FEMS Microbiology Letters, 363, 2016, fnw129

doi: 10.1093/femsle/fnw129
Advance Access Publication Date: 12 May 2016
Research Letter

RESEARCH LETTER – Environmental Microbiology

Uptake and effect of rare earth elements on gene expression in *Methylosinus trichosporium* OB3b

Wenyu Gu[1,†], Muhammad Farhan Ul Haque[1,†], Alan A. DiSpirito[2] and Jeremy D. Semrau[1,*]

[1]Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI 48109-2125, USA and [2]Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011, USA

*Corresponding author: Department of Civil and Environmental Engineering, University of Michigan, 109 EWRE Bldg, 1351 Beal Avenue, Ann Arbor, MI 48109-2125, USA. Tel: +734-764-6487; Fax: +734-763-2275; E-mail: jsemrau@umich.edu
†These individuals contributed equally to this manuscript.
One sentence summary: Rare earth elements control the expression of alternative methanol dehydrogenases in the model methanotroph, *Methylosinus trichosporium* OB3b, providing new strategies for the manipulation of methanotrophic activity for multiple applications.
Editor: Nathan Basiliko

ABSTRACT

It is well known that *Methylosinus trichosporium* OB3b has two forms of methane monooxygenase (MMO) responsible for the initial conversion of methane to methanol, a cytoplasmic (soluble) methane monooxygenase and a membrane-associated (particulate) methane monooxygenase, and that copper strongly regulates expression of these alternative forms of MMO. More recently, it has been discovered that *M. trichosporium* OB3b has multiple types of the methanol dehydrogenase (MeDH), i.e. the Mxa-type MeDH (Mxa-MeDH) and Xox-type MeDH (Xox-MeDH), and the expression of these two forms is regulated by the availability of the rare earth element (REE), cerium. Here, we extend these studies and show that lanthanum, praseodymium, neodymium and samarium also regulate expression of alternative forms of MeDH. The effect of these REEs on MeDH expression, however, was only observed in the absence of copper. Further, a mutant of *M. trichosporium* OB3b, where the Mxa-MeDH was knocked out, was able to grow in the presence of lanthanum, praseodymium and neodymium, but was not able to grow in the presence of samarium. Collectively, these data suggest that multiple levels of gene regulation by metals exist in *M. trichosporium* OB3b, but that copper overrides the effect of other metals by an as yet unknown mechanism.

Keywords: methanotrophy; methanol dehydrogenase; methane monooxygenase; rare earth elements; copper

INTRODUCTION

In methanotrophs and methylotrophs, microbes that utilize methane and/or methanol for growth, it was initially assumed that methanol oxidation was performed in these microbes by a calcium-containing pyrroloquinoline quinone (PQQ)-linked methanol dehydrogenase (MeDH), or Mxa-MeDH (Williams *et al.* 2005). Recent genomic and biochemical evidence indicates, however, an alternative PQQ-MeDH that contains a rare earth element (REE) in its active site also exists (Xox-MeDH), and this form may actually be catalytically superior to Mxa-MeDH (Hibi *et al.* 2011; Keltjens *et al.* 2014; Pol *et al.* 2014; Wu *et al.* 2015). Early data suggested that expression of the alternative forms of MeDH was not regulated by the (un)availability of REEs (Nakagawa *et al.* 2012), but it has been shown that in the presence of cerium, expression of *xoxF*, encoding for the single subunit

Received: 6 April 2016; **Accepted:** 11 May 2016

of Xox-MeDH increased in the α-Proteobacterium, *Methylosinus trichosporium* OB3b, while expression of *mxaF* and *mxaI*, encoding for the large and small subunits of Mxa-MeDH, respectively, goes down (Farhan Ul Haque *et al.* 2015). Subsequently, it was shown that cerium and other REEs (lanthanum, praseodymium and neodymium) exhibited a similar effect on MeDH expression in the methylotroph, *Methylobacterium extorquens* AM1 (Vu *et al.* 2016). It was also later shown that cerium and lanthanum upregulate expression of *xox* genes, while repressing *mxa* genes in the γ-Proteobacterium, *Methylomicrobium buryatense* (Chu and Lidstrom 2016).

