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MINIREVIEW

MINIREVIEW

Interaction between C1-microorganisms and plants: contribution to the global carbon cycle and microbial survival strategies in the phyllosphere

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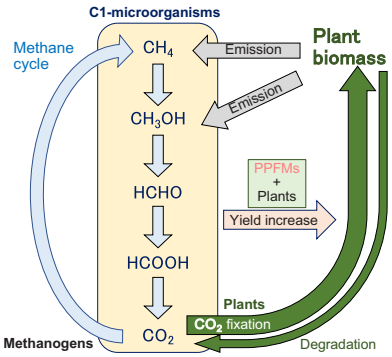
ABSTRACT

C1-microorganisms that can utilize C1-compounds, such as methane and methanol, are ubiquitous in nature, and contribute to drive the global carbon cycle between two major greenhouse gases, CO_2 and methane. Plants emit C1-compounds from their leaves and provide habitats for C1-microorganisms. Among C1-microorganisms, *Methylobacterium* spp., representative of methanol-utilizing methylotrophic bacteria, predominantly colonize the phyllosphere and are known to promote plant growth. This review summarizes the interactions between C1-mircroorganisms and plants that affect not only the fixation of C1-compounds produced by plants but also CO_2 fixation by plants. We also describe our recent understanding of the survival strategy of C1-microorganisms in the phyllosphere and the application of *Methylobacterium* spp. to improve rice crop yield.

Graphical Abstract



C1-microorganisms contribute to drive the global carbon cycle. Positive interactions between C1-microorganisms and plants enhance CO_2 fixation and increase plant biomass.

Keywords: methanol, methylotorph, phyllosphere, *Methylobacterium*, plant growth promotion

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Among compounds that have no carbon-carbon bond, the most oxidized compound CO_2 and the most reduced compound methane are two major greenhouse gases. Methane is the second most abundant greenhouse gas after CO_2, and contributes ~20% to global warming induced by long-lived greenhouse gases since pre-industrial times (Kirschke *et al.* 2013). The global carbon cycling between these two gases is called methane cycle (Figure 1). According to several reports regarding the global methane budget, annual emission and sink of methane are estimated to be 560 Tg and 550 Tg, respectively (Kirschke *et al.* 2013; Saunois *et al.* 2016). Methane is produced by methanogenic archaea in anaerobic environments including those of anthropogenic origins, such as paddy fields. Most of the atmospheric methane (>80%) is oxidized by the hydroxy radical in the troposphere (Guenther 2002). In the methane cycle, the biological oxidation of methane to CO_2 is conducted by a group of microorganisms called C1-microorganisms (methylotrophs) that can utilize reduced C1-compounds, including methane and methanol, as the sole source of carbon and energy. Primary oxidation of methane is performed not only by aerobic methanotrophic bacteria that are methane-utilizing methylotrophs, but also by anaerobic methanotrophic (ANME) archaea (Knief 2019). Methanotrophic bacteria oxidize methane generated in anoxic environments before it reaches the atmosphere as well as the atmospheric methane (Aronson, Allison and Helliker 2013).

C1-microorganisms inhabit various natural environments. Recently much attention has been paid to the above-ground part of plants called phyllosphere as major habitats for C1-microoganisms, since it has been reported that huge amounts of methane and methanol are emitted from living plants (Nemecek-Marshall *et al.* 1995; Fall and Benson 1996; Keppler *et al.* 2006). Among microorganisms living in the phyllosphere, methanol-utilizing methylotrophic bacteria, *Methylobacterium* spp., also known as pink-pigmented facultative methylotrophs (PPFMs), are dominant colonizers and some of them are known to promote the plant growth (Knief *et al.* 2010, 2012; Dourado *et al.* 2015; Kumar *et al.* 2016). While plant-rhizobia and plant-mycorrhizae interactions in the root environments (rhizosphere) have been investigated for a long time, interactions between PPFMs and plants in the phyllosphere have come to be investigated in the last two decades. Furthermore, recently PPFMs are considered to contribute not only to the oxidation process from methane to CO_2 in the global carbon cycle but also to CO_2 fixation by plants (Figure 1).

