Metagenomics of Methanogenic Communities in Rice Paddy: The Importance of *Methanocella*

12

Yahai Lu

Contents

1 Introduction 312
2 Importance of Root-Derived C in Methane Formation 313
3 *Methanocellales* as the Key Methanogens in Rice Rhizosphere 315
3.1 *Methanocellales* on Rice Roots 315
3.2 *Methanocellales* in Rice Rhizosphere 317
4 Metagenomic Insights into *Methanocellales*' Adaptation to Rice Rhizosphere 318
5 Isolation of *Methanocella* Species into Pure Culture 320
6 Comparative Genomics and Comprehensive Understanding of *Methanocellales* 321
6.1 Phylogeny and Taxonomy 321
6.2 Novel Features of Core Metabolisms for Methanogenesis 323
6.3 Carbon Metabolisms 325
6.4 Nitrogen Metabolisms 326
6.5 Sulfur Metabolisms 327
6.6 Understanding of Oxidative Adaptation 327
7 Conclusive Remarks 330
References 331

Abstract

Methane is a potent greenhouse gas in the atmosphere that has shown nearly tripled increase since the preindustrial era. Paddy fields represent an anthropogenic source contributing about 5% of annual global CH_4 emission. It is important to understand the mechanism of CH_4 production and emission in order to understand carbon cycling and develop mitigation technology for CH_4 emissions. In this chapter, I review the research advances of methanogenesis in association with rice roots with an emphasis on the finding and characterization of

Y. Lu (✉)
College of Urban and Environmental Sciences, Peking University, Beijing, China
e-mail: luyh@pku.edu.cn

A. J. M. Stams, D. Z. Sousa (eds.), *Biogenesis of Hydrocarbons*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-319-78108-2_14

Methanocellales methanogens. The importance of root-derived C as a major C source for CH_4 production, the identification of *Methanocellales* as the key methanogens responsible for CH_4 production in rice rhizosphere, and the genomic insights into the adaptation of the *Methanocellales* methanogens to paddy field environments have been discussed. Mechanistic understanding of *Methanocellales* ecophysiology shall not only shed a light on methanogen evolution and ecology but also pave a way towards the development of biotechnology for control of methane emissions from paddy fields.

1 Introduction

Rice is cultivated on approximately 155 million hectares worldwide, accounting for 14% of total arable land (Haefele et al. 2014). More than half of rice cultivation is under irrigated conditions. As a result, paddy fields are one of major anthropogenic sources for atmospheric methane (CH_4), a potent greenhouse gas that has increased from about 715 ppb in the preindustrial times to 1850 ppb in 2015 (Saunois et al. 2016; Schaefer et al. 2016). The Intergovernmental Panel of Climate Change (2013) reported an annual emission of 33–40 Tg CH_4 from the world paddy fields, equivalent to 12.5% of anthropogenic CH_4, or 5.0% of annual global CH_4 emission (IPCC 2013).

A campaign of CH_4 flux measurements and observations in paddy fields initiated in the late 80s of last century. The consensus of numerous measurements from Europe, America, and across Asia revealed that the seasonal pattern of CH_4 emissions from paddy fields comprises two or three peaks, with the first occurring in a few weeks after flooding and the second or third in the later season. The emission of CH_4 is the result of three processes, namely, production, oxidation, and transportation. The production is processed by methanogens living in anoxic niches of paddy fields. Rice plants have the well-developed aerenchyma system that serves as the major conduit for the transportation of CH_4 into the atmosphere. This aerenchyma system also allows the diffusion of O_2 from the atmosphere to the roots and the surrounding soil, i.e., the rhizosphere. Thereby, the roots of rice plants and the rhizosphere become partially oxic and allow aerobic activity and especially the oxidation of CH_4 that consumes on average a half of CH_4 produced in the anoxic soils before emission (Conrad 2004; Liesack et al. 2000).

In correspondence to the seasonal pattern of CH_4 emissions, the production of CH_4 in paddy fields is considered comprising two phases. In the first phase, the soil organic matter, plant residues, and/or manures deposited from previous season provide the substrates for methanogenesis (Cicerone et al. 1992; Sass et al. 1991; Yagi and Minami 1990). In the later phase, the methanogenic substrates results mainly from the newly-generated plant materials, namely, the root exudates and sloughed-off root cells and debris (Holzapfelpschorn et al. 1986; Lindau et al. 1991). These two phases, often overlapping in reality, are assumed to correspond to the first and the second (and/or third) peak of CH_4 emissions from paddy fields (Kimura 1997; Vandergon and Neue 1995). Obviously, the roots of rice plants play very

important roles in CH_4 emissions from paddy fields. In this chapter, I review the research advances of methanogenesis in association with rice roots with an emphasis on the finding and characterization of *Methanocellales* methanogens. Four aspects will be highlighted in particular: (1) the importance of root-derived C as a major C source for CH_4 production in paddy fields; (2) identification of *Methanocellales* as the key methanogens responsible for CH_4 production from the root-derived C; (3) phylogenetic and genomic characterization of the *Methanocellales* methanogens; and (4) mechanistic understanding of ecophysiology of *Methanocellales* in rice rhizosphere.

2 Importance of Root-Derived C in Methane Formation

Rhizosphere is the critical interface in terrestrial ecosystem. Through this interface, plants take up nutrients from soil and in return release photosynthesized products into the soil, feeding soil microbes. Microorganisms in the rhizosphere are actively involved in biogeochemical cycling of C, N, S, Fe, and many other elements (Arth et al. 1998; Lu et al. 2002; Neubauer et al. 2002; Scheid and Stubner 2001). It has been estimated that 30–60% of the net photosynthesized carbon is allocated to the roots, and 40–90% of this fraction is released into soil in the forms of root exudates, sloughed-off cells, and root debris or rhizodeposition to name together (Lynch and Whipps 1990). The rhizodeposition in paddy soils serves as a major carbon source for CH_4 production. By using a ^{13}C tracer approach, Minoda and Kimura (1994) revealed that part of photosynthesized ^{13}C was transported to the rhizosphere, transformed to CH_4, and emitted to the atmosphere just a few hours after the commencement of $^{13}CO_2$ application (Minoda and Kimura 1994). Dannenberg and Conrad (1999) reported that about 3–6% of the assimilated radioactivity ($^{14}CO_2$) by rice plants were emitted as $^{14}CH_4$ within 16 d after labeling (Dannenberg and Conrad 1999).

The rhizodeposition can be separated into different groups such as water-soluble exudates, secretions, lysates, mucilages, sloughed-off cells, decaying root debris, and gases (Bolton Jr. et al. 1993). It can contain all kinds of chemicals found in a plant cell, from sugars, amino acids, organic acids to more complex components such as proteins, polysaccharides, lipids, hormones, and vitamins. To evaluate the effect of rhizodeposition in methanogenesis, a comparative experiment was conducted using acetate and glucose as controls (Lu et al. 2000c). The effect of root exudates was found to be similar to acetate and glucose. But the addition of acetate and glucose yielded a significant priming effect on the decomposition of soil organic matter leading to a higher CH_4 production, while root exudates caused only a moderate priming effect (Lu et al. 2000c).

To directly evaluate the effect of rhizodeposition on CH_4 production and emission, the experiments under in situ conditions were conducted by observing the spatial variation of dissolved organic carbon (DOC) and dissolved CH_4 in soil porewater along with a distance from rice roots (Lu et al. 2000a, b). These studies revealed that DOC in root zone soil, i.e., the rhizosphere, increased substantially

with plant growth while that in the nonroot zone soil did not show significant change. Since no external organic materials were added, the increase of DOC in the root zone soil reflects the release of organic C from plant roots. The maximal concentration of DOC occurred between rice flowering and maturation, in consistence with the observation that root exudation of rice plants reached maxima during these stages (Lu et al. 1999). Dissolved organic C represents a mobile and labile form of soil organic matter and is expected to be easily degradable.

Correspondingly, the dissolved CH_4 in the root zone soil began to increase at the maximum tillering stage of rice plants and reached to the maxima at the maturing stage (Lu et al. 2000a, b). In the nonroot zone, CH_4 concentrations also increased gradually to the levels comparable with those in the root zone. But a lag period of 1–3 weeks was consistently detected. Higher dissolved CH_4 in the root zone soil compared with nonroot zone soil (Fig. 1) suggests that CH_4 in the root zone soil was produced locally from the decomposition of DOC pool derived from plant photosynthesized C. In correspondence with the concentration of dissolved CH_4, the rate of CH_4 emission increased significantly during the period from rice flowering to