One interesting finding was that although cerium controlled expression of alternative forms of MeDH in methanotrophs and methylotrophs, such regulation appears to be over-ridden by the 'copper-switch' in methanotrophs (Farhan Ul Haque *et al.* 2015). That is, some methanotrophs can express either a cytoplasmic (soluble) methane monooxygenase (sMMO) or a membrane-bound (particulate) methane monooxygenase (pMMO). Expression of these two forms is controlled by the availability of copper, with sMMO only expressed when copper is deficient, while pMMO expression increases with increasing copper (Semrau, DiSpirito and Yoon 2010). In *M. trichosporium* OB3b, expression of *xoxF* increased in the presence of cerium regardless of the concentration of copper. In the absence of copper, expression of *mxaF* and *mxaI*, decreased, but cerium had little effect on expression of these genes in the presence of copper. Here, we extend these initial findings to determine if other REEs also serve to regulate expression of alternative forms of the MeDH, and if such regulation is also over-ridden by copper.

MATERIALS AND METHODS

Growth conditions

Wild-type *M. trichosporium* OB3b and the *mxaF*::Gm^r mutant (where the gene encoding for the large subunit of the Mxa-MeDH was disrupted via marker-exchange mutagenesis) were grown in nitrate mineral salt (NMS) medium (Whittenbury, Phillips and Wilkinson 1970) with methane as the only substrate as previously described (Farhan Ul Haque *et al.* 2016). The *mxaF*::Gm^r mutant was maintained with addition of 2.5 μg ml^{-1} gentamicin and 25 μM cerium (as $CeCl_3$). To test the effect of copper and other lanthanides on gene expression, 10 μM copper (as $CuCl_2$) and 25 μM lanthanides (i.e. lanthanum as $LaCl_3$, praseodymium as $Pr(NO_3)_3$, neodymium as $NdCl_3$ and samarium as $Sm(NO_3)_3$) were supplemented to NMS medium when desired. The following metal salts were purchased from Fisher Scientific: $CuCl_2$ (Catalog #C79-500), $CeCl_3 \bullet 7H_2O$ (Catalog #AC211820250), $LaCl_3$ (Catalog #AC315230100), $Pr(NO_3)_3 \bullet 5H_2O$ (Catalog #AC194590500) and $Sm(NO_3)_3 \bullet 6H_2O$ (Catalog #AC316150250), while $NdCl_3 \bullet 6H_2O$ was purchased from Sigma Aldrich (Catalog #289183). Cultures were grown in triplicate and to late exponential phase before harvesting for analysis of gene expression and metal distribution. All glassware for culturing experiments was acid-washed overnight in 3 M nitric acid before use.

Metal measurements

After grown to late exponential phase, 20 ml cultures were harvested for metal analysis as described previously (Farhan Ul Haque *et al.* 2015, 2016). Briefly, cells were centrifuged and cell pellets were washed once in 10 ml fresh NMS medium before being re-suspended in 1 ml of fresh NMS medium for storage at –80°C. To analyze metal concentration associated with biomass, samples were thawed at room temperature and 1 ml of 70% (vol/vol) HNO_3 was added to cell suspension and digested for 2 h at 95°C with inversion every 15 min. Digested cell suspensions were diluted with fresh NMS medium and nitric acid to achieve a final concentration of 2% nitric acid and loaded to an inductively coupled plasma mass spectrometry (ICP-MS 7900; Agilent Technologies, Santa Clara, CA) for metal measurements. The ICP standards of copper (Catalog #SC194), praseodymium (Catalog #PPR1KN), neodymium (Catalog #PND1KN-100) and samarium (Catalog #PSM1KN-100) were purchased from Fisher Scientific. The standard of lanthanum was purchased from Sigma Aldrich (Catalog #11523). The 1000 ppm stocks of standards were serially diluted to create standard curves at desired range with correlation coefficient of >0.99. Protein concentrations were converted from cell densities (as OD_{600}) measured by Genesys 20 Visible spectrophotometer (Spectronic Unicam, Waltham, MA). The correlation was obtained using Bradford assay (Bio-Rad Laboratories) as descried previously (Semrau *et al.* 2013). The background levels of copper and lanthanides in the growth medium were measured and found to be: copper = 8.0 ± 0.6 nM, lanthanum = 4 ± 2 nM, praseodymium = 2.3 ± 0.07 nM, neodymium 2.4 ± 0.2 nM, samarium = 2.3 ± 0.07 nM.