In this minireview, we summarize the carbon cycle mediated by C1-microorganisms and plants and describe the current understanding of the survival strategies of PPFMs in the phyllosphere. Finally, we also discuss the application of PPFMs to improve rice crop yield.

Emission of C1-compounds from plants and plant colonization of C1-microorganisms

Methanol that originates from methylesters in the plant cell wall constituent pectin is emitted from plant leaves and its annual emission is estimated to be 100 Tg (Fall and Benson 1996; Galbally and Kirstine 2002; Henrot *et al.* 2017). The concentration of methanol emitted from plants to the air phase has been reported to fluctuate depending on the opening and closing of stomata (Nemecek-Marshall *et al.* 1995). But we reported that the concentration of methanol available for microorganisms on the surface of *Arabidopsis thaliana* leaves oscillates during the daily light-dark cycle (Kawaguchi *et al.* 2011). By using a cell-based methanol sensor of the methylotrophic yeast *Candida boidinii* in which the fluorescent protein is expressed under the methanol-induced gene promoter, we showed that the methanol concentration in the phyllosphere was higher in the dark period and lower in the light period, which was opposite to that of atmospheric methanol. We think that methanol, which is accumulated in the spongy parenchyma of the leaf during stomatal closing in the dark period, diffuses to the surface of the leaf.

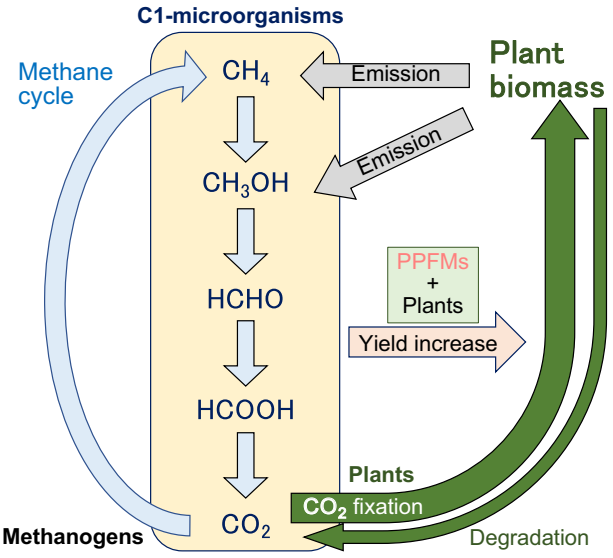

Figure 1. The global carbon cycle mediated by C1-miroorganisms and plants. Methane is generated from CO_2 by methanogens and C1-microorganisms including methanotrophs and methanol-utilizing methylotrophs oxidize methane and other C1 compounds to CO_2. This cycle is known as methane cycle. Recently, C1-microorganisms in the phyllosphere were found to utilize methane and methanol produced by plants. Positive interactions between PPFMs and plants enhance CO_2 fixation and increase plant biomass (yield increase).

The global leaf area is estimated to be twice as large as the surface of the earth and provides habitats for bacterial populations of 10^{26}–10^{27} cells, as well as for lower numbers of archaea and fungi (Lindow and Brandl 2003). In such a huge phyllospheric environment, methanol-utilizing *Methylobacterium* is a major genus among phyllospheric bacteria; eg, this genus has been shown to be the most dominant in both dicots and monocots, such as soybean, clover, and rice (Delmotte *et al.* 2009; Knief *et al.* 2012).

Methane emission from plants was first reported in 2006 (Keppler *et al.* 2006). In addition to the methane emitted via aerenchyma from soil environments, some plants aerobically produce methane, which is assumed to be formed during the synthesis of pectin methyl ester groups along with photosynthesis (Aulakh *et al.* 2000; Bruhn *et al.* 2012). Annual emission of methane from plants is estimated to be 10-69 Tg (Kirschbaum *et al.* 2006; Parsons *et al.* 2006; Butenhoff and Khalil 2007). According to some previous reports, metagenomic and metaproteomic analyses did not detect methanotrophs on leaves of soybean, clover, *A. thaliana,* and so on (Yang *et al.* 2001; Delmotte *et al.* 2009), but other studies detected small populations on leaves of rice, soybean, and so on (Finkel *et al.* 2011; Ikeda *et al.* 2011; Knief *et al.* 2012). Recently we demonstrated that methanotrophs could be cultivated from 12% of the phyllosphere samples (Iguchi *et al.* 2012). Furthermore, we found that both submerged and floating aquatic plants serve as a niche for methanotrophs and that these hydrophytes associated with methanotrophs have higher methane oxidation activity than emergent parts of plants (Yoshida *et al.* 2014; Iguchi *et al.* 2019).

