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A methyltransferase gene from arbuscular mycorrhizal fungi involved in arsenic methylation and volatilization

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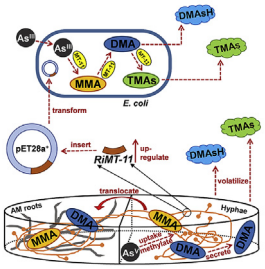
HIGHLIGHTS

- An arsenite methyltransferase gene *RiMT-11* was cloned and expressed in *E. coli* (Δ*ars*).
- As methylation and volatilization was measured in AM symbioses under axenic condition.
- *RiMT-11* conferred *E. coli* arsenite resistance by As methylation and volatilization.
- Methylated and gaseous As was detected from AM symbioses.
- *RiMT-11* in extraradical mycelium was highly induced by arsenate addition.

GRAPHICAL ABSTRACT



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ABSTRACT

Arbuscular mycorrhizal fungi (AMF), ubiquitous symbiotic fungi associated with the majority of terrestrial plants, were demonstrated to play important roles in arsenic (As) translocation and transformation in the plant-soil continuum, and substantially influence plant As tolerance. However, the direct involvement of AMF in As methylation and volatilization and their molecular mechanisms remain unsolved. Here, an arsenite methyltransferase gene *RiMT-11* was identified and characterized from AM fungus *Rhizophagus irregularis*. Heterologous expression of *RiMT-11* enhanced arsenite resistance of *E. coli* (Δ*ars*) through methylating As into monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and ultimately volatile trimethyl arsine (TMAs). In a two-compartment *in vitro* monoxenic cultivation system, methylated and volatile As were also detected from AM symbioses with arsenate addition, accompanied by strong up-regulation of *RiMT-11* expression in extraradical hyphae. The present study provided direct evidence and illustrated an underlying mechanism of As methylation and volatilization by AMF, leading to a deeper insight into the role of AMF in As biogeochemical cycling.

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1. Introduction

Arsenic (As) is a naturally pervasive element and widely used in agriculture, livestock, electronics, industry, metallurgy and medicine (Mandal and Suzuki, 2002). Arsenic contamination, especially in soil and water, has raised worldwide attention due to the excessive anthropogenic release of As to the environment and its severe toxicity to organisms and ecosystems (Moreno-Jimenez et al., 2012; Rodriguez-Lado et al., 2013). Inorganic As, mainly existing in pentavalent arsenate [As(V)] and trivalent arsenite [As(III)], is the predominant forms of As in soils while it can be transformed into various organic forms by the ubiquitous biological methylation. Methylated As, mainly in the forms of pentavalent monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO), is reported to be less toxic than inorganic As(III) and As(V) (Campbell and Nordstrom, 2014). Although some trivalent methylated As even exhibited higher toxicity than inorganic As, they were usually the intermediates in the methylation process, or would rapidly be oxidized to pentavalent forms (Challenger, 1945; Hayakawa et al., 2005). Furthermore, volatile As, such as dimethyl arsine (DMAsH) and trimethyl arsine (TMAs), as the final products in As methylation, plays a crucial role in the global As cycling (Wang et al., 2014a). Hence, As methylation is acknowledged to be an important biological detoxification process (Akter et al., 2005).

As omnipresent soil fungi, arbuscular mycorrhizal fungi (AMF) exist in every continent worldwide and can form intimate symbioses with most of the terrestrial plants (Davison et al., 2015; Barbosa et al., 2017). AMF survive on carbon offered by host plants and they in return provide hosts with water and minerals especially when plants cannot acquire easily, suggesting that they make up a crucial niche in ecosystems (Pearson and Jakobsen, 1993; Li et al., 2013; Jiang et al., 2017). Moreover, AM symbioses were also proved to enhance plant resistance to soil contamination by heavy metals, such as As (Dong et al., 2008), Cr (Wu et al., 2015), Cd (Chen et al., 2004a), Pb (Chen et al., 2005b), Cu (Chen et al., 2007b), Zn (Chen et al., 2004b) and U (Chen et al., 2005a), etc.