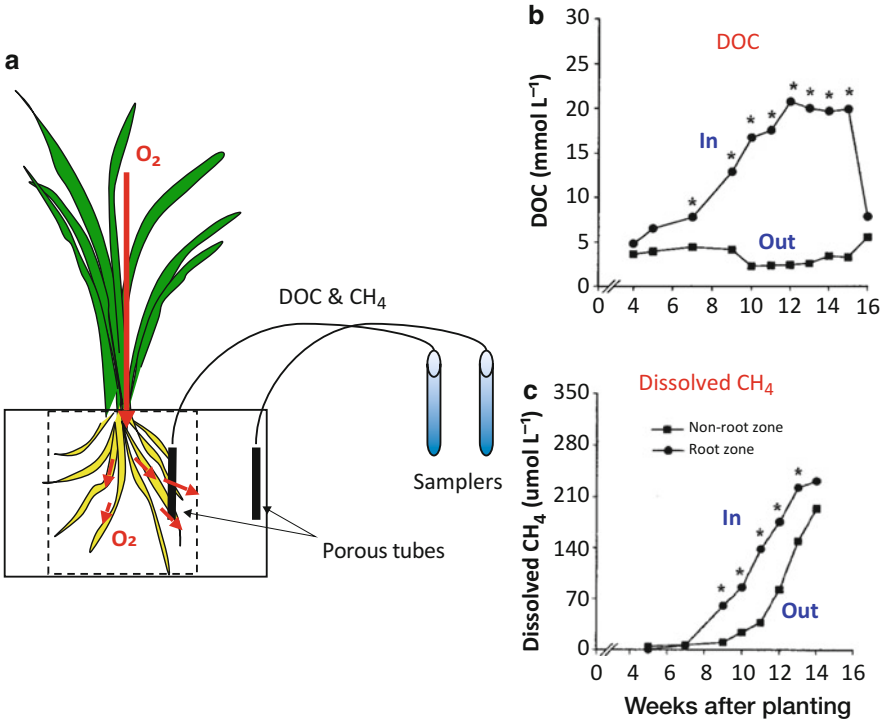

Fig. 1 Rice-plant microcosm for observing the dissolved organic C (DOC and dissolved CH_4 in paddy soil. (**a**) Two sampling ceramic tubes are buried vertically beneath the soil surface with one close to root zone (i.e., rhizosphere) and another outside root zone; (**b**) Seasonal change of DOC in root zone (In) and outside root zone (Out); (**c**) Seasonal change of dissolved CH_4 in root zone (In) and outside root zone (Out). (Taken from Lu et al. 2000b)

maturation. The statistical analyses revealed significant positive linear correlations between the porewater DOC, dissolved CH_4, and the rate of CH_4 emission over the growing season of rice plants (Lu et al. 2000a, b). These results support that the late season peaks of CH_4 emission are due to the supply of plant-borne C through rhizodeposition (Neue et al. 1997).

Collectively, the pioneering studies demonstrated that the DOC pool in the rice rhizosphere was continuously enriched by plant-borne C during plant growth and this DOC pool is easily available for methanogenesis. The rice rhizosphere is probably a very important place for methanogenic activity. This finding however is apparently in conflict with the conventional theory that methanogens are known to be strictly anaerobic while the root surface and the closely connected rhizosphere is partly oxic due to O_2 leaks from the plants. It remains elusive why certain methanogens can survive and even thrive in the rice rhizosphere.

3 *Methanocellales* as the Key Methanogens in Rice Rhizosphere

Two hypotheses were proposed to explain the activity of methanogenesis on rice roots and the rhizosphere: (i) methanogens colonizing rice roots are probably O_2 resistant; (ii) they may develop a spatial strategy, inhabiting where O_2 does not exist, for example, the old root segments where O_2 release is lacking (Conrad 2004; Grosskopf et al. 1998b). Indeed, the community composition and activity of methanogenic archaea in association with rice roots differ greatly from those in the soil distant from roots. Specifically, the CO_2 reduction pathway was found to be prevalent in CH_4 production in rice root preparations (Chin et al. 2004; Conrad and Klose 1999, 2000; Lehmann-Richter et al. 1999), whereas the aceticlastic pathway usually accounted for over 65% of total CH_4 production in the anoxic bulk soil (Conrad 1999; Conrad et al. 2002; Wind et al. 1999). Both environmental detection and enrichment cultivation from the excised rice roots revealed a dominance of an uncultured archaeal linage Rice Cluster I (Grosskopf et al. 1998b; Lehmann-Richter et al. 1999), which was later characterized as hydrogenotrophic methanogens and finally isolated into pure culture as a novel methanogen order *Methanocellales* (Lu and Lu 2012b; Sakai et al. 2008, 2010). The *Methanosaeta* spp. that often dominated in the bulk soil (Chin et al. 1999; Fey and Conrad 2003; Grosskopf et al. 1998a) was rarely detected on rice roots (Chin et al. 2004). These preliminary studies suggest that a very different population of methanogens are selected by rice roots. To identify the active methanogenic organisms responsible for CH_4 production in rice rhizosphere, several experiments using molecular and isotopic labeling approaches were conducted (Lu and Conrad 2005; Lu et al. 2005).

3.1 *Methanocellales* on Rice Roots

In an incubation experiment using the excised rice roots as inoculants, the ^{13}C fully labeled CO_2 was applied with H_2 or N_2 in the headspace of incubation vessels

(Lu et al. 2005). Two pH buffer systems based on carbonate or phosphate were prepared for incubation. The conditions thus created included the combination of buffer system phosphate (P) or carbonate (C) and the headspace composition H_2 or N_2. The $^{13}CH_4$ was detected immediately after the anaerobic incubation of rice roots, indicating the readily activity of methanogens from rice roots. The production of CH_4, however, was faster in C buffer than in P buffer. Strikingly, the rate of CH_4 production was greater with N_2 than with H_2 in the headspace during the initiation period of methanogenesis. An estimate based on ^{13}C labeling under C–N_2 combination indicated that approximately 100% and 65% of CH_4 were produced from CO_2 reduction during the early and late periods, respectively. These estimates were consistent with previous reports showing the prevalence of hydrogenotrophic methanogenesis on rice roots (Conrad and Klose 1999; Conrad et al. 2002; Lehmann-Richter et al. 1999). The higher CH_4 production under C–N_2 compared to C–H_2 combination indicates that the supply of H_2 resulted in a negative effect on CO_2-reducing methanogenesis in the incubations. This was somewhat surprising as H_2 was the energy source for hydrogenotrophic methanogens to reduce CO_2 for CH_4 production.

The analysis of archaeal 16S rRNA gene abundances revealed a significant difference in community composition among different conditions. Under C–N_2 condition, the *Methanocellales*, the yet-uncultured archaeal lineage by the time, showed a significant increase of 16S rRNA gene abundances in the ^{13}C-labeled DNA, indicating that these methanogens were more active than others. Apparently, the *Methanocellales* were responsible for CH_4 production from H_2/CO_2, the dominant pathway of CH_4 production in rice root preparations. The relative abundance of *Methanosarcinaceae* also increased in the late stage, indicating the increasing contribution of acetate-dependent methanogenesis towards the end of incubation (Chin et al. 2004; Conrad et al. 2002). When H_2 was supplied (i.e., under C–H_2), the *Methanosarcinaceae* became exclusively dominated, whereas *Methanocellales* were detected only at low abundance. The high H_2 condition apparently favored the growth of hydrogenotrophic *Methanosarcina* spp. over *Methanocellales*. Under P buffer conditions, the *Methanobacteriaceae* and *Methanosarcinaceae* were selected, while *Methanocellales* were present only marginally.

The *Methanocellales* had been repeatedly detected in different environments including rice roots, anoxic rice soils (Chin et al. 2004; Grosskopf et al. 1998a; b; Lueders and Friedrich 2000), and wetlands (Galand et al. 2002; Jurgens et al. 2000; Sizova et al. 2003). Little had been known however about their physiology. The above DNA-SIP experiment revealed that these methanogens were remarkably suppressed when H_2 was supplied to either P or C buffer systems. A previous enrichment study showed that phosphate was not toxic to *Methanocellales* (Lehmann-Richter et al. 1999). Therefore, application of H_2 appeared the only reason for the depression of *Methanocellales* in root preparations. This finding increased the clouding in understanding methanogenesis associated with rice roots. It was speculated that *Methanocellales* were probably adapted to low H_2 condition and were less selective under the artificially H_2-enriched conditions. It has been reported that the H_2 partial pressure can indeed regulate the expression of genes involved in methanogenesis (Luo et al. 2002) that can vary depending on methanogen identity.

3.2 *Methanocellales* in Rice Rhizosphere

The unculturability of vast microbial species in environments demands culture-independent approaches to understand their activity and functioning. The development of stable isotope probing (SIP) in combination with molecular fingerprinting based on DNA and RNA provided such a powerful approach (Lu and Conrad 2005). This technique has been used to detect methanogens in rice root preparations as described above (Lu et al. 2005). To identify the active methanogens in rice rhizosphere under in situ conditions, RNA-SIP approach was applied to in an intact rice-soil system, in which rice plants were supplied with the ^{13}C-labeled CO_2 for plant photosynthesis and the photosynthesized ^{13}C was tracked for its distribution from the plant top to the rhizosphere and the assimilation by soils microbes.