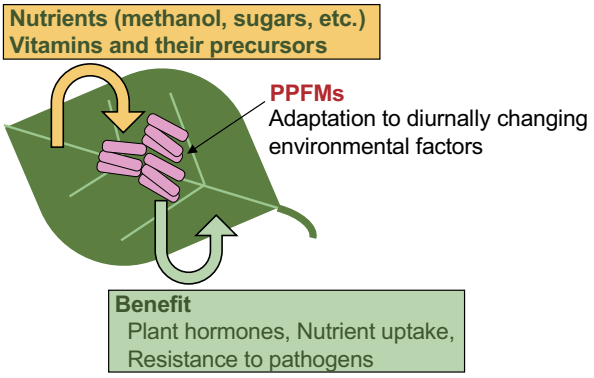

Figure 2. Interactions between PPFMs and plants in the phyllosphere. PPFMs utilize nutrients such as methanol as a carbon source and other cofactors such as vitamins. PPFMs provide benefit to plants by producing plant hormones, enhancing nutrient uptake of plants, and inducing resistance to pathogens. PPFMs have various cellular functions for adapting to diurnally changing environmental factors in the phyllosphere.

Distribution of PPFMs in the phyllosphere and species level specificity between PPFMs and plants

Although a number of PPFM strains have been isolated from plant-related materials, the species-level distribution of PPFMs in the phyllosphere and the species-level specificity between PPFMs and plants have not been well-understood until recently. We investigated the distribution of PPFMs on the leaves of various vegetables, and revealed that the number of PPFMs on the leaves differed among the plants although they were grown at the same farm (ca. 100 m^2) (Mizuno *et al.* 2012). Thus, the plant species affect the population size of PPFMs on leaves. Furthermore, we found that PPFMs were highly abundant on leaves of green perilla [*Perilla frutescens viridis* (Makino) Makino] and red perilla [*Perilla frutescens crispa* (Thunb.) Makino]. The PPFMs isolated from red perilla seeds harvested in different years and those isolated from red perilla leaves planted at four geographically different places in Japan had 16S rRNA sequences identical to that of the representative strain *Methylobacterium* sp. OR01 isolated from red perilla seeds (Mizuno *et al.* 2013). These results indicate the geographically independent species-level specific PPFM-perilla plant associations. We also confirmed the direct transmission of *Methylobacterium* sp. OR01 from red perilla seeds to their leaves by using the antibiotics-resistant strain OR01 (Mizuno *et al.* 2013).

Nutrient sources for PPFMs in the phyllosphere

In the phyllosphere, PPFMs utilize compounds supplied by plants as their nutrient sources for growth and survival (Figure 2). The ability of PPFMs to utilize methanol is thought to be one of the reasons why these are the dominant bacteria colonizing the phyllosphere. In previous studies using the representative model strain *Methylobacterium extorquens* AM1, [which has recently been re-classified as *Methylorubrum extorquens* (Green and Ardley 2018)], mutant strains lacking *mxaF* or *xoxF*, which encode the large subunit of Ca^{2+}-dependent methanol dehydrogenase (MDH) or a lanthanides-dependent MDH, respectively, were shown to be less competitive than the wild-type strain for colonizing plant leaves (Sy *et al.* 2005; Schmidt *et al.* 2010). These results suggest that the ability to utilize methanol as a carbon source is advantageous for PPFMs for growth and survival in the phyllosphere. On the other hand, these mutant strains were still able to colonize plant leaves, indicating that PPFMs utilize other carbon sources besides methanol in the phyllosphere. Indeed, the presence of sugar compounds including glucose on the leaf surface has been reported (Mercier and Lindow 2000).