Nucleic acid extraction and cDNA synthesis

Actively growing cultures of *M. trichosporium* OB3b wild type under different conditions were harvested at the late exponential phase for Total DNA and RNA extractions. Total DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) from cell pellets of 10 ml culture centrifuged at 5000 × *g* for 10 min, according to manufacturer's instruction. Total RNA was extracted following an already described protocol (Semrau *et al.* 2013). Briefly, cultures (9 ml) were harvested by adding 1 ml of stop solution (5% buffer equilibrated phenol [pH 7.3] in ethanol) and centrifugation at 5000 × *g* for 10 min at 4°C. Cell pellets were re-suspended in 0.75 ml of extraction buffer. The samples were transferred to 2-ml screw caped plastic tubes already containing: 0.5 g of 0.1-mm zirconia-silica beads (Biospec Products), 35 μl of 20% sodium dodecyl sulfate and 35 μl of 20% sarkosyl. The samples were homogenized in a mini-bead beater (1 min at 4800 rpm). Total RNA was recovered from the samples and subjected to DNase treatments as described previously (Semrau *et al.* 2013). The RNA samples were further purified using RNA Clean & Concentrator-5 (Zymo Reseach, Irvine, CA) according to manufacturer's instructions. PCR amplification of the 16S rRNA gene was performed to check for any contamination of DNA and further DNase treatments were performed until RNA was free of any DNA contamination. Quantities of purified RNA were measured using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). RNA samples were stored at –80°C and used for cDNA synthesis within 2 days of purification. First strand cDNAs were synthesized from DNA-free total RNAs (500 ng) using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Quantitative PCR analyses

Quantitative PCR (qPCR) and reverse transcription-quantitative PCR analyses were performed to quantify the expression of various genes in *M. trichosporium* OB3b wild-type cultures grown under different concentrations of copper and REEs. Already designed and tested gene-specific primers (Farhan Ul Haque *et al.* 2015) were used for qPCR amplifications. qPCR reactions were performed in 96-well reaction PCR plates in a volume of 20 μl

Table 1. Rare earth element (REE) uptake by *M. trichosporium* O3b wild type and *mxaF*::Gm^r mutant as a function of the growth concentration of copper and REE. Values presented are mean ± standard deviation of triplicate cultures.

Strain	Growth condition		nmole REE • (mg protein)$^{-1}$	% REE uptake
Wild type	0 μM Cu	0 μM REE	0.2 ± 0.3*	–
		25 μM La	80.9 ± 4.7	96.1 ± 1.4
		25 μM Pr	94.6 ± 10.3	95.6 ± 1.7
		25 μM Nd	94.2 ± 16.3	98.4 ± 0.4
		25 μM Sm	92.3 ± 14.8	93.0 ± 3.6
	10 μM Cu	0 μM REE	0.1 ± 0.021*	–
		25 μM La	60.6 ± 6.5	91.9 ± 0.7
		25 μM Pr	78.3 ± 9.4	94.6 ± 6.4
		25 μM Nd	87.4 ± 5.9	98.3 ± 1.4
		25 μM Sm	84.6 ± 16.1	97.6 ± 0.9
mxaF::Gm^r	0 μM Cu	0 μM REE	NG	–
		25 μM La	76.4 ± 3.8	91.8 ± 0.2
		25 μM Pr	78.0 ± 5.7	89.0 ± 1.4
		25 μM Nd	89.7 ± 9.7	96.5 ± 1.6
		25 μM Sm	NG	–
	10 μM Cu	0 μM REE	NG	–
		25 μM La	51.9 ± 1.4	64.7 ± 0.7
		25 μM Pr	59.4 ± 3.0	81.2 ± 1.0
		25 μM Nd	67.0 ± 9.3	84.8 ± 1.2
		25 μM Sm	NG	–