In this plant-soil microcosm, CH_4 in soil pore water as well as that emitted into the air was found to be rapidly labeled with ^{13}C (Lu and Conrad 2005), suggesting that methanogenesis in the rice rhizosphere was active and closely linked to plant photosynthesis under in situ conditions. The ^{13}C labeled RNA retrieved from rice rhizosphere revealed a signature fingerprint associated with methanogenic archaea (Fig. 2). Specifically, a characteristic terminal restriction fragment (394-bp) was significantly enriched with ^{13}C out of seven fragments belonging to different archaeal lineages (Lu and Conrad 2005). By comparison, no specific signature fingerprint was revealed in the control microcosm without $^{13}CO_2$. Undoubtedly, the methanogenic archaeal lineage characterized by the signature fragment 394 bp

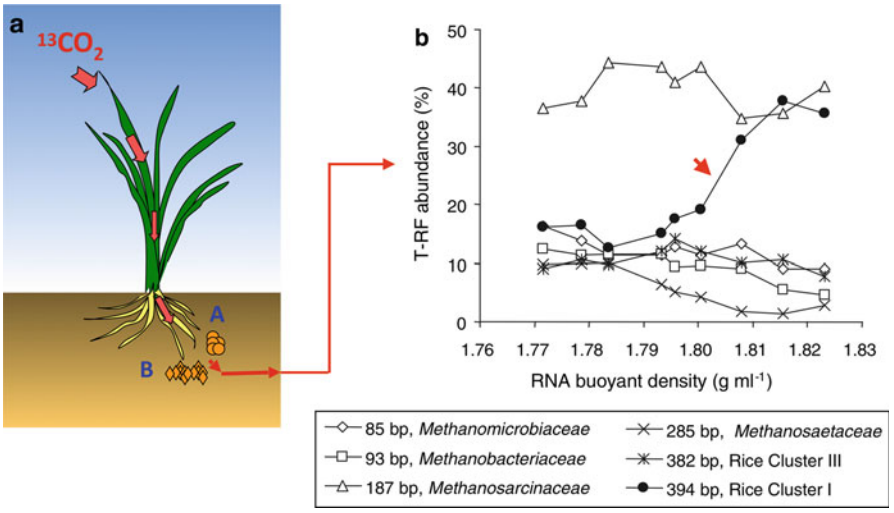

Fig. 2 Rice-plant microcosm for RNA-SIP detection of active methanogens in rice rhizosphere. (**a**) Rice plants were fed with $^{13}CO_2$ in a closed chamber and microbial RNA were extracted from rice rhizosphere for RNA-SIP dissection; (**b**) Fingerprinting of the density resolved RNA revealed that a signature fragment (394 bp), representative of Methanocellales (Rice Cluster I), was 13C labeled. (Taken from Lu and Conrad 2005)

assimilated the ^{13}C derived from organic substances that were deposited into the rhizosphere after photosynthesis. To characterize phylogenetic affiliation of this active methanogen in rice rhizosphere, the 16S RNA clone libraries were constructed, which revealed that out of seven methanogen lineages, the *Methanocellales* methanogens (i.e., uncultured RC-I by that time) was characterized with the signature fragment 394-bp (Lu and Conrad 2005). Thus, the *Methanocellales* were identified as the most active methanogens in rice rhizosphere where the release of organic substrates and O_2 leaks occur simultaneously. These results are in line with earlier studies showing that CH_4 production in excised rice root preparations is mainly due to the activity of *Methanocellales* (Lehmann-Richter et al. 1999; Lu et al. 2005; Lueders et al. 2001). Given the fact that paddy fields are an important source of methane emission (Conrad 2009; IPCC 2013) and plant-photosynthesized carbon provides a major source for CH_4 production in paddy soil (Lu et al. 2000a, b; Minoda and Kimura 1994), the identification of *Methanocellales* as the key player in rice rhizosphere opens a new window for further investigation and deeper understanding of methanogenesis in paddy fields.

4 Metagenomic Insights into *Methanocellales*' Adaptation to Rice Rhizosphere

After the discovery of *Methanocellales* as the key player of CH_4 production in paddy soils, it was highly demanding to elucidate the physiological mechanisms of their activity, particularly in a way associated with rice roots. Due to the nature of difficulty in isolating them into pure cultures, enrichment cultivations were intensively tried in the Max-Planck Institute for Terrestrial Microbiology that finally resulted in an enrichment, named MRE50, in which *Methanocellales* were the only archaeal component (Erkel et al. 2005). This enrichment was then served as a genomic source for constructing fosmid clone library in order to pinpoint the *Methanocellales* metagenome (Erkel et al. 2006). A complete genome sequence of a single *Methanocellales* representative (RC-I_{MRE50}) was reconstructed that offers the path to look into the putative metabolic capacity of *Methanocellales* methanogens.

The RC-I_{MRE50} genome has a size of about 3.18 Mb with 3103 predicted coding sequences. The genome reveals a series of unique features for energy conservation, biosynthesis, C and N metabolisms that are distinct from many known methanogens (Erkel et al. 2006). The central energy metabolism with CH_4 production from CO_2 reduction appears related to the hydrogenotrophic *Methanosarcina*, containing a membrane-bound hydrogenase with cytochrome b, a trait found only in the members of *Methanosarcinales* by the time (Thauer 1998). However, unlike *Methanosarcina* spp., RC-I_{MRE50} also encodes a system of using formate and formaldehyde for methanogenic growth, which is the typical trait of obligately hydrogenotrophic methanogens (Erkel et al. 2006). RC-I_{MRE50} harbors adenosine 5′-monophosphate-forming acetyl-coenzyme A (CoA) synthetase (ACS) for acetate assimilation and the carbon monoxide dehydrogenase complex

for acetyl-CoA biosynthesis from CO_2 that are common to most obligately hydrogenotrophic methanogens. But RC-I_{MRE50} additionally encodes a membrane-bound pyrophosphatase that can help these methanogens to recover a portion of the energy invested in acetate activation, which is not available in other methanogens that use ACS for acetate assimilation.

The pyruvate metabolism encoded in RC-I_{MRE50} includes ethanol production from acetaldehyde, acetoin production from acetolactate, and two pathways for acetyl-CoA formation from pyruvate. Most anaerobes including methanogens use the pyruvate-ferredoxin oxidoreductase that is oxygen-sensitive for the decarboxylation of pyruvate and acetyl-CoA production. By comparison, aerobes usually use the pyruvate dehydrogenase (PDH) for similar function. Interestingly, the RC-I_{MRE50} genome encodes both pathways. The PDH complex has been typically found in aerobic and facultatively anaerobic microorganisms but is lacking in all known methanogens by the time. It was therefore speculated that RC-I_{MRE50} likely uses the glycolytic pathway to survive the oxic periods (Erkel et al. 2006). Energy for maintenance may result from pyruvate and acetate production. Reducing equivalents generated from glucose and pyruvate oxidation can be recycled through the fermentation of pyruvate to ethanol. The allosteric control of the glycolytic pathway may allow RC-I_{MRE50} to respond quickly to the environmental changes in redox states.

The RC-I_{MRE50} genome appeared to contain biosynthetic pathways for all amino acids except glutamate (Erkel et al. 2006). But the glutamate synthesis was later found to be present in the genome analysis of *Methanocella* pure cultures (see details below). Nevertheless, RC-I_{MRE50} encodes a candidate ABC-type glutamate import system. The ability of RC-I_{MRE50} to take up glutamate from environments and to incorporate it into enzyme synthesis was experimentally confirmed (Erkel et al. 2006). This feature might confer an advantage for *Methanocellales* to live near rice roots as glutamate may be available in root exudates and/or decomposing plant root materials. Besides the glutamate uptake, RC-I_{MRE50} genome reveals two additional mechanisms for nitrogen acquisition via ammonium assimilation and dinitrogen fixation (nitrogenase). These combined traits indicate the metabolic flexibility of RC-I_{MRE50} in nitrogen acquisition. In addition, RC-I_{MRE50} also reveals an unique sulfur assimilation through the reduction of sulfate to sulfide. It contains genes coding for sulfurylase and adenylylsulfate kinase that are lacking in all methanogen genomes sequenced by the time. Most methanogens depend on sulfite, sulfide, or sulfur-containing amino acids as sulfur source for assimilation. The ability of RC-I_{MRE50} to use sulfate may confer *Methanocellales* another advantage to adapt the rhizospheric environment, where sulfate instead of the reduced sulfur forms may be available due to oxic conditions.

Since O_2 is diffused from the top of rice plants down to roots and released into the rhizosphere, the transient anoxic/oxic conditions prevail on root surface and in the rhizosphere soil. In addition, paddy fields often experience wet-dry cycling due to field management requirement (Liu et al. 2015). The key for methanogens to inhabit rice rhizosphere is therefore dependent on the capacity of resisting oxidative stresses. Aerotolerant systems were previously found in the aceticlastic *Methanosarcina* spp. The obligately hydrogenotrophic methanogens however acquire only a limited set of

antioxidant enzymes. Strikingly, the RC-I_{MRE50} genome encodes multiple sets of genes coding for antioxidant enzymes, including the mono-functional large subunit heme catalase that is most ancient and robust of all known catalases (Chelikani et al. 2004). Three different reactive oxygen species (ROS) scavengers are present that can be used to remove both external and internal superoxide anions. In particular, the exogenous superoxide anions can be scavenged by a periplasmic Cu, Zn-dependent superoxide dismutase (SodC) (Fournier et al. 2003), while the cytoplasmic superoxide anions be removed by two types of superoxide reductase (SOR) containing rubredoxin and desulfoferrodoxin, respectively. SORs are considered the most important oxygen defense systems in anaerobes (Jenney et al. 1999), especially under strong oxygen exposure (Fournier et al. 2003). In addition, the RC-I_{MRE50} also encodes bacterial-type enzymatic systems with repair mechanisms for oxidative lesions of DNA, such as formamidopyrimidine-DNA glycosylase (MutM), 3-methyladenine-DNA glycosylase (MPG), and the Holliday junction resolvasome (RuvABC) (Erkel et al. 2006). Possessing these multiple antioxidant and repair systems confers *Methanocellales* the extraordinary ability to be aerotolerant. Thus, *Methanocellales* are genetically equipped with competitive advantages over obligately hydrogenotrophic methanogens in the rice rhizosphere. Together with the potentials of acquiring alternate sulfur and nitrogen nutritions, *Methanocellales* appear to have evolved the methanogenic life well-fitting to the rice rhizosphere.

