Biological Function of Lanthanide in Plant-Symbiotic Bacteria: Lanthanide-Dependent Methanol Oxidation System

Viagian Pastawan¹, Nanung Agus Fitriyanto² and Tomoyuki Nakagawa¹,³*

¹ The United Graduate School of Agricultural Science, Gifu University, Tokai National Higher Education and Research System, 1-1 Yanagido, Gifu 501-1193, Japan
² Faculty of Animal Science, Universitas Gadjah Mada, Jl. Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia
³ Faculty of Applied Biological Sciences, Gifu University, Tokai National Higher Education and Research System, 1-1 Yanagido, Gifu 501-1193, Japan

ABSTRACT

In plant-symbiotic bacteria, such as some methylotrophic bacteria and rhizobia, a novel type of pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenase (MDH) was recently identified. This MDH, named XoxF encoded by the xox cluster, requires lanthanide (Ln) as a cofactor. Moreover, there is steady indication that these plant symbiotic bacteria strains possess some Ln-dependent cell functions: the strains are able to recognize Ln species under growth conditions, to uptake Ln species into the cell, and to regulate their Ln-dependent methanol metabolisms based on the particular Ln species present. In this review, we focus on the molecular mechanisms involved in Ln-dependent methanol metabolism and Ln-utilizing systems in the plant-symbiotic bacteria, and discuss the physiological roles of these Ln-dependent systems for the plant-symbiotic bacteria in the phyllosphere and rhizosphere.

Keywords
lanthanide, methanol utilization, methylotrophs, plant symbiosis, rhizobia, XoxF

1. Introduction

Lanthanide (Ln), which are a group of metals with atomic numbers 57 to 71 (Fig. 1), are widely associated with many primary and secondary minerals, such as phosphates, carbonates, fluorides, and silicates, especially pegmatites, granites, and related metamorphic and igneous rocks in natural environments (Tyler and Olsson 2005).

The Ln species have unique chemical and physical properties that set them apart from other elements, and they show relatively similar features each other. For example, each Ln has the similar ionic radius, although the ionic radius of the Ln decreases with increasing atomic number (Aide, 2019). This is called as “Ln Contraction”. The Ln is also defined as elements having partially to filled 4f orbital ground state electronic configurations, resulting from having three electrons removed from their d, s and f orbitals (Aide, 2019). The Ln species exhibit considerable ionic bonding character, and act as Lewis acids in the Ln³⁺ state (Lee, 1992; Aide, 2019). On the other hand, each Ln species also have subtle and distinct differences in electronic properties, i.e., magnetic, electrical or optical properties. From these properties, Ln species are one of the indispensable materials involved in the development of modern electronic applications, and many other high technology industries, and thus have been called as “vitamins for modern industry” (Balaram, 2019).
Ln species are also abundantly distributed in field soils and plants. In the soil, concentrations of Ln species having relatively smaller atomic weight, namely La$^{3+}$, Ce$^{3+}$, Pr$^{3+}$, and Nd$^{3+}$, are generally greater than those of the heavier Ln species (Aide, 2019). In this review, we defined these four elements as light-Ln (L-Ln), and among L-Ln species, Ce$^{3+}$ is the most abundant in the soil, with a concentration of over 60 mg/kg of soil (Aide, 2019). Therefore, all living things are accessible to Ln species, especially, microorganisms inhabiting the soil and cohabiting with plants, as they, along with plants, appear to be constantly affected by L-Ln.

Recently, Ln-dependent cellular responses and metabolic systems have been revealed in several plants and plant-symbiotic and soil microorganisms (Table 1). In particular, since the epochal discovery of Ln-dependent methanol dehydrogenase (MDH) in plant symbiotic bacteria from the *Methylobacterium* and *Bradyrhizobium* groups (Hibi *et al.*, 2011; Fitriyanto *et al.*, 2011a; Nakagawa *et al.*, 2012), the Ln-dependent MDHs and Ln-utilizing systems have been reported in these plant-symbiotic bacteria, along with methanotrophs (Pol *et al.*, 2014; Chu and Lidstrom, 2016; Vekeman *et al.*, 2016; Kato *et al.*, 2020).