NG: no growth.
*Measured as the sum of La, Pr, Nd and Sm.

containing 0.8 μl cDNA/DNA, 1 × iTaq Universal SYBR Green Supermix (Bio-Rad), 0.5 μM of each of forward and reverse primers and nuclease-free sterile water (Fisher Scientific, Pittsburgh, PA). CFX Connect Real Time PCR Detection System (Bio-Rad, Hercules, CA) was used to run a three step qPCR program consisting of an initial denaturation at 95°C for 3 min and 40 cycles of denaturation (95°C for 20 s), annealing (58°C for 20 s) and extension (68°C for 30 s). To confirm the specificity, qPCR products were subjected to melting curve analysis with temperature ranging from 65°C to 95°C after the completion of amplification cycles. The threshold amplification cycle (CT) values were then imported from CFX Manager Software (Bio-Rad, Hercules, CA) into Microsoft Excel to quantify the expression of different genes. Calibration curves based on plasmid preparations with known copy numbers for each gene were used to calculate the gene transcripts per ng RNA and copy numbers per ng of DNA. The average ratios of transcript number to copy number determined from the RNA and DNA extracted from the same culture at the same growth point, were represented as a measure of expression levels.

RESULTS

Methylosinus trichosporium OB3b wild type grew in the presence of every tested REE (lanthanum, praseodymium, neodymium and samarium) both in the presence and absence of copper (data not shown). More than 90% of the added REE was found to be associated with biomass (Table 1), indicating that methanotrophs appear to have a mechanism (as yet uncharacterized) for the uptake of these metals. The *mxaF*::Gm^r mutant of *M. trichosporium* OB3b, able only to express Xox-MeDH, also was able to grow in the presence of lanthanum, praseodymium, neodymium, but not samarium (data not shown), and again, most of the added REE was cell-associated (Table 1).

Quantitative PCR was then used to determine if the 'copper-switch' was still operative under these conditions, and if, as found earlier for cerium, expression of *mxaF* and *mxaI* was not affected by the presence of other REEs in the presence of copper. As can be seen in Fig. 1, expression of *pmoA* and *mmoX* in *M. trichosporium* OB3b wild type was not affected by the presence of any REE either in the absence or presence of copper, but the 'copper-switch' was readily apparent, i.e. *pmoA* expression increased in the presence of copper while *mmoX* expression decreased. Expression of *mxaF* and *mxaI*, however, was significantly reduced ($P < 0.05$) in the absence of copper when an REE was also present (Fig. 2). In the presence of copper, expression of *mxaF* and *mxaI* was not affected by the presence of any REE. Finally, expression of *xoxF1* and *xoxF2* increased in the presence of most REEs regardless of the presence or absence of copper; the only exception was expression of *xoxF2* in the presence of samarium (Fig. 3).

DISCUSSION

Here, we extend an earlier finding that cerium serves to regulate expression of alternative methanol dehydrogenases in *M. trichosporium* OB3b (Farhan Ul Haque *et al.* 2015) and show that other REEs, i.e. lanthanum, praseodymium, neodymium and samarium, also induce expression of Xox-MeDH and repress expression of Mxa-MeDH. Further, as found for cerium, such control was only evident in the absence of copper, i.e. the 'copper-switch' appears to be of primary importance in controlling expression of genes involved in the conversion of not only methane to methanol, but also methanol to formaldehyde.

Although samarium did affect expression of *mxaF* and *mxaI* in the absence of copper in *M. trichosporium* OB3b wild type, its impact was comparatively less than that of lanthanum, praseodymium and neodymium (Fig. 2), and samarium also caused the lowest increase in expression of *xoxF1* and *xoxF2* (Fig. 3). Further, the *mxaF*::Gm^r mutant was unable to grow in the presence of samarium. Similar findings have been reported

