chistoserdova, L. (2016). A synthetic ecology perspective: how well does behavior of model organisms in the laboratory predict microbial activities in natural habitats? Front. Microbiol. *7*, 946.

Yu, Z., Beck, D.A.C., and 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. https://doi.org/10.3389/fmicb.2017.02392

Zheng, Y., Huang, J., Zhao, F., and Chistoserdova, L. (2018). Physiological effect of XoxG(4) on lanthanide-dependent methanotrophy. MBio *9*, e02430–17.