In this review, we will focus on the cell functions and Ln-utilizing systems in plant-symbiotic bacteria, with a special emphasis on the molecular mechanisms of dependent methanol metabolisms based on Ln species in the *Methylobacterium* and *Bradyrhizobium* groups, and their physiological roles in plant-symbiotic bacteria in the phyllosphere and rhizosphere.

2. Ln-dependent methanol metabolism in the methylotrophic bacteria

Methylotrophs are microorganisms capable of utilizing C$_1$ compounds, such as methanol, methane, and methylamine, as a sole carbon source for their growth. They are ubiquitous in nature; in fact, some of methylotrophic bacteria are well-known plant epiphytes (Leveau, 2006; Mizuno *et al.*, 2012; Okumura *et al.*, 2016). Among them, the *Methylobacterium* group, such as the genera *Methylobacterium* and *Methylorubrum* (formerly genus *Methylomonas*),...
Methylobacterium [Green and Ardley, 2018]), an aerobic facultative methylotrophic α-proteobacterium, is one of the most abundant bacterial genera in the phyllosphere (Knief et al., 2008; 2010; 2012).

Table 1: List of some Ln-dependent cellular responses and metabolic systems reported in plants, and plant-symbiotic and soil microorganisms

| Organisms             | Phenotypes                                      | References              |
|-----------------------|-------------------------------------------------|-------------------------|
| Plants                |                                                 |                         |
| Maize                 | Growth promotion effects, Increase in antioxidative capacity, Increase in seed germination | d’Aquino et al., 2009  |
| Durum wheat           | Growth promotion effects, Increase in antioxidative capacity, Increase in seed germination | Hong et al., 2017       |
| Rice                  | Increase in seed germination                    | Fashui et al., 2000     |
|                       | Root growth promotion effects                   | Liu et al., 2013        |
| Microorganisms        |                                                 |                         |
| *Streptomyces* spp.   | Overproduction of antibiotics                   | Kawai et al., 2007      |
| *Methylobacterium* spp. | Ln-dependent methanol dehydrogenase              | Hibi et al., 2011       |
|                       | Methanol metabolism                              | Nakagawa et al., 2012   |
| *Bradyrhizobium* spp. | Ln-dependent methanol dehydrogenase              | Fitriyanto et al., 2011a|
|                       | Methanol metabolism                              | Wang et al., 2019       |
|                       | Methanol metabolism                              | Pastawan et al., 2020   |
|                       | Exopolysaccharide production                     | Fitriyanto et al., 2011b|
| *Methylacidiphilum* fumariolicum | Methane metabolism                           | Pol et al., 2014       |
| *Methylomicrobium* spp. | Methane metabolism                          | Fitriyanto et al., 2011a|
| *Pseudomonas* putida   | Glycerol metabolism                             | Pol et al., 2014        |
|                       | Mn-dependent methanol dehydrogenase              | Pastawan et al., 2020   |
|                       | Methane metabolism                              | Pastawan et al., 2020   |
|                       | Exopolysaccharide production                     | Pastawan et al., 2020   |
|                       | Methane metabolism                              | Pastawan et al., 2020   |
|                       | Exopolysaccharide production                     | Pastawan et al., 2020   |

For methanol metabolism of the *Methylobacterium* group, the first step is methanol oxidation catalyzed by pyrroloquinoline quinone (PQQ)-dependent MDH in periplasm (Fig. 2A). The PQQ-dependent MDH, MxaFI encoded by the *mxa* cluster, is widely distributed in strains belonging to the *Methylobacterium* group (Anthony, 2004; Chistoserdova, 2011; Chistoserdova and Lidstrom, 2013) and has been assumed to be the key and essential enzyme in methanol metabolism of the strains because an *mxaF* deficient mutant strain of *Mr. extorquens* AM1 is unable to grow on methanol under laboratory conditions (Nunn and Lidstrom, 1986a; 1986b). Moreover, since MxaFI requires Ca²⁺ as a cofactor (Anthony and Williams, 2003), it was believed that Ca²⁺ was an exclusive essential factor for methanol metabolism by the *Methylobacterium* group.