Some trace cofactors such as vitamins have also been reported to be present on the leaf surface (Gargallo-Garriga *et al.* 2016), and can be utilized by PPFMs in the phyllosphere (Rodionov *et al.* 2009). Recently, a number of PPFMs isolated from living plant samples, including *Methylobacterium* sp. OR01, were found to require B vitamins for their growth on a minimal medium, and most B vitamin-auxotrophic PPFMs required pantothenate (vitamin B5) (Yoshida *et al.* 2019). Further analysis revealed that *Methylobacterium* sp. OR01 could not synthesize β-alanine, which is one of the precursors of pantothenate biosynthesis. β-Alanine and its biosynthetic precursors, spermine, spermidine, 5,6-dihydrouracil, *N*-carbamoyl-β-alanine, and 3-hydroxypropanoate, restored the growth of *Methylobacterium* sp. OR01 in minimal medium. This strain could colonize leaves of *A. thaliana* cultivated on a plant medium without pantothenate or its precursors, and furthermore, pantothenate, β-alanine and several precursor compounds were detected in the slight wash solution of *A. thaliana* leaves. These results suggest that pantothenate-auxotrophic PPFMs colonize the phyllosphere by utilizing not only pantothenate, but also β-alanine and some other precursors produced by the host plants.

When the plant colonization ability between the pantothenate auxotrophic *Methylobacterium* sp. OR01 and *M. extorquens* AM1, which is prototrophic for not only pantothenate but also other B vitamins, were compared, *Methylobacterium* sp. OR01 was found to dominate over the non-auxotrophic strain AM1 on *A. thaliana* leaves (Yoshida *et al.* 2019). The auxotrophic *Methylobacterium* sp. OR01 can save the energy costs of the biosynthesis of pantothenate or β-alanine. Thus, the fitness advantage of the auxotrophic strain increased more than that of the prototrophic strain. This hypothesis is supported by the recent report that half of the bacterial strains isolated from *A. thaliana* leaves had auxotrophic requirements for biotin, niacin, pantothenate, and/or thiamine (Ryback, Bortfeld-Miller and Vorholt 2022).

Survival strategies of PPFMs to adapt to phyllosphere environments

The phyllosphere is thought to be a harsh environment and PPFMs in the phyllosphere are exposed to various kinds of environmental factors, such as diurnal temperature change, UV radiation, drought, osmotic pressure, reactive oxygen species (ROS), and low nutrients. As described above, methanol concentration on the leaf surface oscillates diurnally. Therefore, PPFMs must have some survival strategies to adapt to these diurnally changing environmental factors (Figure 2). One such strategy is to regulate stress-response genes. It was reported that PhyR, which is a general stress response regulator, is involved in plant colonization of *M. extorquens* AM1 and other α-proteobacteria (Gourion, Rossignol and Vorholt 2006; Gourion, Francez-Charlot and Vorholt 2008; Gourion *et al.* 2009). PhyR was first identified as a more abundantly produced protein by the proteome analysis of *M. extorquens* AM1 in the phyllosphere than in the rhizosphere. The *phyR* mutant strain was shown to be deficient in its plant colonization ability and was also shown to increase sensitivity to various stresses, such as heat, UV light, osmolarity, and ROS (Gourion, Rossignol and Vorholt 2006; Gourion, Francez-Charlot and Vorholt 2008). Thus, PhyR appears to be essential for plant colonization and the general stress response system regulated by PhyR might contribute to enhanced fitness in phyllosphere

environments. We also revealed that PhyR was involved in resistance to heat shock and UV light in a methanotroph, *Methylosinus* sp. B4S isolated from a plant leaf (Iguchi *et al.* 2013).

Not only nutrients available for PPFMs, but also environmental conditions such as temperature and sun light diurnally change in the phyllosphere. We have investigated the physiological role of *M. extorquens* AM1 KaiC proteins, which are homologues of the component of circadian clock generator in cyanobacteria (Iguchi *et al.* 2018). KaiC proteins in cyanobacteria have both autokinase and autophosphatase activities and the phosphorylation level of KaiC exhibits an environment-independent oscillation with a 24 h period (Johnson, Mori and Xu 2008; Johnson *et al.* 2017). The Kai protein complex (KaiA, KaiB, and KaiC) regulates global gene expression through downstream regulators such as LabA. *M. extorquens* AM1 has two KaiC homologues, KaiC1 and KaiC2, in which serine residues corresponding to the phosphorylation sites of the cyanobacterial KaiC are conserved. We tested competitive colonization between the wild-type and gene-disrupted strains on *A. thaliana* and revealed that KaiC2 and LabA are necessary for optimal colonization of *M. extorquens* AM1 in the phyllosphere (Iguchi *et al.* 2018). In addition, the phosphorylation-defective mutant KaiC2m was unable to restore the colonization ability of the Δ*kaiC2* strain, indicating that phosphoregulation of KaiC2 is important for colonization on plants.