Two universal mechanisms well demonstrated for the enhanced heavy metal tolerance by mycorrhizal symbiosis were “growth dilution effect” and “mycorrhizal immobilization”, signifying that AMF could improve plant P nutrition, leading to growth promotion and finally heavy metal dilution in plant tissues, or AMF could immobilize metals within plant root, inhibiting them from transferring to shoots (Chen et al., 2007a; Dong et al., 2008; Wu et al., 2015). As seen in reports, AMF can also colonize plants in natural As-contaminated soils (Sun et al., 2016) and alleviate As phytotoxicity (Zhang et al., 2015b). However, the underlying mechanisms of AMF resisting As contamination still remain scarce. Ultra et al. (2007) detected DMA in the mycorrhizosphere of sunflowers, giving the first evidence of AMF potentially involving in As transformation. Therewith, Yu et al. (2009) found AMF inoculation could increase DMA concentration in maize shoots. Recent studies discovered that DMA was only detectable in shoots of mycorrhizal alfafa (Zhang et al., 2015b) and AMF colonization increased the proportion of organic As in rice grains especially under flooded condition (Li et al., 2016; Zhang et al., 2016). These findings suggested that AMF are highly possible to engage in the As methylation, thus offering a new pathway for these fungi to alleviate As phytotoxicity. Nevertheless, none of these experiments was conducted under sterile condition and the potential effects of soil microbe were not negligible. Many microbes owning the capacity of As methylation, such as *Rhodopseudomonas*, *Lenzites* and *Trichophyton*, also release gaseous As into the atmosphere (Chen et al., 2017a; Qin et al., 2006). Several As methyltransferase genes have been identified and characterized in different kingdoms, including bacteria, archaea, fungi, algae and animals (Lin et al., 2002; Hamdi et al., 2012; Wang et al., 2014b; Zhang et al., 2015a; Guo et al., 2016; Verma et al., 2016). Very recently, Maldonado-Mendoza and Harrison (2018) identified a methyltransferase gene *RiMT-11* from AMF *R. irregularis* and proved that *RiMT-11* was strongly induced by arsenate. However, up to date, contribution of this gene to As transformation in AMF and the direct evidence of AMF involving in As methylation and volatilization remain unsolved, although their methylation ability seems definite.

The present study therefore aimed to provide evidences of As methylation and volatilization by AMF and gain better understanding of the role of AMF in As transformation and As biogeochemical cycling. We chose *Rhizophagus irregularis* DAOM 197198 as a model AMF because of its available genome database and well-demonstrated physiological functions. We cloned the methyltransferase gene *RiMT-11* from *R. irregularis* DAOM 197198, and verified the gene function by heterologous expression in mutant *Escherichia coli*. Besides, a two-compartment root-organ cultivation system was adopted to examine the expression of *RiMT-11* and the capacity of *R. irregularis* to methylate and volatilize As *in situ* under axenic condition.

2. Materials and methods

2.1. Biological materials and cultivations

The AM symbiosis was established and cultivated using the methods described by St-Arnaud et al. (1996). A petri plate with two compartments, named as “root compartment” (RC) and “hyphal compartment” (HC) respectively, was used for monoxenic culture of AM fungus (Fig. S1, sample). *Agrobacterium rhizogenes* (Ri T-DNA)-transformed carrot (*Daucus carota*) roots inoculated with *R. irregularis* were cultured in RC filled with minimal (M) medium solidified by 0.4% (w/v) phytagel (Sigma-Aldrich, Ronkonkoma, New York, USA) (St-Arnaud et al., 1996). AM symbioses were incubated at 25 °C in the dark for 8 weeks till it overspread the full RC. Afterwards, liquid M medium without sucrose was added to HC, followed by a further 4-week incubation till extraradical mycelium crossed the central wall and grew into HC, in which roots were clipped to ensure only hyphae could grow.

Escherichia coli was incubated in LB medium at 37 °C with shaking. Strain JM109 (TAKARA Biotechnology Co. Ltd, Dalian, China) was used for plasmid replication. Strain AW3110 (DE3, *Δars*), of which As-resistant operon, *ars,* was knocked out, and the corresponding wild type strain W3110 were used for the functional verification of *RiMT-11* (Carlin et al., 1995).

2.2. Cloning of the methyltransferase gene and heterologous expression in E. coli

The putative *RiMT-11* gene sequence was obtained from the genome database of *R. irregularis* DAOM 197198 by comparing with other known methyltransferase genes (*arsM*) using Basic Local Alignment Search Tool (BLAST). These *arsM* genes were aligned and constructed a phylogenetic tree using DNAMAN 8.0 (Lynnan Corp, PointeClaire, QC, Canada) and MEGA 7.0.14 (Kumar et al., 2016) by using the Neighbor-Joining (NJ) method respectively. 0.05 g fresh hyphae from HC were harvested for total RNA extraction using TRIZOL (Invitrogen, Grand Island, New York, USA). Complementary DNA (cDNA) was synthesized from the RNA sample using a PrimeScript® RT Reagent Kit (TAKARA Biotechnology Co. Ltd, Dalian, China). The full-length coding sequence containing ATG start codon and TAA stop codon was PCR amplified using high-fidelity KOD-Plus DNA polymerase (TOYOBO Life Science, Osaka, Japan) and the primer pairs, *RiMT*-111F & *RiMT*-111R (Table S1). PCR product was cloned into pGEM-T Easy vector (Promega Corp., Madison,