In 2011, however, Hibi et al. (2011) reported that *Mb. radiotolerans* NBRC 15690 possessed Ln-dependent MDH. Until this finding, there had been no reports on the enzyme requiring Ln species as a cofactor. Moreover, *xoxF*, the gene encoding Ln dependent MDH, was identified on the genomes of *Mr. extorquens* AM1 (Nakagawa et al., 2012), and then the *MxaF*-deficient mutant strain ΔmxaF from strain AM1 was also discovered to possess the ability to grow on methanol with La³⁺ (Nakagawa et al., 2012). These findings indicate that XoxF, rather than MxaFI, may be a main MDH in methanol metabolism for the cells grown in natural environments.

Since these reports, Ln-dependent MDH has garnered significant attention, and many scientists have focused on discovering vital phenomena dependent on Ln species in plant-symbiotic bacteria, the *Methylobacterium* group.

2.1 XoxF: Ln-dependent MDH in the methylotrophic bacteria

As mentioned above, nearly all strains in the *Methylobacterium* group have at least two types of MDHs, such as MxaFI, a Ca²⁺-dependent MDH, and XoxF, an Ln-dependent MDH (Fig. 2A).
In the case of *Mr. extorquens* AM1, MxaFI is encoded on the *mxa* cluster (Fig. 2B) and the gene cluster includes several genes for methanol oxidation; *mxaF* and *mxaI*, encoding subunits α and β of MDH, respectively (Nunn and Lidstrom, 1986a; 1986b); *mxaG*, encoding cytochrome _c_₅, which is electron acceptor for MxaFI (Amaratunga *et al*., 1997); *mxaJ*, encoding a putative chaperone for MxaFI (Featherston *et al*., 2019); *mxaA*, encoding a putative chaperone for Ca²⁺ insertion to MxaF (Morris *et al*., 1995); and many other genes of unknown function (Anderson *et al*., 1990; Toyama *et al*., 1998). On the other hand, XoxF is encoded on the *xox1* cluster (Chistoserdova and Lidstrom 1997). The gene composition of the *xox1* cluster is very simple compared to that of the *mxa* cluster: *xoxF*, encoding Ln-dependent MDH (Nakagawa *et al*., 2012); *xoxG*, encoding cytochrome _c_₅ (Chistoserdova and Lidstrom 1997); and *xoxJ*, encoding a putative chaperone for XoxF (Featherston *et al*., 2019). In the genome of *Mr. extorquens* AM1, there is another gene cluster for PQQ-dependent MDHs, i.e., the *xox2* cluster (Vuilleumier *et al*., 2009), but its function is still unknown.

On the other hand, the homology of the amino acid sequence between MxaF and XoxF in strain AM1 is about 50%, and MxaFI is a hetero tetramer consisting of α- and β-subunits, while XoxF is a dimer of only the α-subunit (Nakagawa *et al*., 2012). MxaFI and the XoxF contain Ca²⁺ and Ln in their active sites, respectively, together with PQQ (Anthony & Williams, 2003; Cotruvo Jr, 2019). In a methanol oxidation reaction catalyzed by MxaF and XoxF, the metal-ligated C5 carbonyl of the PQQ accepts the electron extracted from methanol, and the electron is then transferred to a XoxG (cytochrome _c_₅) (Cotruvo Jr, 2019).