Methylobacterium extorquens AM1 exhibits temperature-dependent UV resistance (TDR). The survival ratio of the wild-type strain after UV treatment has been shown to increase with increasing growth temperatures (24-32 °C) (Iguchi *et al.* 2018). Further analyses revealed that the TDR phenotype was positively regulated by KaiC1 and negatively regulated by KaiC2. Based on the analyses of KaiC1 and KaiC2 protein levels and their phosphorylation status at different temperatures, we concluded that the amount of KaiC proteins and the phosphorylation state of KaiC2 control the UV resistance pathway in an integrated manner according to the growth temperature, thus allowing cells to adapt to changing environmental conditions.

Positive interaction between plant and PPFMs: improvement of rice crop yield in paddy fields

Some PPFMs are known to enhance seedling growth and total biomass of various plants. Plant growth promotion by PPFMs is thought to be achieved by the following characteristics of PPFMs (Figure 2) (Dourado *et al.* 2015; Yurimoto, Shiraishi and Sakai 2021); (1) they produce phytohormones, such as auxins and cytokinins, and the inhibitor of ethylene biosynthesis (ie, 1-aminocyclopropane-1-carboxylate (ACC) deaminase) (Ortiz-Castro *et al.* 2009). (2) they induce systemic plant resistance against pathogens and diseases (Madhaiyan *et al.* 2004). (3) they also facilitate improvements in uptake of plant nutrients with their involvement in functions such as siderophore production, phosphate solubilization, and N_2 fixation (Kumar *et al.* 2019). There have been scattered reports on improvement on yield by treatment with PPFMs (via seed inoculation or foliar spraying) under laboratory conditions or pot-scale cultivation, particularly for vegetables (Abanda-Nkpwatt *et al.* 2006; Madhaiyan *et al.* 2006; Ryu *et al.* 2006; Meena *et al.* 2012). However, improvement of crop yields by inoculation of PPFMs at the field level has not been well investigated. Recently, foliar spraying of PPFMs was found to improve rice crop yields in a commercial paddy field (Yurimoto *et al.* 2021). The crop yield of the sake-brewing rice cultivar Hakutsurunishiki was improved by foliar spraying of PPFMs in a commercial paddy field for over a 5-year period. Interestingly, foliar spraying of not only living cells but also killed cells or a cell wall polysaccharide fraction gave positive effects on the rice crop yield (Figure 3a). After optimization of the timing of PPFM inoculation, a one-time foliar spray of killed PPFM cells after the heading date was found to be effective in increasing the rate of ripening (Figure 3b) and crop yield (16% increase in the unit yield, Figure 3c). We also observed the greenization of rice seedling leaves by PPFM spraying, possibly due to the increase in plant chlorophyll content leading to an enhancement in photosynthetic activity. The mechanism of how PPFMs affect the rice crop yield after the heading date is still unclear; however, we speculate that a direct interaction of PPFM cell wall components with the plant might stimulate the plant cells to enhance photosynthetic activity during the translocation stage of rice growth (Yurimoto *et al.* 2021).