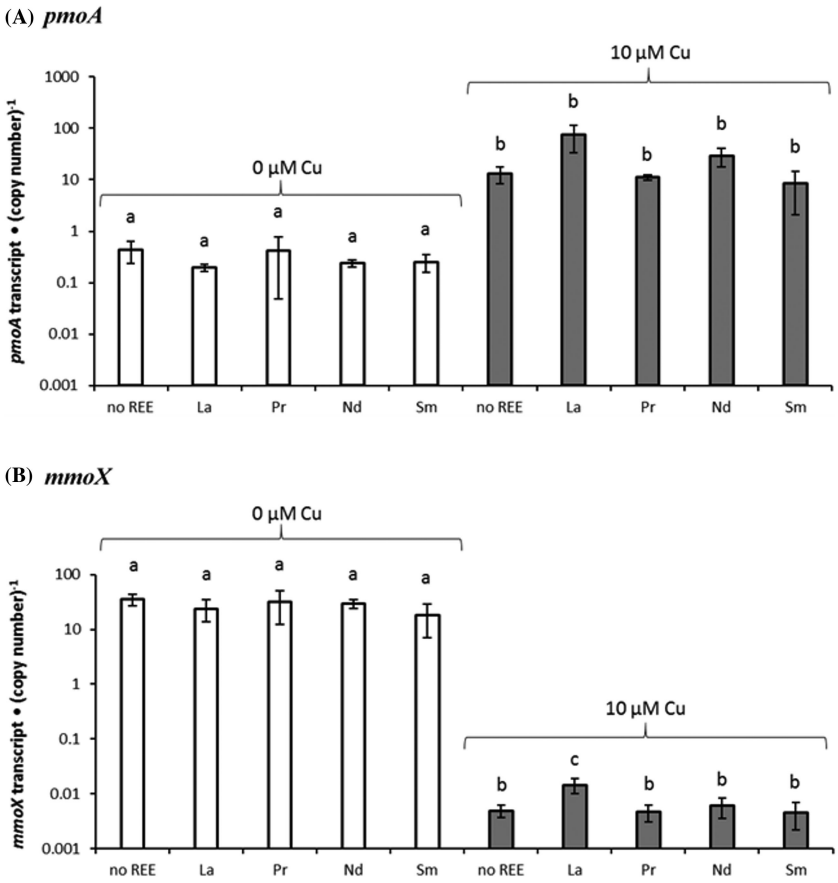

Figure 1. Expression analyses of *pmoA* (**A**) and *mmoX* (**B**) in *M. trichosporium* OB3b wild type grown in the absence of any rare earth element (no REE) or in the presence of 25 μM Lanthanum (La), Praseodymium (Pr), Neodymium (Nd) or Samarium (Sm) with either 0 μM or 10 μM copper. Errors bars represent the standard deviation of triplicate cultures. The values for columns within each plot labeled by different letters are significantly different ($P < 0.05$).

in *M. extorquens* AM1, where samarium was found to have relatively weaker effects on growth in Mxa-MeDH mutant strains (Vu *et al.* 2016). It was suggested that the relatively greater ionic radius of samarium versus other REEs may either limit uptake of samarium and/or insertion into Xox-MeDH. The ionic radius of an element, however, depends on a number of factors, including oxidation state and coordination number, and the ionic radii of the REEs have great overlap (Shannon 1976). Our finding that samarium was effectively taken up by both *M. trichosporium* OB3b wild type and *mxaF*::Gm^r mutant suggests that reduced effect of samarium compared to other REEs is not due to limited bioavailability, but rather a reduced ability to correctly insert samarium into Xox-MeDH, i.e. poor coordination with residues that stabilize its localization within Xox-MeDH as well as ensure its effective coordination with PQQ for electron transfer. It is recommended that Xox-MeDH in more methanotrophs be structurally characterized to better determine the nature of the metal-binding site in Xox-MeDH; to date only the Xox-MeDH from *Methylacidiphilum fumariolicum* SolV, an acidophilic methanotroph of the Verrucomicrobia, has been studied to any great extent (Pol *et al.* 2014).