In particular, XoxF is able to utilize only L-Ln species, _i.e._, La³⁺, Ce³⁺, Pr³⁺, and Nd³⁺, as a cofactor, and it chooses La³⁺ preferentially over Nd³⁺ (Wang *et al*., 2020). Moreover, it is known that Ln species as a cofactor affect several enzymatic properties of XoxF, such as the dye-linked MDH activity, thermal stability, and the interaction with XoxG (Featherston *et al*., 2019; Good *et al*., 2019; Wang *et al*., 2020). In short, the preference of XoxF for L-Ln species has a definitive effect on its methanol-oxidizing activity, and L-Ln species existing in growth conditions.
environments affect the methylotrophic growth of strains in the *Methyllobacterium* group (Vu *et al*., 2016; Wang *et al*., 2020).

### 2.2 Ln-uptake system and activation of XoxF in the methylotrophic bacteria

Since XoxFs require Ln species as a cofactor in the periplasm, the strains in the *Methyllobacterium* group must uptake Ln species into the cell (at least into the periplasm). Indeed, the strains, together with methanotrophs, are able to uptake Ln species into the cell (Gu *et al*., 2014; Cotruvo Jr, 2019). In other words, these bacteria strains must possess some Ln-uptake systems.

As shown in Fig. 3, an Ln-uptake system has already been postulated in methylotrophic and methanotrophic bacteria (Cotruvo Jr, 2019; Featherston *et al*., 2019; Groom *et al*., 2019; Ochsner *et al*., 2019; Roszczenko-Jasińska *et al*., 2019; Cotruvo Jr *et al*., 2018). In this system, the cells are believed to uptake Ln species bonded by a lanthanophore, and the Ln species is transferred into the cell using a TonB-dependent system, which is suspected to be similar to siderophore- and chalcophore-mediated iron and copper uptake (Daumann, 2019). The TonB-dependent system for iron and copper comprises specific outer- and inner-membrane transporters, a periplasmic binding protein, and an enzyme for siderophore degradation for iron release (Moeck and Coulton, 1998; Noinaj *et al*., 2010; Kenney and Rosenzweig, 2018). In strain AM1, the TonB-dependent system for Ln species is encoded by the *lut* cluster (Roszczenko-Jasińska *et al*., 2019), the gene cluster is conserved widely in the *Methyllobacterium* group. In the TonB dependent system for Ln species in the *Methyllobacterium* group, LutH is the TonB dependent receptor, and LutE and LutF are the ATPase and membrane components of the ABC transporter, respectively. Moreover, the *lutH* deficient mutant strain could not grow on methanol/Ln, but LutE or LutF is not essential for Ln-dependent methanol growth (Ochsner *et al*., 2019; Roszczenko-Jasińska *et al*., 2019). Since XoxF, which is post translationally transferred from cytosol, needs to be activated in the periplasm (Fig. 3), LutH is needed for transport of Ln into the periplasm from outside of the cell (Ochsner *et al*., 2019; Roszczenko-Jasińska *et al*., 2019).

![Figure 3: Schematic representation of putative Ln-uptake and activation systems for XoxF in *Mr. extorquens* AM1](image_url)

The lanthanophore complexed with Ln is recognized by the outer membrane TonB-dependent receptor (LunH), which is a gated porin with an N-terminal plug domain, and LunH transports the lanthanophore into the periplasm. In the periplasm, Ln is released from the lanthanophore, and Ln is used for activation of XoxF. Active form of XoxF catalyzes methanol oxidation with XoxG, which is cytochrome c<sub>1</sub>, electron acceptor for XoxF. XoxJ is the binding protein with XoxF, and lanmodulin (LanM) is a highly selective Ln-binding protein in the periplasm. On the other hand, Ln is transported into the cytosol by the ABC transporter consisting of LutE, which is the ATPase, and LutF, which is membrane components of the transporter.
In periplasm, it has been speculated that XoxJ interacts with a partially folded apo-XoxF and aids in cofactor insertion in the activation step of XoxF in the periplasm (Fig. 3) (Featherston et al., 2019). Mature XoxF catalyzes methanol oxidation with XoxG, which is cytochrome c₁, electron acceptor for XoxF, in periplasm (Fig. 3). Moreover, lanmodulin (LanM), a highly selective Ln-binding protein, has already been identified (Cotruvo Jr et al., 2018; Cook et al., 2019), but the function of LanM in the activation of XoxF is unknown.