5 Isolation of *Methanocella* Species into Pure Culture

Despite the metagenomic insights into their adaptation to rice rhizosphere and more generally to oxic conditions, deeper understanding of their physiology and ecology is impossible without isolation of *Methanocellales* into pure culture. The efforts to isolate them therefore have never been stopped though the difficulty. The first pure culture of *Methanocella* were obtained from a Japanese rice field soil using a syntrophic cultivation approach. The formal order name, *Methanocellales*, was then given based on the phylogeny of this pure culture, and the strain itself was named as *Methanocella paludicola* strain SANAE^T (Sakai et al. 2007, 2008). The second isolate, a thermophilic methanogen, *Methanocella arvoryzae* strain MRE50^T, was later purified from the enrichment established for the metagenomic investigation (Lueders et al. 2001; Sakai et al. 2010). The isolation of these two strains would have offered a chance to address many ecophysiology questions. Unfortunately, despite the successful isolation of strains SANAE^T and MRE50^T, the maintenance and cultivation of these strains in lab require some extraordinary techniques, which impede the further investigations. Therefore, more isolates particularly with the fast-growing trait are still needed. Such a strain, *Methanocella conradii* strain HZ254^T, named after Ralf Conrad, a pioneering scientist on this methanogen lineage, was finally obtained from a Chinese paddy field soil (Lu and Lu 2012b). A moderate high temperature has been an effective strategy to isolate this strain, in line with early enrichment studies (Fey et al. 2001; Peng et al. 2008).

The third strain was phylogenetically closer to *M. paludicola* SANAE^T (16S rRNA gene similarity of 95.0% and mcrA gene similarity of 87.5%) than to *M. arvoryzae* MRE50^T (92.4% and 86.5% for the 16S rRNA and mcrA similarity, respectively) (Fig. 3) (Lu and Lu 2012b). Though three strains share some common phenotypic features, such as the rod-shaped morphology, they differ in formate utilization, flagellum formation, temperature optimum, pH range, and salinity susceptibility. In contrast to the phylogenetic relationship, strain HZ254^T seems to be closer to MRE50^T than SANAE^T in major phenotypic traits including temperature optimum, flagellum formation, and salinity susceptibility. The 16S rRNA gene sequence divergence of 5% between HZ254^T and SANAE^T implies that strain HZ254^T potentially represents a new genus instead of new species (Lu and Lu 2012b).

6 Comparative Genomics and Comprehensive Understanding of *Methanocellales*

6.1 Phylogeny and Taxonomy

Thus far three *Methanocella* strains have been available, namely *M. paludicola* SANAE^T, *M. arvoryzae* MRE50^T and *M. conradii* HZ254^T (Lu and Lu 2012b; Sakai et al. 2008, 2010). Though they have been classified together as a genus *Methanocella*, they could potentially represent multiple genera and even families due to low similarities of their 16S rRNA and mcrA genes (Lu and Lu 2012b; Sakai et al. 2010). In consistence with the analyses of 16S rRNA and mcrA, the global nucleotide identities calculated based on whole genome alignments suggest that *M. conradii* and *M. paludicola* are more closely related each other than to *M. arvoryzae*. The phylogenetic trees constructed based on multiple markers (i.e., 16S rRNA, mcrA and ribosomal proteins) also placed *M. conradii* closer to *M. paludicola* than to *M. arvoryzae* (Borrel et al. 2013; Lu and Lu 2012b). The Average Amino Identity (AAI) that can be more relevant to infer genetic relationship at high taxonomic levels indicated that *M. conradii* and *M. paludicola* together represent a genus, while *M. arvoryzae* alone represents a separate genus (Lyu and Lu 2015), according to the consensus criterion of AAI (Konstantinidis and Tiedje 2007).

Before the proposal of order *Methanocellales*, methanogens had been classified into five characterized orders, i.e., *Methanopyrales*, *Methanococcales*, *Methanobacteriales*, *Methanomicrobiales,* and *Methanosarcinales* (Liu and Whitman 2008). Comparative genomic analyses have grouped these orders into Class I (consisting of *Methanopyrales*, *Methanococcales* and *Methanobacteriales*), Class II (the *Methanomicrobiales*), and Class III (the *Methanosarcinales*) methanogens, respectively (Anderson et al. 2009). Phylogenetically, the *Methanocellales* can be placed between Class II and III methanogens (Lu and Lu 2012b; Sakai et al. 2008, 2010). Although the physiological relationships remain unclear, *Methanocellales* do share some ecological features with either Class II or III. For instances, both *Methanocellales* and Class II methanogens are detected in rice soils and wetlands where H_2 partial pressure is low (1–10 pa), whereas *Methanocellales* also share

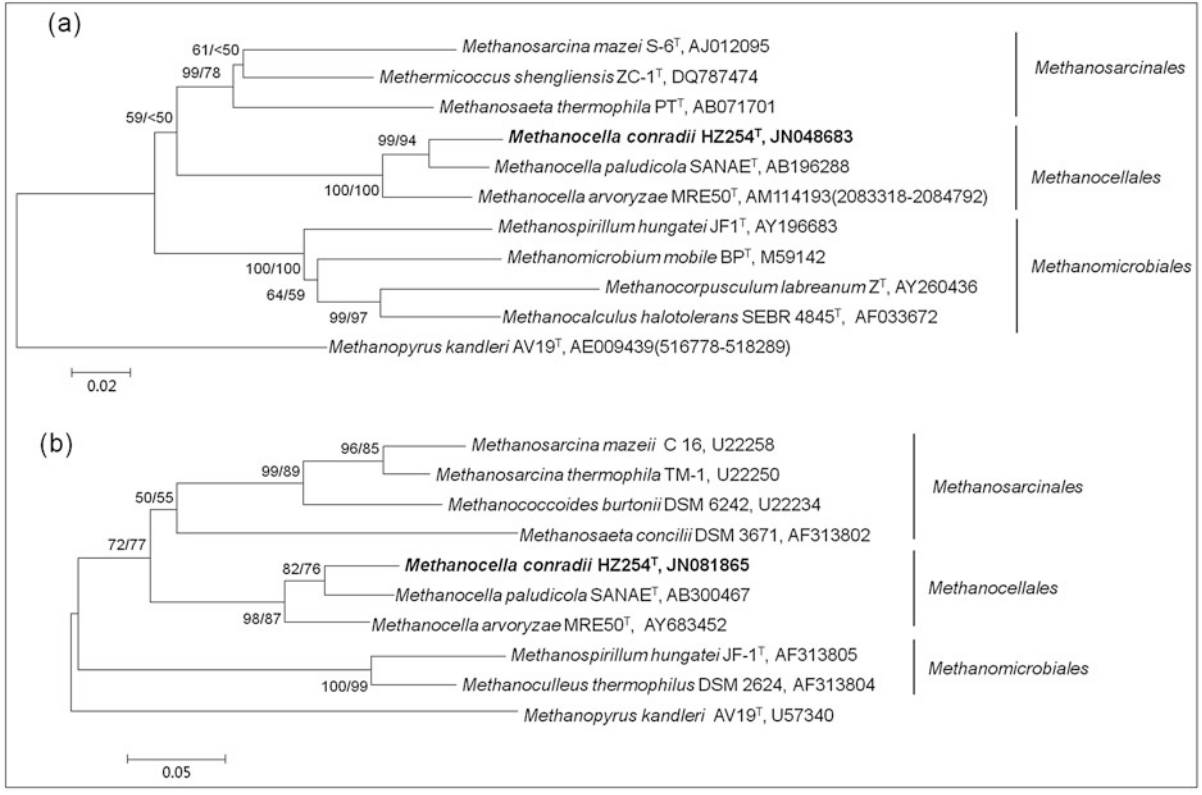

Fig. 3 Phylogeny of Methanocellales based on (**a**) 16 S rRNA genes and (**b**) Deduced methyl-coenzyme reductase subunit A amino acid sequence (McrA). (Taken from Lu and Lu 2012b)

common habitats with Class III methanogens, such as upland soils where aeration and desiccation occur periodically (Angel et al. 2012; Angel et al. 2011; Aschenbach et al. 2013; Conrad et al. 2006).

Genome sequences of three *Methanocella* strains and their comparative analysis offer an opportunity to elucidate the basic ecophysiology traits of this novel type of methanogens. A detailed reannotation of SANAE^T and MRE50^T was performed using the same annotation pipeline used for the third strain HZ254^T to ensure the consistency in comparison (Lyu and Lu 2015). The reannotation of SANAE^T and MRE50^T genomes revealed several new genes, pseudogenes, and some CRISPR region(s) that were not identified previously. Analyses of COG, Pfam, and TIGRfam classifications also revealed more functional insights into many genes not assigned before. Whole genome alignments revealed the extensive rearrangements of genomic regions among three strains. Three *Methanocella* strains share a core genome comprised of 1187–1245 ortholog groups, depending on the threshold set for amino acid identity (Lyu and Lu 2015). More orthologs are shared between *M. conradii* and *M. paludicola* than to *M. arvoryzae*, consistent with the phylogenetic relationship among them.