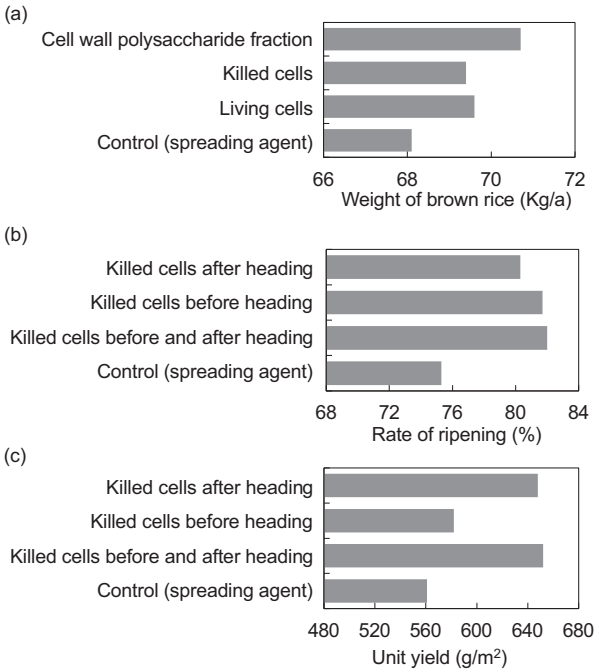

Figure 3. Summary of the effects of PPFM treatment on rice crop yields (cultivar Hakutsurunishiki). Graphs were replotted from the previously reported data (Yurimoto *et al.* 2021). (a) The weight of brown rice in a commercial paddy field in 2017 following the indicated treatments. (b and c) The rate of ripening (b) and the unit yield (c) in 2018 after the indicated treatments.

Conclusion and future perspectives

In this review, we described our recent understanding of the interaction between C1-microorganisms and plants, particularly on the survival strategies of PPFMs in the phyllosphere and improvement of rice crop yield by them. Positive interactions between PPFMs and plants affect the global carbon cycle both through fixation of C1-compounds produced by plants and CO_2 fixation by plant photosynthesis (Figure 1). We still need to better understand the basis of symbiotic interactions between methylotrophs and plants at the molecular level.

The practical use of the positive interactions between PPFMs and plants will lead to development of new technologies both in agriculture and in environmental sustainability. Since PPFM cells can be cultivated at high-cell density with methanol as a carbon source (Schrader *et al.* 2009), which can be derived from methane or renewable biomass, it is easy to prepare large amounts of cells for use in the field. Application of PPFMs to agriculture has

the potential to reduce CO_2 emission. Thus, the prospects of C1-microorganisms playing extremely important roles in the global carbon cycle are high.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abanda-Nkpwatt D, Musch M, Tschiersch J *et al*. Molecular interaction between *Methylobacterium extorquens* and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site. *J Exp Bot* 2006;**57**:4025-32.

Aronson EL, Allison SD, Helliker BR. Environmental impacts on the diversity of methane-cycling microbes and their resultant function. *Frontiers in Microbiology* 2013;**4**:225.

Aulakh MS, Wassmann R, Rennenberg H *et al*. Pattern and amount of aerenchyma relate to variable methane transport capacity of different rice cultivars. *Plant Biol* 2000;**2**:182-94.

Bruhn D, Moller IM, Mikkelsen TN *et al*. Terrestrial plant methane production and emission. *Physiol Plant* 2012;**144**:201-9.

Butenhoff CL, Khalil MAK. Global methane emissions from terrestrial plants. *Environ Sci Technol* 2007;**41**:4032-47.

Delmotte N, Knief C, Chaffron S *et al*. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proc Natl Acad Sci* 2009;**106**:16428-33.

Dourado MN, Camargo Neves AA, Santos DS *et al*. Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic *Methylobacterium* spp. *Biomed Res Int* 2015;**2015**:909016.

Fall R, Benson AA. Leaf methanol - the simplest natural product from plants. *Trends Plant Sci* 1996;**1**:296-301.

Finkel OM, Burch AY, Lindow SE *et al*. Geographical location determines the population structure in phyllosphere microbial communities of a salt-excreting desert tree. *Appl Environ Microbiol* 2011;**77**:7647-55.

Galbally IE, Kirstine W. The production of methanol by flowering plants and the global cycle of methanol. *J Atmos Chem* 2002;**43**:195-229.

Gargallo-Garriga A, Sardans J, Perez-Trujillo M *et al*. Shifts in plant foliar and floral metabolomes in response to the suppression of the associated microbiota. *BMC Plant Biol* 2016;**16**:78.

Gourion B, Francez-Charlot A, Vorholt JA. PhyR is involved in the general stress response of *Methylobacterium extorquens* AM1. *J Bacteriol* 2008;**190**:1027-35.

Gourion B, Rossignol M, Vorholt JA. A proteomic study of *Methylobacterium extorquens* reveals a response regulator essential for epiphytic growth. *Proc Natl Acad Sci* 2006;**103**:13186-91.