In this manner, molecular mechanisms for the Ln-uptake system and activation of XoxF have gradually been clarified, but many unclear aspects remain.

2.3 Regulation of methanol metabolism by Ln species in the methylotrophic bacteria

Many strains in the Methylobacterium group can recognize the presence of Ln species under growth conditions, and they can select a suitable MDH gene, which must be expressed, according to the presence of the particular Ln (Nakagawa et al., 2012; Vu et al., 2016). In methanol growth without L-Ln species, strain AM1 induces Ca²⁺-dependent MDH, MxaF₁, but under the presence of L-Ln species, the strain dominantly expresses the xoxF₁, instead of the mxaF₁ gene (Nakagawa et al., 2012; Vu et al., 2016). This gene regulation based on L-Ln species is called the “Ln switch,” and the gene regulation is widely observed in many strains in not only the Methylobacterium group but also in methanotrophs (Chu et al., 2016; Masuda et al., 2018; Lv and Tani, 2018; Zang et al., 2018).

To date, many researchers have focused on the Ln switch, and some factors participating in the system have been already identified. As shown in Fig. 4A, it was reported that a two-component system, MxbDM, regulates the expression of the mxa cluster, and another two-component system, MxcQE, also regulates the expression of mxbDM (Springer et al., 1997). The MxcE, MxaB, and MxbM are all required for expression of the mxa cluster but only MxbM is required for the repression of the xox₁ cluster (Morris and Lidstrom, 1992; Springer et al., 1997; Springer et al., 1998; Skovran et al., 2011). On the other hand, XoxF₁ is required for expression of the mxa cluster (Skovran et al., 2011; Masuda et al., 2018). Moreover, Skovran et al. postulate a molecular mechanism for the Ln switch: the apo-XoxF may function as a cellular sensor of Ln in the periplasm, interacting with one or both of the sensor kinases MxcQ and MxbD (Fig. 4A) (Vu et al., 2016; Skovran et al., 2019). In this hypothesis, under growth conditions

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**Figure 4:** Putative Ln switch: regulation mechanism for gene expression of the mxa and xox clusters in Mr. extorquens AM1

(A) In the absence of Ln on growth conditions, apo-XoxF activates expression of the mxa cluster and represses expression of the xox₁ cluster, using two-component systems, MxcQE and MxbDM.

(B) In the presence of Ln on growth conditions, XoxF is active form as MDH in the periplasm, and then the active form does not interact with the two-component systems. Thus, expression of the mxa cluster is repressed and expression of the xox₁ cluster is activated.
without Ln, MxbD and/or MxcQ together with apo-XoxF could signal to the MxbM and/or MxcE response regulators to activate mxa expression and repress xox1 expression (Vu et al., 2016). When Ln is present under the growth conditions, XoxF is activated by Ln, and signaling by these two component systems is canceled, resulting in the repression of the mxa cluster and activation of the xox1 cluster (Fig. 4B) (Vu et al., 2016).

In the case of methanotroph, another regulator for the Ln switch was identified: the histidine kinase MxaY controls the Ln switch in Methylomicrobium buryatense 5GB1C (Chu et al., 2016). There have been no reports on orthologous genes in strain AM1, and Skovran et al. (2019) mentioned that the MxbD in strain AM1 and the MxaY in strain 5GB1C may play analogous roles in the Ln switch as the main Ln sensors, and that the MxaY may directly sense L-Ln species while the MxbD may instead sense apo-XoxF.

In this way, the outline of the Ln switch has gradually become clear, but the detailed mechanism remains largely unknown.