6.2 Novel Features of Core Metabolisms for Methanogenesis

All three strains possess a complete gene set for the typical hydrogenotrophic methanogenesis characterized as the closed Wolfe cycle (Thauer 2012). The major differences among three genomes are the copy numbers of several genes in the pathway, specifically the genes coding for the B subunit of F420-reducing hydrogenase (*frhB*), the D subunit of F420-nonreducing hydrogenase (*mvhD*), and the E subunit of energy-converting hydrogenase (*echE*). The ecological insights into these differences have yet to be evaluated. Two novel features, however, were identified that are shared by all three strains (Lyu and Lu 2015). The first is the gene organization related to the Wolfe cycle and the second is the presence of a putative [NiFe] hydrogenase complex that was not found in other methanogens.

Hydrogenotrophic methanogens are known to employ a multienzyme complex to perform the flavin-based electron bifurcation for the energy conservation from oxidation of H_2 or formate (Costa et al. 2010; Lie et al. 2012). This complex consists of formylmethanofuran dehydrogenase (Fwd), heterodisulfide reductase (Hdr), and Mvh (Fwd/Mvh/Hdr in short). The formate dehydrogenase (Fdh) may also join with the formation of the Fwd/Mvh/Fdh/Hdr supercomplex. In Class I methanogens, though the formation and functioning of Fwd/Mvh(Fdh)/Hdr multienzyme complex, the genes coding for these components are located separately in their genomes (Hendrickson et al. 2004; Kaster et al. 2011; Thauer et al. 2010). In contrast, *Methanocella* as well as many of Class II methanogens organize most of those genes into large gene clusters. A 10-gene cluster consisting of whole sets of *fwd* and *hdr* genes and a gene for the subunit D of Mvh (*mvhD*) was identified in all three *Methanocella* stains (Mtc_2477–2468, MCPlv_2811–2802, and MRE50lv_2189–2180) (Lyu and Lu 2015). There exists even a second larger gene cluster comprising the above 10 genes

together with two *fdh* genes in *M. arvoryzae* and *M. paludicola* (MCPlv_1593–1604 and MRE50lv_0274–0285). This unique organization of large gene cluster may facilitate the assembly of multienzyme complex with less biological cost and preventing the transcriptional resource waste (Anderson et al. 2009; Lie et al. 2012). The inclusion of *fdh* in the gene cluster may allow *M. arvoryzae* and *M. paludicola* to grow on formate as the sole carbon and energy source, whereas *M. conradii* is not known to have this ability (Lu and Lu 2012b).

Methanocella seem to be exceptionally adapted at low H_2 concentrations. This feature is initially illustrated in root preparation experiment (Lu et al. 2005). The isolation of the strain *M. paludicola* SANAE^T by using the syntrophic coculture technique confirmed that low H_2 condition favors *Methanocella* over other hydrogenotrophic methanogens (Sakai et al. 2007). More evidences are illustrated with the detection of *Methanocellales* in association with different bacteria syntrophs that syntrophically oxidize short-chain fatty acids in paddy soils (Gan et al. 2012; Liu et al. 2011; Lueders et al. 2004; Rui et al. 2011). Therefore, though the Class I methanogens use the similar Wolfe cycle and perform the flavin-based electron bifurcation for the core metabolisms, *Methanocellales* appear to possess a specific capacity to perform these functions at H_2 level close to the thermodynamic limit. The reason for this unique feature is possibly related to the presence of the large gene cluster coding for Fwd/Mvh/Hdr complex, which can confer a better efficiency in energy conservation through facilitating the assembly of multienzyme complex for electron bifurcation. Gene clustering is considered a common strategy used by prokaryotes to increase efficiency in forming protein complexes (Sneppen et al. 2010). A global transcriptional analysis for HZ254^T indeed illustrated the elevated expression of this gene cluster under limited H_2 condition in syntrophic coculture compared with high H_2 in monoculture (Liu et al. 2014).

The second unique feature of *Methanocella* genomes is the presence of a putative [NiFe] hydrogenase complex. The coding genes for this complex are organized into a 8-gene cluster (Mtc_0479–0486, MRE50lv_2279–2272 and MCPlv_2682–2674) including *echE* (energy-converting hydrogenase subunit E) and *hdrB* homologs (heterodisulfide reductase subunit B) (Lyu and Lu 2015). The *EchE* homologs possess the [NiFe] binding motifs and are phylogenetically more closely related to the bacterial Coo hydrogenase (carbon monoxide-induced hydrogenase) in the sulfate-reducing bacteria than to the canonical Ech hydrogenase in methanogens. A significant divergence from Coo and Ech is that the novel hydrogenase does not encode the Na^+/H^+ translocating subunit (i.e., CooM or EchA), while all other subunits essential for the oxidation of H_2 and electron transfer are present (Lyu and Lu 2015). Similar to the phylogeny of Ech, the HdrB homologs are phylogenetically more closely related to homologs in sulfate-reducing prokaryotes than to those in methanogens. Compared to the canonical form that catalyzes CoB-S-S-CoM reduction in methanogens, HdrB homologs in sulfate-reducing prokaryotes are involved in sulfite reduction and presumably reduce the intramolecular disulfide bridge of the DsrC (Dissimilatory sulfite reductase subunit C) (Grein et al. 2013). Based on phylogeny and traits described above, the novel hydrogenase is tentatively named as the Disulfide Reducing Hydrogenase (Drh) complex (Lyu and Lu 2015).

Due to the absence of the Na^+/H^+ translocating subunit, Drh would be unable to conserve energy from H_2 oxidation. It was speculated that the HdrB subunit in the Drh complex may use the disulfide of an unknown enzyme or compound as the electron acceptor (Aslund et al. 1997).

Methanocellales appears to have exceptional aerotolerant abilities, and all three strains encode a substantial number of genes involved in antioxidant resistance (Erkel et al. 2006) (and see below for further information). However, a robust antioxidant system would need to consume a number of reducing equivalents (Imlay 2008). The Wolfe cycle is unlikely to provide such a source, because its activity shall be severely repressed under oxic conditions. Given the close phylogenetic relationship of Drh to Coo and Fhl (formate-hydrogen lyase) that are known to be involved in CO detoxification and stress resistance (Bonam et al. 1989; Rossmann et al. 1991), Drh in *Methanocella* is probably involved in the antioxidant tolerance. It has been revealed that methanogens tend to develop their antioxidant systems around thioredoxins using the thio/disulfide redox cycling mechanism (Susanti et al. 2014). The oxidation of thio mosaics into disulfide in cells would be expected under air exposure. *Methanocella* perhaps use Drh to couple the H_2 oxidation (i.e., electron supply) to thio/disulfide redox cycling (i.e., via the HdrB) and channel the electrons into repairing machinery for oxidation damages.

6.3 Carbon Metabolisms

All genes for the Embden-Meyerhof-Parnas (EMP) pathway except hexokinase or glucokinase are present in three *Methanocella* strains, indicating that they are able to convert glucose-1-phosphate into pyruvate via glycolysis. The presence of *ppsA* (phosphoenolpyruvate synthase) and *suhB* (D-fructose 1,6-bisphosphatase) indicates that they also have the ability of synthesizing glucose-1-phosphate from pyruvate through gluconeogenesis that may further lead to the synthesis of glycogen, a reserve material in many methanogens (Yu et al. 1994). Thus, under certain circumstances *Methanocellales* may use gluconeogenesis to store energy and switch to glycolysis under starvation.

Pyruvate plays a pivotal role in cellular chemistry. *Methanocella* appear to have diverse pathways for pyruvate metabolisms. Firstly, all three strains could reversibly oxidize pyruvate to acetyl-CoA using pyruvate ferredoxin oxidoreductase (Por) and/or pyruvate dehydrogenase (Pdh). Acetyl-CoA can then be converted to acetate by acetyl-CoA synthase (Acd) or vice versa by acetyl-CoA synthetase (Acs). The presence of Ppa (inorganic pyrophosphatase) would allow *Methanocella* to recover a portion of energy via proton translocation during the acetate activation for biosynthesis. Though physiological tests indicate that acetate is needed for growth by all three strains (Lu and Lu 2012b; Sakai et al. 2008, 2010), *M. arvoryzae* may use the Codh/Acd (CO dehydrogenase/acetyl-CoA synthase) for autotrophy. As indicated earlier, Pdh is known to operate mainly in aerobic and facultatively anaerobic microorganisms while Por is oxygen sensitive. Comparative genomic analysis confirms that Pdh is present in all three strains (Lyu and Lu 2015). Possessing of

both For and Pdh by *Methanocella* possibly offers them an adaptive strategy to the alternating anoxic/oxic conditions. Specifically, Pdh is probably activated for pyruvate metabolism under oxic conditions (Erkel et al. 2006; Sakai et al. 2011). Secondly, all three strains possess the coding genes for acetolactate synthase, which could be used in biosynthesis of branched-chain amino acids from pyruvate (Bowen et al. 1997). A third potential pathway of pyruvate metabolism probably uses Pdc (pyruvate decarboxylase) to ferment pyruvate into either ethanol to recycle NAD or into acetate to generate reduced ferredoxin, which however was detected only in *M. arvoryzae* and the annotation for the coding genes was putative due to the low identity to known *pdc*. Further experimental studies are necessary to verify different pathways of pyruvate metabolisms in *Methanocella*.