Although it is now apparent that methanotrophs strongly respond to the presence of REEs, nothing is known as to how these metals are sensed, collected or regulate gene expression. A review of the literature reveals that the question of uptake of REEs is actually poorly understood, likely due to the fact it was initially believed that REEs were nonessential as they had no established biological function (Nakamura, Tagamio and Uchida 2006). Early studies found that cerium uptake varies widely, with some bacteria taking up >80% of added cerium (e.g. *Escherichia coli* and *Aerobacter aerogenes*) while others take up <25% (e.g. *Serratia marcescens* and *Pseudomonas vulgaris*). In fact, uptake can vary widely within specific genera, i.e. *Streptomyces flavovirens* has been shown to take up >75% of added cerium while *S. viridoflavus* collects <25% (Johnson and Kyker 1961). It has been argued that such uptake is largely passive (Johnson and Kyker 1961), but others have found that microbes can actively oxidize cerium (Moffett 1990). This result, coupled with the finding that REEs control the expression and activity of MeDH in methanotrophs, and that most of the added REEs are associated with biomass, however, suggest an uptake mechanism for REEs exists in at least some microbes.

Equally unknown is the basis by which REEs control gene expression in methanotrophs. At this time, expression of only four genes in *M. trichosporium* OB3b has been shown to be affected by the presence of REEs, i.e. *mxaF* and *mxaI* (which are co-expressed on the same polycistronic transcript), and *xoxF1* and *xoxF2* (which have different transcripts). Scanning of the promoter regions of these genes indicates that there is one unique sequence found in all: CGA(T/C)(G/A)TGACC. This sequence has also been found in the promoter regions of over a dozen genes in the genome of *M. trichosporium* OB3b. The majority of these

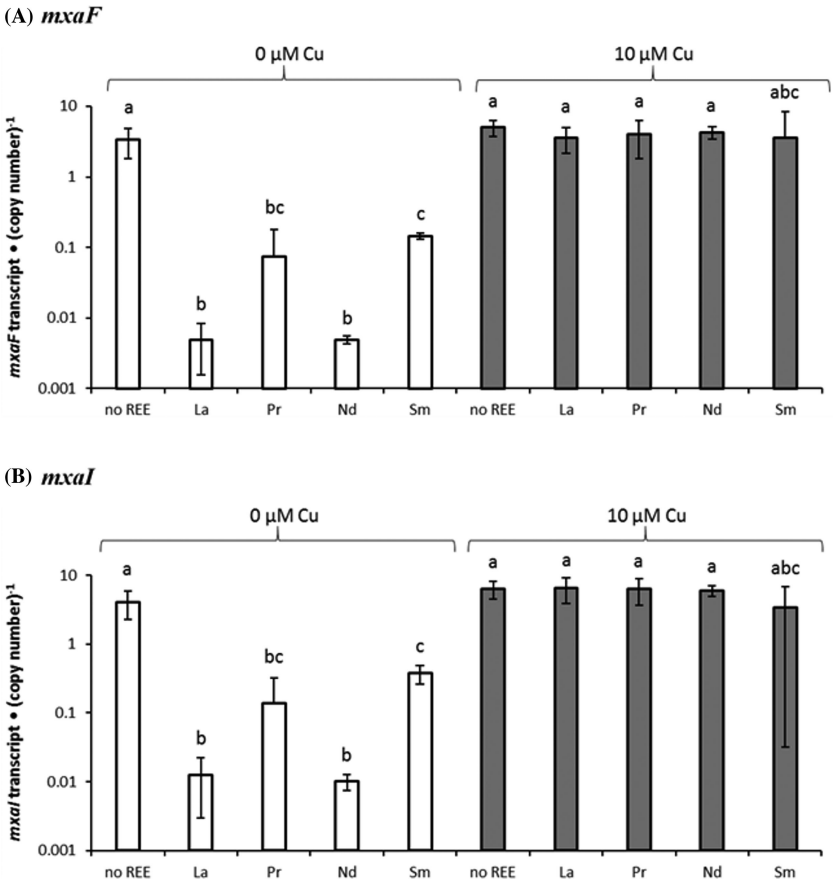

Figure 2. Expression analyses of *mxaF* (**A**) and *mxaI* (**B**) in the *M. trichosporium* OB3b wild type grown in the absence of any rare earth element (no REE) or in the presence of 25 μM Lanthanum (La), Praseodymium (Pr), Neodymium (Nd) or Samarium (Sm) with either 0 μM or 10 μM copper. Errors bars represent the standard deviation of triplicate cultures. Lower error bars are not visible for Pr with 0 μM Cu for both *mxaF* and *mxaI* and Sm with 10 μM Cu for *mxaF* as the standard deviation is larger than the mean. The values for columns within each plot labeled by different letters are significantly different ($P < 0.05$).