3. Ln-dependent methanol metabolism in genus Bradyrhizobium

It is well known that the XoxF encoding genes are widely distributed not only in methylotrophic bacteria species, but also in non-methylotrophic species including rhizobia (Sudtachat et al., 2009; Keltjens et al., 2014; Chistoserdova and Kalyuzhnaya, 2018). Moreover, XoxFs can be phylogenetically classified into five major clades (XoxF1–XoxF5), and XoxFs from rhizobia are categorized into clade XoxF5 together with XoxFs in Methylobacterium groups (Keltjens et al., 2014; Chistoserdova and Kalyuzhnaya, 2018).

Since Fitriyanto et al. (2011a) reported that Bradyrhizobium sp. Ce-3 (= MAFF211645) grown on methanol with Ce3+ possessed Ln-dependent MDH, the enzymatic properties of XoxFs including the recombinant form have been indicated in genus Bradyrhizobium (Wang et al., 2019; Huang et al., 2019), and the physiological functions of XoxF and the Ln-dependent methanol metabolic pathway, in which XoxF participates, have become one of the targets for the study in the field of C1 microbiology.

3.1 XoxF: Ln-dependent MDH in genus Bradyrhizobium

It had been known that the xoxF-like gene appeared on genomes of strains in genus Bradyrhizobium (Kaneko et al., 2002; Sudtachat et al., 2009), and, naturally, B. diazoefficiens USDA110 (synonym of B. japonicum [Delamuta et al., 2013]) also has the xoxF gene (mxaF); blr6213, which had been a putative ortholog of mxaF of strain AM1 (73.3% amino acid sequence homology), in its genome. However, the strain showed little MDH activity under laboratory conditions (Sudtachat et al., 2009). Therefore, the xoxF gene in the genome of USDA110 had been considered to be one of the unknown functional genes or pseudogenes.

Recently, strains Bradyrhizobium sp. Ce-3 and USDA110 grown on methanol with L-Ln species possessed Ln-dependent MDH (Fitriyanto et al., 2011a; Wang et al., 2019) and showed methanol utilization with supplementation of L-Ln species in the methanol medium (Wang et al., 2019; Pastawan et al., 2020). Both purified Ln-dependent MDHs exhibited sufficient methanol-oxidizing activities and contained L-Ln species as a cofactor in their subunits, similarly to XoxFs from strains in the Methylobacterium group (Fitriyanto et al., 2011a; Wang et al., 2019; Huang et al., 2019). Moreover, in the solution structure of the XoxF from strain USDA110 was analyzed by small angle X-ray scattering (SAXS) analysis, suggesting that XoxF adopts a dimer structure in solution (Wang et al., 2019).

Genes encoding Ln-dependent MDHs in Bradyrhizobium strains were also identified (Fitriyanto et al., 2011a; Wang et al., 2019; Pastawan et al., 2020). In the case of USDA110, Ln-dependent MDHs were encoded by the xoxF gene (blr6213), and XoxF is induced only by methanol with L-Ln species, such as La3+, Ce3+, Pr3+, and Nd3+ (Wang et al., 2019; Pastawan et al., 2020). Moreover, in Bradyrhizobium sp. strain Ce-3, the xoxF gene was also expressed...
only by methanol with L-Ln species (Pastawan et al., 2020). Therefore, strains in genus *Bradyrhizobium* can recognize the presence of methanol with L-Ln, and the strains induce XoxF for some forms of methanol metabolisms in an L-Ln dependent manner.

### 3.2 Ln-dependent methanol metabolism in the rhizobia

In rhizobia, Ln-dependent MDH is encoded by the *xox* cluster (Sudtachat et al., 2009; Pastawan et al., 2020), and the gene cluster was conserved widely in the rhizobia through the clades Ia, Ib and II (Fig. 5). However, the constituent genes on the *xox* cluster in the rhizobia are different from those on the *xoxI* cluster of strains in the *Methylobacterium* group (Fig. 2 and 5); the *xox* cluster in the rhizobia is composed of four genes: *fldA*, encoding glutathione-dependent formaldehyde dehydrogenase, and *gfaA*, encoding S-(hydroxymethyl) glutathione synthase, except for *xoxF* and *xoxG* (Fig. 5). It seems that the difference in gene contents on the *xox* clusters between the rhizobia and the *Methylobacterium* group may be responsible for the difference in physiological functions between both *xox* clusters.