Initial metagenomic and genomic surveys indicated that only the coding genes for isocitrate dehydrogenase and fumarase were present in *Methanocella*, leading to the assumption that neither the oxidative nor the reductive tricarboxylic acid (TCA) cycle was operated in *Methanocellales* (Erkel et al. 2006; Lu and Lu 2012a; Sakai et al. 2011). Due to the possible lacking of 2-oxoglutarate (2-OG) that is needed in glutamate synthesis, *Methanocella* may need to acquire glutamate from environments. A careful manual annotation of three *Methanocella* genomes, however, revealed that all three strains possess the (Re)-type citrate synthase homologs (Mtc_1389, MRE50lv_1257, and MCPlv_0455), sharing an identity of ~33% to that of *Clostridium kluyveri* (Lyu and Lu 2015). The manual annotation also identified a putative aconitase in all three strains encoded by two genes belonging to COG1679 and COG1786. These two genes located in a same cluster would presumably produce the functional motifs in one type of aconitate hydratase, aconitase A. Two types of aconitate hydratase are known: aconitase A widespread in all three domains of life while aconitase B found only in Proteobacteria (Makarova and Koonin 2003). Collectively, the manual reannotation suggests that *Methanocella* encode the nonconventional citrate synthase and aconitate hydratase, and together with the isocitrate dehydrogenase, a partial oxidative TCA from citrate to 2-oxoglutarate (2-OG) would be possible for *Methanocella* (Lyu and Lu 2015).

6.4 Nitrogen Metabolisms

Methanocella encode diverse nitrogen assimilation and regulation systems with a few differences among three strains (Lyu and Lu 2015). They all encode Amt (ammonia transporter) for ammonia uptake, which can then be assimilated via the GS (glutamine synthetase) and GOGAT (glutamate synthase) systems. GDH (glutamate dehydrogenase) that usually operates at high ammonium concentration is also present in *M. arvoryzae* and *M. paludicola*, increasing their flexibility for ammonium assimilation. At least one amino acid ABC transporter is identified in each strain, allowing them to uptake organic nitrogen sources. A complete *nif* operon for nitrogen fixation is present in *M. conradii* and *M. arvoryzae*, but not in *M. paludicola* (Lyu and Lu 2015). Thus, nitrogen fixation may operate in some but not all *Methanocellales* methanogens.

A 2-OG (2-oxoglutarate) based nitrogen regulation system is predicted in three strains (Lyu and Lu 2015). This system senses nitrogen level using 2-OG as a trigger as having been revealed in *Methanococcus* and *Methanosarcina* (Leigh and Dodsworth 2007). When nitrogen is limiting, 2-OG accumulates that removes the inhibitory effects of GlnK (nitrogen regulatory protein P-II) on Amt and GS and of NifI1I2 on Nif (nitrogenase), hence promoting both ammonium uptake and N_2 fixation. In addition, the enhancement of GS activity by 2-OG accelerates nitrogen assimilation. In addition, NrpR is also found in three strains. NrpR is a transcription repressor that is found mainly in Archaea (Lie et al. 2007; Lie and Leigh 2007). In nitrogen-starved cells, 2-OG would prevent NrpR from binding to the operators in the promoter regions of *nif* and *glnA*, hence facilitating transcription of these nitrogen assimilation genes. Though the identification of the 2-OG based nitrogen regulatory system, whether it functions and plays a role in N nutrition has yet to be determined by experimental studies.

6.5 Sulfur Metabolisms

The metagenomic analysis of RC-I_{MRE50} has revealed the presence of a complete set of genes for sulfate assimilation, namely, the *cysC* (adenylylsulfate kinase), *cysH* (PAPS reductase), and sulfite reductase (Erkel et al. 2006). This prediction is reconfirmed in the genomes of *M. arvoryzae* and *M. paludicola* (Sakai et al. 2011). But the gene coding for sulfite reductase is missing in *M. conradii* (Lu and Lu 2012b). Nevertheless, all three strains encode a PiT family transporter for the uptake of phosphate or sulfate, and *M. arvoryzae* additionally encodes a putative sulfate permease. Thus, at least *M. arvoryzae* and *M. paludicola* are likely able to use sulfate as a sulfur source. For FeS cluster assembly, sulfite is often the only sulfur source for many methanogens due to the lack of cysteine desulfurase, whereas the genes coding for this enzyme are present in *Methanocella* (Lyu and Lu 2015). In addition, three strains encode two iron sulfur assembly systems which enable them to explore alternative sulfur sources for FeS synthesis. The first uses ApbC type FeS carrier and SufBCD type synthesis system, which is present predominantly in Class I methanogens with sulfide as sulfur source, while the second uses the A-type FeS carrier and IscSU synthesis system with cysteine as sulfur source (Liu et al. 2012). This may allow *Methanocella* to switch between two systems in concert with redox changes in environment, using sulfide at low and cysteine at high redox potentials, securing sulfur nutrition. The putative use of sulfate and the presence of two iron sulfur assembly systems reinforce the adaptation of *Methanocellales* to oxidative conditions.

6.6 Understanding of Oxidative Adaptation

Methanogenic analysis has indicated that *Methanocellales* contain multiple sets of genes coding for antioxidant systems that is the key for surviving and thriving in

alternate anoxic/oxic habitats like rice rhizosphere. To confirm this capacity, an extensive comparative genomic analysis was conducted for three strains (Lyu and Lu 2018). Theoretically, three lines of antioxidant strategies could have been evolved in microbes to defend the oxygenation challenge: (i) avoiding the production of reactive oxygen species (ROS), (ii) reducing accumulation of ROS within the cell, and (iii) repairing self for ROS damage. Studies have revealed that these strategies are essential for both aerobes and anaerobes to survive oxidative stress (Imlay 2008, 2015). The comparative genomic analysis therefore has been focused on identifying these strategies in *Methanocella* genomes (Lyu and Lu 2018).

The methanogenesis pathway where redox reactions are most active inside the cell of methanogens is assumably the main place for ROS production. Specifically, the flavin-based electron bifurcation system that requires the formation of flavosemiquinone could react with oxygen to form O_2^- and H_2O_2 (Buckel and Thauer 2013). This electron bifurcation mechanism has been proposed to operate in *Methanocella* (Liu et al. 2014; Liu and Lu 2018). The comparative genomic analyses indicate that the number of [4Fe-4S] motifs involved in the electron bifurcation-based methanogenesis was reduced by about 70% in *Methanocella* compared to the Class I methanogens (Lyu and Lu 2018). This change in electron transfer machinery could reduce the chance for HO· production through the Fenton reaction.

The second major strategy lies on the capacity of O_2/ROS elimination that is catalyzed by a variety of antioxidant enzymes in microbes. The enzymes known to reduce O_2 to H_2O and transform H_2O_2 and O_2^- to less toxic O_2 have been characterized (Imlay 2008). Many of O_2/ROS eliminations depend on redox reactions and require reducing power to proceed. Small redox proteins play an important role in supplying such a reducing power (Lu and Holmgren 2014). These proteins also serve as a buffering system to keep cellular redox system from becoming over oxidized (Susanti et al. 2014). Though the presence of O_2/ROS elimination enzymes in many methanogens, *Methanocella* possess statistically more genes encoding these enzymes than the Class I (hydrogenotrophic) methanogen counterparts (Lyu and Lu 2018). These observations suggest that *Methanocella* are equipped with a higher capacity for O_2/ROS elimination (Fig. 4).

A closer examination of the O_2/ROS elimination systems indicates the evolutionary robustness of this elevated capacity in *Methanocellales*. First, NO/O_2 reductase is more abundant than $F_{420}H_2$ oxidase in *Methanocella*. Both enzymes can oxidize O_2 into H_2O, but the latter is deactivated when cells are exposed to air (Seedorf et al. 2004), while the former has a higher *Km* for O_2 (Silaghi-Dumitrescu et al. 2005). In addition, NO/O_2 reductase detoxifies NO, a product of denitrification that can be produced at the oxic-anoxic interface (Kluber and Conrad 1998). A shift from $F_{420}H_2$ oxidase to NO/O_2 reductase could suggest an evolutional adaptation of *Methanocellales* to the severer oxidative conditions. Second, rubredoxin and thioredoxin are the major small redox proteins found in methanogens. Thioredoxin operates at much lower redox potentials than rubredoxin, transferring electrons at around -300 to -120 mV versus 0 ± 100 mV, respectively (Aslund et al. 1997; Lin et al. 2005). In comparison with other hydrogenotrophic methanogens,