encode for proteins of unknown function, but one is annotated as a TonB-dependent transporter (data not shown). It is tempting to speculate that this sequence may serve as binding site for some regulatory protein that differentially regulates expression of genes encoding for Mxa- versus Xox-MeDH (and perhaps other proteins) in response to REEs, and the TonB-dependent transporter may be involved in their uptake.

It should be stressed that although *mxaF* and *mxaI* expression decreased in the presence of REEs, this was only in the absence of copper. In the presence of copper, *mxaF* and *mxaI* expression was not reduced with the concurrent addition of an REE, although *xoxF1* and *xoxF2* expression did increase. These data indicate that the *mxa* operon is controlled by both REEs and copper, but that copper is the primary regulator. Scanning of the promoter regions of genes clearly shown to be upregulated with respect to copper, e.g. *pmoA*, *mxaF* and *mxaI* finds one consensus sequence: TATT(G/T)CA(C/T)GT. Further, examination of the entire genome of *M. trichosporium* OB3b indicates that this sequence is found in the promoter region of many genes encoding for proteins of unknown function. It is tempting to speculate that this sequence is important for the copper regulation of these genes. It is also interesting to note that the *mxa* operon was the only operon found to have both putative copper and cerium regulatory sequences.

In conclusion, we demonstrate that a range of REEs affect the expression of alternative methanol dehydrogenases in *M. trichosporium* OB3b, but such control is only apparent in the absence of copper. Further, growth of the *mxaF*::Gm^r mutant of *M. trichosporium* OB3b was not observed in the presence of samarium, but did occur in the in the presence of lanthanum, praseodymium and neodymium, suggesting that these metals enable Xox-MeDH to become active, but samarium cannot.

FUNDING

The work was supported by the Office of Science (Biological and Environmental Research), US Department of Energy, Grant #DE-SC0006630 to JDS and AAD.

Conflict of interest. None declared.