Gourion B, Sulser S, Frunzke J *et al*. The PhyR-s^{EcfG} signalling cascade is involved in stress response and symbiotic efficiency in *Bradyrhizobium japonicum*. *Mol Microbiol* 2009;**73**:291-305.

Green PN, Ardley JK. Review of the genus *Methylobacterium* and closely related organisms: a proposal that some*Methylobacterium* species be reclassified into a new genus, *Methylorubrum* gen. nov. *Int J Syst Evol Microbiol* 2018;**68**:2727-48.

Guenther A. The contribution of reactive carbon emissions from vegetation to the carbon balance of terrestrial ecosystems. *Chemosphere* 2002;**49**:837-44.

Henrot AJ, Stanelle T, Schroder S *et al*. Implementation of the MEGAN (v2.1) biogenic emission model in the ECHAM6-HAMMOZ chemistry climate model. *Geosci Model Dev* 2017;**10**:903-26.

Iguchi H, Sato I, Sakakibara M *et al*. Distribution of methanotrophs in the phyllosphere. *Biosci Biotechnol Biochem* 2012;**76**:1580-3.

Iguchi H, Sato I, Yurimoto H *et al*. Stress resistance and C1 metabolism involved in plant colonization of a methanotroph *methylosinus* sp. B4S. *Arch Microbiol* 2013;**195**:717-26.

Iguchi H, Umeda R, Taga H *et al*. Community composition and methane oxidation activity of methanotrophs associated with duckweeds in a fresh water lake. *J Biosci Bioeng* 2019;**128**:450-5.

Iguchi H, Yoshida Y, Fujisawa K *et al*. KaiC family proteins integratively control temperature-dependent UV resistance in *Methylobacterium extorquens* AM1. *Environ Microbiol Rep* 2018;**10**:634-43.

Ikeda S, Anda M, Inaba S *et al*. Autoregulation of nodulation interferes with impacts of nitrogen fertilization levels on the leaf-associated bacterial community in soybeans. *Appl Environ Microbiol* 2011;**77**:1973-80.

Johnson CH, Mori T, Xu Y. A cyanobacterial circadian clockwork. *Curr Biol* 2008;**18**:R816-25.

Johnson CH, Zhao C, Xu Y *et al*. Timing the day: what makes bacterial clocks tick? *Nat Rev Microbiol* 2017;**15**:232-42.

Kawaguchi K, Yurimoto H, Oku M *et al*. Yeast methylotrophy and autophagy in a methanol-oscillating environment on growing *Arabidopsis thaliana* leaves. *PLoS One* 2011;**6**:e25257.

Keppler F, Hamilton JTG, Brass M *et al*. Methane emissions from terrestrial plants under aerobic conditions. *Nature* 2006;**439**:187-91.

Kirschbaum MUF, Bruhn D, Etheridge DM *et al*. A comment on the quantitative significance of aerobic methane release by plants. *Funct Plant Biol* 2006;**33**:521-30.

Kirschke S, Bousquet P, Ciais P *et al*. Three decades of global methane sources and sinks. *Nat Geosci* 2013;**6**:813-23.

Knief C. Diversity of methane cycling microorganisms in soils and their relation to oxygen. *Curr Issues Mol Biol* 2019;**33**:23-56.

Knief C, Delmotte N, Chaffron S *et al*. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *ISME J* 2012;**6**:1378-90.

Knief C, Ramette A, Frances L *et al*. Site and plant species are important determinants of the *Methylobacterium* community composition in the plant phyllosphere. *ISME J* 2010;**4**:719-28.

Kumar M, Tomar RS, Lade H *et al*. Methylotrophic bacteria in sustainable agriculture. *World J Microbiol Biotechnol* 2016;**32**:120.

Kumar M, Kour D, Yadav AN *et al*. Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. *Biologia (Bratisl)* 2019;**74**:287-308.

Lindow SE, Brandl MT. Microbiology of the phyllosphere. *Appl Environ Microbiol* 2003;**69**:1875-83.

Madhaiyan M, Poonguzhali S, Senthilkumar M *et al*. Growth promotion and induction of systemic resistance in rice cultivar

co-47 (*Oryza sativa* L.) by *methylobacterium* spp. *Bot Bull Acad Sinica* 2004;**45**:315-24.