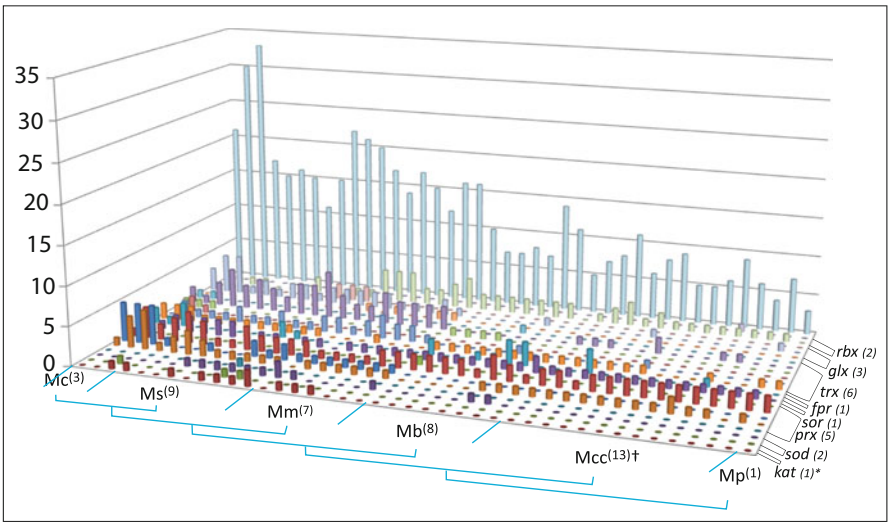

Fig. 4 Methanocellales contain on average highest numbers of genes encoding antioxidant systems. Included for the comparative analysis are three genomes of Methanocellales [Mc(3)], nine genomes of Methanosarcinales [Ms(9)], seven genomes of Methanomicrobiales [Mm(7)], eight genomes of Methanobacteriales [Mb(8)], thirteen genomes of Methanococcales [Mcc(13+)] and one genome of Methanopyrales [Mp(1)]. The coding genes for analysis consist of catalase (kat), superoxide dismutase (sod), peroxiredoxin (prx), superoxide reductase (sor), F420H2 oxidase (fpr), thioredoxin (trx), glutaredoxin system (glx) and rubredoxin (rbx). The number in parentheses indicates the COGs of the respective genes

Methanocella contain similar number of rubredoxin proteins, but the thioredoxin proteins are substantially increased (Lyu and Lu 2018), indicating a potential enhancement of the redox buffering system in *Methanocellales*. Third, transmembrane thioredoxin proteins are present in *Methanocella*, but rare in other hydrogenotrophic methanogens (Table 2). In addition to the presence of thioredoxin domain both in the cytoplasmic and periplasmic side, these transmembrane proteins have two or three cysteine residues in the transmembrane region. These transmembrane thioredoxin proteins may enable electron shuffle between the cytoplasmic and periplasmic spaces, which may help with redox recovery around the cellular membranes under oxidative stress. Fourth, while the classical hydrogenotrophic methanogens appear to use $F_{420}H_2$ to regenerate the reduced thioredoxin, *Methanocellales* probably use NADPH or NADH. NADPH or NADH are more stable electron carriers than $F_{420}H_2$ in an oxygenated Earth environments. These changes in oxidant-detoxifying systems of *Methanocellales* appear systematic and holistic.

The third strategy for oxidative tolerances is the self-repairing. Metagenomic analysis already revealed the repairing system is enriched in *Methanocellales* (Erkel et al. 2006). The analysis of pure culture genomes expanded these mechanisms with more details (Lyu and Lu 2018). ROS once formed can cause extensive damages to

cell components. For instances, DNA mutation or dysfunction may occur due to the oxidation of purines and pyrimidines (Dalhus et al. 2009). The membrane lipids can be oxidized into phospholipid hydroperoxides (PLOOH). The proteins containing sulfur amino acids can be deformed with the formation of disulfide bonds or methionine sulfoxide, leading to disorder of protein structures (Manevich et al. 2002). In addition, ROS may disrupt the iron-sulfur (FeS) clusters which are the prosthetic groups of many enzymes in methanogens. Genes coding for DNA base repairing and S–S or S=O group-reducing enzymes were moderately or strongly enriched in *Methanocella* compared with the Class I methanogens (Lyu and Lu 2018). The enrichment of cytoplasmic S–S reduction enzymes in *Methanocella* is consistent with the elevated abundance of thioredoxins relative to other hydrogenotrophic methanogens. The genes coding for PLOOH reduction (peroxiredoxins) are also enriched in *Methanocella*.

7 Conclusive Remarks

Methanocellales represent a novel type of methanogens initially discovered with DNA fingerprinting of paddy soils. These methanogens were often detected in rice rhizosphere or in association with rice roots. Earlier studies demonstrated that a considerable fraction of the plant-photosynthesized C is allocated to rice roots, released into rice rhizosphere and thereby the DOC pool serves as a major carbon source for methanogenesis, leading to the seasonal maxima of CH_4 emissions. Strikingly, methanogenesis appears to occur close to rice roots. This methanogenic activity was not very expected because rice plants have a well-developed aerenchyma system where O_2 can diffuse from the plant top to roots and released into the rhizosphere. As a result, rice roots and rhizosphere are partly oxic. The dilemma of active methanogenesis in the rice rhizosphere and the nature of strictly anaerobic lifestyle of methanogens causes a huge curiosity to look into the biological logic and mechanism.

Due to the nature of difficult-to-cultivation, intensive studies using molecular techniques were conducted with a focus on the ecophysiology of methanogens in paddy soils. Meanwhile, multiple efforts for enrichment and cultivation were undergone. Strikingly, molecular techniques including DNA-SIP approach revealed that albeit as hydrogenotrophic methanogens *Methanocellales* dominated over other methanogens when H_2 partial pressure was low, indicating that out of the vast methanogenic populations in paddy soil *Methanocellales* might be better adapted under low H_2 condition. This trait is possibly a reason why they escaped the isolation albeit existing widespread in environments. The exploration under in situ conditions using RNA-SIP technology revealed that *Methanocellales* play the key role in CH_4 production in rice rhizosphere. Further studies were then focused on why they can adapt to low H_2 condition and thrive in the rhizosphere where O_2 leaks can occur.

Metagenomic investigation revealed a series of traits that support the adaptation of *Methanocellales* to rhizosphere environment. They possess multiple sets of antioxidant systems and repair systems. They are versatile to assimilate various

sources of N and S and they may activate different core metabolisms to facilitate biosynthesis and survival during environment shift to oxic conditions. After continuous efforts for years, three strains were finally isolated into pure culture. Extensive genomic analyses were conducted to reveal the taxonomic, evolutional, and ecological properties of *Methanocellales*. The phylogenetic analyses using multiple marker genes in combination with genome alignment and AAI analyses consistently suggest that *M. conradii* and *M. paludicola* are closely related each other and together can be classified as a new genus while *M. arvoryzae* may belong to another genus. Comparative genomic analyses reveal that metabolic features for *Methanocellales* appear to be more diverse than previously predicted from metagenomic investigation. Three strains share close resemblance as well as novel features on the core metabolisms, such as specialization in utilizing H_2 at low concentrations. For the adaptation to oxic condition that is key for their activity in rice rhizosphere, at least three general evolutionary mechanisms have been acquired and enriched in *Methanocellales*. The first is the usage of enzymes producing less ROS in the central methanogenesis pathway, particularly the flavin-based electron bifurcation system has been modified from classical hydrogenotrophic methanogens toward a less possibility of ROS production. The second is the expansion and diversification upon a core antioxidant system for the O_2/ROS elimination. And the third is the occurrence of multiple self-repairing pathways from O_2/ROS damages. Further studies are necessary to explore these novel genomic features, which would not only contribute to a deeper understanding of *Methanocellales* and methanogens in general but pave a way towards the development of biotechnology for control of methane emissions from paddy fields.

Acknowledgments This work was financially supported by the National Natural Science Foundation (41630857) and the National Key Research and Development Program of China (2016YFD0200306).

References

Anderson I, Ulrich LE, Lupa B, Susanti D, Porat I, Hooper SD, Lykidis A, Sieprawska-Lupa M, Dharmarajan L, Goltsman E et al (2009) Genomic characterization of methanomicrobiales reveals three classes of methanogens. PLoS One 4:e5797

Angel R, Matthies D, Conrad R (2011) Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PLoS One 6:e20453

Angel R, Claus P, Conrad R (2012) Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J 6:847–862

Arth I, Frenzel P, Conrad R (1998) Denitrification coupled to nitrification in the rhizosphere of rice. Soil Biol Biochem 30:509–515

Aschenbach K, Conrad R, Rehakova K, Dolezal J, Janatkova K, Angel R (2013) Methanogens at the top of the world: occurrence and potential activity of methanogens in newly deglaciated soils in high-altitude cold deserts in the Western Himalayas. Front Microbiol 4:359

Aslund F, Berndt KD, Holmgren A (1997) Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein-protein redox equilibria. J Biol Chem 272:30780–30786

Bolton H Jr, Fredrickson JK, Elliott LF (1993) Microbial ecology of the rhizosphere, p. 27–63. In Metting FB (ed.), Soil Microbial Ecology. Marcel Dekker, New York, NY

Bonam D, Lehman L, Roberts GP, Ludden PW (1989) Regulation of carbon-monoxide dehydrogenase and hydrogenase in Rhodospirillum-rubrum – effects of CO and oxygen on synthesis and activity. J Bacteriol 171:3102–3107

Borrel G, O'Toole PW, Harris HMB, Peyret P, Brugere JF, Gribaldo S (2013) Phylogenomic data support a seventh order of Methylotrophic methanogens and provide insights into the evolution of Methanogenesis. Genome Biol Evol 5:1769–1780

Bowen TL, Union J, Tumbula DL, Whitman WB (1997) Cloning and phylogenetic analysis of the genes encoding acetohydroxyacid synthase from the archaeon Methanococcus aeolicus. Gene 188:77–84