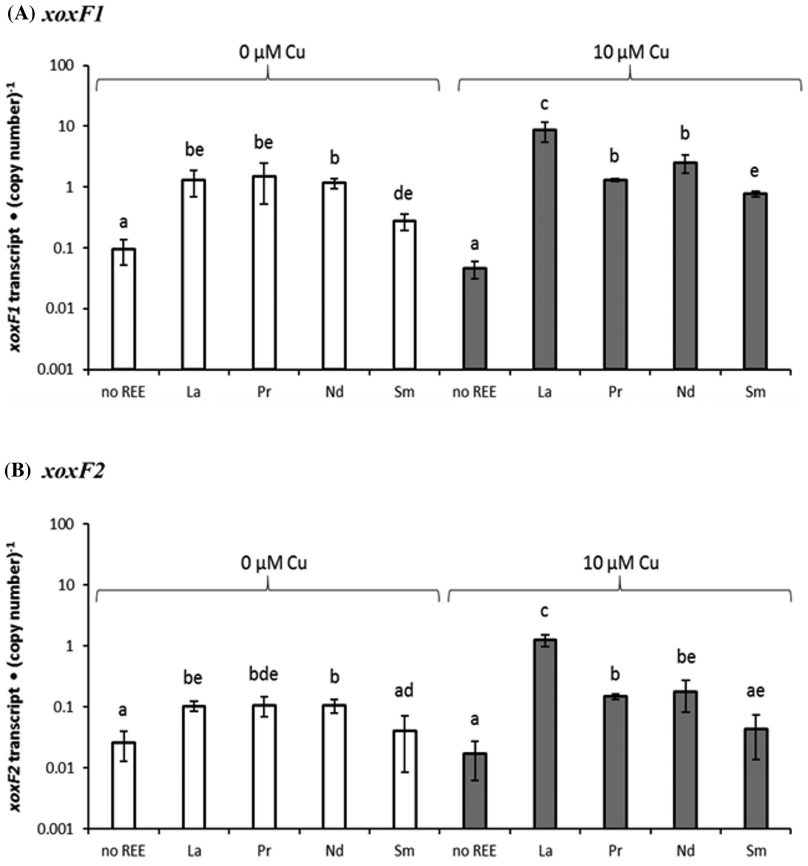

Figure 3. Expression analyses of *xoxF1* (**A**) and *xoxF2* (**B**) in the *M. trichosporium* OB3b wild type grown in the absence of any rare earth element (no REE) or in the presence of 25 μM Lanthanum (La), Praseodymium (Pr), Neodymium (Nd) or Samarium (Sm) with either 0 μM or 10 μM copper. Errors bars represent the standard deviation of triplicate cultures. The values for columns within each plot labeled by different letters are significantly different ($P < 0.05$).

REFERENCES

Chu F, Lidstrom ME. XoxF acts as the predominant methanol dehydrogenase in the type I methanotroph *Methylomicrobium buryatense*. *J Bacteriol* 2016, DOI: 10.1128/JB.00959-15.

Farhan Ul Haque M, Gu W, DiSpirito AA *et al*. Marker Exchange Mutagenesis of *mxaF*, encoding the large subunit of the Mxa methanol dehydrogenase, in *Methylosinus trichosporium* OB3b. *Appl Environ Microb* 2016;**82**:1549–55.

Farhan Ul Haque M, Kalidass B, Bandow N *et al*. Cerium regulates expression of alternative methanol dehydrogenases in *Methylosinus trichosporium* OB3b. *Appl Environ Microb* 2015;**81**:7546–52.

Hibi Y, Asai K, Arafuka H *et al*. Molecular structure of La3+-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. *J Biosci Bioeng* 2011;**111**: 547–9.

Johnson GT, Kyker GC. Fission-product and cerium uptake by bacteria, yeasts, and molds. *J Bacteriol* 1961;**81**: 733–40.

Keltjens JT, Pol A, Reimann J *et al*. PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. *Appl Microbiol Biot* 2014;**98**:6163–83.

Moffett JW. Microbially mediated cerium oxidation in sea water. *Nature* 1990;**345**:421–3.

Nakagawa T, Mitsui R, Tutani A *et al*. A catalytic role of XoxF1 as La^{3+}-dependent methanol dehydrogenase in *Methylobacterium extorquens* strain AM1. *PLoS One* 2012;**7**: e50480.

Nakamura Y, Tagamio K, Uchida S. Effect of nutrient uptake by plant roots on the fate of REEs in soil. *J Alloys Compd* 2006;**408–412**:413–6.

Pol A, Barends TRM, Dietl A *et al*. Rare earth metals are essential for methanotrophic life in volcanic mudpots. *Environ Microbiol* 2014;**16**:255–64.

Semrau JD, DiSpirito AA, Yoon S. Methanotrophs and copper. *FEMS Microbiol Rev* 2010;**34**:496–531.

Semrau JD, Jagadevan S, DiSpirito AA *et al*. Methanobactin and MmoD work in concert to act as the 'copper-switch' in methanotrophs. *Environ Microbiol* 2013;**15**:3077–86.

Shannon RD. Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. *Acta Crystallogr* 1976;**A32**:751–67.

Vu HN, Subuyi GA, Vijayakumar S *et al*. Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. *J Bacteriol* 2016, DOI: 10.1128/JB.00937-15.

Whittenbury R, Phillips KC, Wilkinson JF. Enrichment, isolation and some properties of methane-utilizing bacteria. *J Gen Microbiol* 1970;**61**:205–18.

Williams PA, Coates L, Mohammed F *et al*. The atomic resolution structure of methanol dehydrogenase from *Methylobacterium extorquens*. *Acta Crystallogr* 2005;**D61**:75–9.

Wu ML, Wessels HJCT, Pol A *et al*. XoxF-type methanol dehydrogenase from the anaerobic methanotroph '*Candidatus* Methylomirabilis oxyfera'. *Appl Environ Microb* 2015;**81**: 1442–51.