On genomes of the rhizobia, the complete methanol oxidation pathway is encoded by the *xox* cluster, although genes for formate dehydrogenase are located on another loci (Pastawan et al., 2020). However, not all genes for the methanol assimilation pathway were found on the genomes of rhizobia, except for *Mb. nodulans* and some strains, which are methylotrophic rhizobia (Sy et al., 2001; Jourand et al., 2004; Grossi et al., 2020). In other words, nearly all the rhizobia are unable to assimilate methanol as a carbon source, and it seems that the rhizobia may utilize methanol as part of an energy supplying system for some main forms of metabolisms, which has not been identified until now.

![Figure 5: The xox clusters encoding MDH in genus Bradyrhizobium](image)

The percentage values on the genes indicate homology at the deduced amino acid sequences with the homologous genes on the *xox* cluster of strain Ce-3.
4. Conclusion

As described in this paper, Ln-dependent XoxF is widely distributed in plant symbiotic bacteria, such as methylotrophic bacteria inhabiting the phyllosphere and rhizobia inhabiting in the rhizosphere. In the phyllosphere, strains in the *Methylobacterium* group assimilate methanol emitted from leaves and then provide PQQ and some plant hormone-like functional compounds, such as cytokinins and auxins, to their host plants (Fig. 6) (Dourado *et al*., 2015). It was reported that some strains in the *Methylobacterium* group could promote growth of host plants (Abanda-Nkpwatt *et al*., 2006; Tani *et al*., 2015; Grossi *et al*., 2020).

On plant leaves, XoxF is one of the most abundant proteins from plant symbiotic bacteria. This was especially true in the *Arabidopsis* sample, in which XoxF was even detected exclusively and no MxaF was detectable (Delmotte *et al*., 2009). This indicates that strains belonging to the *Methylobacterium* group assimilate methanol emitted from leaves using only the Ln-dependent methanol metabolic pathway, at least on the *Arabidopsis*; namely, it seems that XoxF and Ln are indispensable key factors for plant symbiosis of the *Methylobacterium* group on the phyllosphere (Fig. 6). Up to now, the enzymatic properties of XoxF in vitro have nearly all been clarified, but details of molecular mechanisms for the Ln switch, Ln-uptake system and activation of XoxF have not been completely identified.

In the rhizosphere, genus *Bradyrhizobium* forms nodule on the roots of leguminous plants, and fixes nitrogen into ammonia for the host plant in these nodules (Fig. 6). XoxF is the sole MDH in the genomes of genus *Bradyrhizobium*, with the exception of some strains (Pastawan *et al*., 2020). The Ln-dependent methanol oxidation pathway encoded by the *xox* cluster is induced only by methanol, which is emitted from plant roots (Pastawan *et al*., 2020). Moreover, *Bradyrhizobium* sp. Ce-3 produces a large amount of the rhamnan, exo-polysaccharide (EPS) consisting of L-rhamnose, in the presence of L-Ln (Fitriyanto *et al*., 2011b). Most rhizobia produce a variety of EPSs, and it has been hypothesized that they might play roles in bacterium-plant interactions (González & Marketon, 2003). Therefore, we believe that the Ln-dependent methanol oxidation pathway, Ln-induced EPSs, and the Ln-utilizing system play some important physiological roles in the plant-symbiosis (and nodulation) of the rhizobia (Fig. 6)

![Figure 6: Plant symbiosis of methylotrophic bacteria and rhizobia based on L-Ln species in the phyllosphere and rhizosphere](image-url)
In this manner, it seems that these plant symbiotic bacteria make effective use of L-Ln species in their symbiosis with plants. However, their molecular mechanisms have not been completely identified, and it is expected that research on the Ln-utilization system in plant symbiotic bacteria will reveal the mechanisms involved in the symbiosis of Ln-utilizing bacteria with their host plants.

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