Madhaiyan M, Poonguzhali S, Sundaram SP *et al*. A new insight into foliar applied methanol influencing phylloplane methylotrophic dynamics and growth promotion of cotton (*Gossypium hirsutum* L.) and sugarcane (*Saccharum officinarum* l.). *Environ Exp Bot* 2006;**57**:168-76.

Meena KK, Kumar M, Kalyuzhnaya MG et *al*. Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormone. *Antonie Van Leeuwenhoek* 2012;**101**:777-86.

Mercier J, Lindow SE. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Appl Environ Microbiol* 2000;**66**:369-74.

Mizuno M, Yurimoto H, Iguchi H *et al*. Dominant colonization and inheritance of *Methylobacterium* sp. strain OR01 on perilla plants. *Biosci Biotechnol Biochem* 2013;**77**:1533-8.

Mizuno M, Yurimoto H, Yoshida N *et al*. Distribution of pink-pigmented facultative methylotrophs on leaves of vegetables. *Biosci Biotechnol Biochem* 2012;**76**:578-80.

Nemecek-Marshall M, Macdonald RC, Franzen FJ *et al*. Methanol emission from leaves: enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development. *Plant Physiol* 1995;**108**:1359-68.

Ortiz-Castro R, Contreras-Cornejo HA, Macias-Rodriguez L *et al*. The role of microbial signals in plant growth and development. *Plant Signaling & Behavior* 2009;**4**:701-12.

Parsons AJ, Newton PCD, Clark H *et al*. Scaling methane emissions from vegetation. *Trends Ecol Evol* 2006;**21**:423-4.

Rodionov DA, Hebbeln P, Eudes A *et al*. A novel class of modular transporters for vitamins in prokaryotes. *J Bacteriol* 2009;**191**:42-51.

Ryback B, Bortfeld-Miller M, Vorholt JA. Metabolic adaptation to vitamin auxotrophy by leaf-associated bacteria. *ISME J* 2022;**16**:2712-24.

Ryu J, Madhaiyan M, Poonguzhali S *et al*. Plant growth substances produced by *Methylobacterium* spp. and their effect on tomato (*Lycopersicon esculentum* L.) and red pepper (*Capsicum annuum* L.) growth. *J Microbiol Biotechnol* 2006;**16**:1622-8.

Saunois M, Bousquet P, Poulter B *et al*. The global methane budget 2000-2012. *Earth System Science Data* 2016;**8**:697-751.

Schmidt S, Christen P, Kiefer P *et al*. Functional investigation of methanol dehydrogenase-like protein XoxF in *Methylobacterium extorquens* AM1. *Microbiology* 2010;**156**:2575-86.

Schrader J, Schilling M, Holtmann D *et al*. Methanol-based industrial biotechnology: current status and future perspectives of methylotrophic bacteria. *Trends Biotechnol* 2009;**27**:107-15.

Sy A, Timmers AC, Knief C *et al*. Methylotrophic metabolism is advantageous for Methylobacterium extorquens during colonization of *Medicago truncatula* under competitive conditions. *Appl Environ Microbiol* 2005;**71**:7245-52.

Yang CH, Crowley DE, Borneman J *et al*. Microbial phyllosphere populations are more complex than previously realized. *Proc Natl Acad Sci* 2001;**98**:3889-94.

Yoshida N, Iguchi H, Yurimoto H *et al*. Aquatic plant surface as a niche for methanotrophs. *Front Microbiol* 2014;**5**:30.

Yoshida Y, Iguchi H, Sakai Y *et al*. Pantothenate auxotrophy of *Methylobacterium* spp. isolated from living plants. *Biosci Biotechnol Biochem* 2019;**83**:569-77.

Yurimoto H, Shiraishi K, Sakai Y. Physiology of methylotrophs living in the phyllosphere. *Microorganisms* 2021;**9**:809.

Yurimoto H, Iguchi H, Di Thien DT *et al*. Methanol bioeconomy: promotion of rice crop yield in paddy fields with microbial cells prepared from natural gas-derived C1 compound. *Microb Biotechnol* 2021;**14**:1385-96.