Buckel W, Thauer RK (2013) Energy conservation via electron bifurcating ferredoxin reduction and proton/Na(+) translocating ferredoxin oxidation. Biochim Biophys Acta 1827:94–113

Chelikani P, Fita I, Loewen PC (2004) Diversity of structures and properties among catalases. Cell Mol Life Sci 61:192–208

Chin KJ, Lukow T, Conrad R (1999) Effect of temperature on structure and function of the methanogenic archaeal community in an anoxic rice field soil. Appl Environ Microbiol 65:2341–2349

Chin KJ, Lueders T, Friedrich MW, Klose M, Conrad R (2004) Archaeal community structure and pathway of methane formation on rice roots. Microb Ecol 47:59–67

Cicerone RJ, Delwiche CC, Tyler SC, Zimmerman PR (1992) Methane emissions from California rice paddies with varied treatments. Glob Biogeochem Cycles 6:233–248

Conrad R (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiol Ecol 28:193–202

Conrad R (2004) Methanogenic microbial communities associated with aquatic plants. In: Varma A, Abbott L, Werner D, Hampp R (eds) Plant surface microbiology. Springer-Verlag, Berlin, pp 35–50

Conrad R (2009) The global methane cycle: recent advances in understanding the microbial processes involved. Environ Microbiol Rep 1:285–292

Conrad R, Klose M (1999) Anaerobic conversion of carbon dioxide to methane, acetate and propionate on washed rice roots. FEMS Microbiol Ecol 30:147–155

Conrad R, Klose M (2000) Selective inhibition of reactions involved in methanogenesis and fatty acid production on rice roots. FEMS Microbiol Ecol 34:27–34

Conrad R, Klose M, Claus P (2002) Pathway of CH_4 formation in anoxic rice field soil and rice roots determined by C-13-stable isotope fractionation. Chemosphere 47:797–806

Conrad R, Erkel C, Liesack W (2006) Rice Cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. Curr Opin Biotechnol 17:262–267

Costa KC, Wong PM, Wang TS, Lie TJ, Dodsworth JA, Swanson I, Burn JA, Hackett M, Leigh JA (2010) Protein complexing in a methanogen suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase. Proc Natl Acad Sci USA 107:11050–11055

Dalhus B, Laerdahl JK, Backe PH, Bjoras M (2009) DNA base repair – recognition and initiation of catalysis. FEMS Microbiol Rev 33:1044–1078

Dannenberg S, Conrad R (1999) Effect of rice plants on methane production and rhizospheric metabolism in paddy soil. Biogeochemistry 45:53–71

Erkel C, Kemnitz D, Kube M, Ricke P, Chin KJ, Dedysh S, Reinhardt R, Conrad R, Liesack W (2005) Retrieval of first genome data for rice cluster I methanogens by a combination of cultivation and molecular techniques. FEMS Microbiol Ecol 53:187–204

Erkel C, Kube M, Reinhardt R, Liesack W (2006) Genome of Rice Cluster I archaea-the key methane producers in the rice rhizosphere. Science 313:370–372

Fey A, Conrad R (2003) Effect of temperature on the rate limiting step in the methanogenic degradation pathway in rice field soil. Soil Biol Biochem 35:1–8

Fey A, Chin KJ, Conrad R (2001) Thermophilic methanogens in rice field soil. Environ Microbiol 3:295–303

Fournier M, Zhang Y, Wildschut JD, Dolla A, Voordouw JK, Schriemer DC, Voordouw G (2003) Function of oxygen resistance proteins in the anaerobic, sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough. J Bacteriol 185:71–79

Galand PE, Saarnio S, Fritze H, Yrjala K (2002) Depth related diversity of methanogen Archaea in Finnish oligotrophic fen. FEMS Microbiol Ecol 42:441–449

Gan Y, Qiu Q, Liu P, Rui J, Lu Y (2012) Syntrophic oxidation of propionate in rice field soil at 15 and 30 degrees C under methanogenic conditions. Appl Environ Microbiol 78:4923–4932

Grein F, Ramos AR, Venceslau SS, Pereira IAC (2013) Unifying concepts in anaerobic respiration: insights from dissimilatory sulfur metabolism. BBA-Bioenergetics 1827:145–160

Grosskopf R, Janssen PH, Liesack W (1998a) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 64:960–969

Grosskopf R, Stubner S, Liesack W (1998b) Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. Appl Environ Microbiol 64:4983–4989

Haefele SM, Nelson A, Hijmans RJ (2014) Soil quality and constraints in global rice production. Geoderma 235–236:250–259

Hendrickson EL, Kaul R, Zhou Y, Bovee D, Chapman P, Chung J, Conway de Macario E, Dodsworth JA, Gillett W, Graham DE et al (2004) Complete genome sequence of the genetically tractable hydrogenotrophic methanogen Methanococcus maripaludis. J Bacteriol 186:6956–6969

Holzapfelpschorn A, Conrad R, Seiler W (1986) Effects of vegetation on the emission of methane from submerged paddy soil. Plant Soil 92:223–233

Imlay JA (2008) Cellular defenses against superoxide and hydrogen peroxide. Annu Rev Biochem 77:755–776

Imlay JA (2015) Diagnosing oxidative stress in bacteria: not as easy as you might think. Curr Opin Microbiol 24C:124–131

IPCC (2013) Summary for policymakers. In: Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom

Jenney FE, Verhagen MFJM, Cui XY, Adams MWW (1999) Anaerobic microbes: oxygen detoxification without superoxide dismutase. Science 286:306–309

Jurgens G, Glockner FO, Amann R, Saano A, Montonen L, Likolammi M, Munster U (2000) Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent in situ hybridization. FEMS Microbiol Ecol 34:45–56

Kaster AK, Goenrich M, Seedorf H, Liesegang H, Wollherr A, Gottschalk G, Thauer RK (2011) More than 200 genes required for methane formation from H_2 and CO_2 and energy conservation are present in Methanothermobacter marburgensis and Methanothermobacter thermautotrophicus. Archaea 2011:973848

Kimura M (1997) Sources of methane emitted from paddy fields. Nutr Cycl Agroecosyst 49:153–161

Kluber HD, Conrad R (1998) Inhibitory effects of nitrate, nitrite, NO and N_2O on methanogenesis by Methanosarcina barkeri and Methanobacterium bryantii. FEMS Microbiol Ecol 25:331–339

Konstantinidis KT, Tiedje JM (2007) Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead. Curr Opin Microbiol 10:504–509

Lehmann-Richter S, Grosskopf R, Liesack W, Frenzel P, Conrad R (1999) Methanogenic archaea and CO_2-dependent methanogenesis on washed rice roots. Environ Microbiol 1:159–166

Leigh JA, Dodsworth JA (2007) Nitrogen regulation in bacteria and archaea. Annu Rev Microbiol 61:349–377

Lie TJ, Leigh JA (2007) Genetic screen for regulatory mutations in Methanococcus maripaludis and its use in identification of induction-deficient mutants of the eulyarchaeal repressor NrpR. Appl Environ Microbiol 73:6595–6600

Lie TJ, Dodsworth JA, Nickle DC, Leigh JA (2007) Diverse homologues of the archaeal repressor NrpR function similarly in nitrogen regulation. FEMS Microbiol Lett 271:281–288

Lie TJ, Costa KC, Lupa B, Korpole S, Whitman WB, Leigh JA (2012) Essential anaplerotic role for the energy-converting hydrogenase Eha in hydrogenotrophic methanogenesis. Proc Natl Acad Sci USA 109:15473–15478

Liesack W, Schnell S, Revsbech NP (2000) Microbiology of flooded rice paddies. FEMS Microbiol Rev 24:625–645

Lin IJ, Gebel EB, Machonkin TE, Westler WM, Markley JL (2005) Changes in hydrogen-bond strengths explain reduction potentials in 10 rubredoxin variants. Proc Natl Acad Sci USA 102:14581–14586

Lindau CW, Bollich PK, Delaune RD, Patrick WH, Law VJ (1991) Effect of urea fertilizer and environmental-factors on CH_4 emissions from a Louisiana, USA Rice Field. Plant Soil 136:195–203

Liu PF, Lu YH (2018) Concerted metabolic shifts give new insights into the syntrophic mechanism between propionate-fermenting Pelotomaculum thermopropionicum and hydrogenotrophic *Methanocella conradii*. Front Microbiol 9:1551

Liu YC, Whitman WB (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci 1125:171–189

Liu P, Qiu Q, Lu Y (2011) Syntrophomonadaceae-affiliated species as active butyrate-utilizing syntrophs in paddy field soil. Appl Environ Microbiol 77:3884–3887

Liu YC, Beer LL, Whitman WB (2012) Methanogens: a window into ancient sulfur metabolism. Trends Microbiol 20:251–258

Liu P, Yang YX, Lv Z, Lu Y (2014) Response of a rice paddy methanogen to syntrophic growth as revealed by transcriptional analyses. Appl Environ Microbiol 80:9

Liu DY, Ishikawa H, Nishida M, Tsuchiya K, Takahashi T, Kimura M, Asakawa S (2015) Effect of paddy-upland rotation on methanogenic archaeal community structure in paddy field soil. Microb Ecol 69:160–168

Lu Y, Conrad R (2005) In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. Science 309:3

Lu J, Holmgren A (2014) The thioredoxin antioxidant system. Free Radic Biol Med 66:75–87

Lu Z, Lu Y (2012a) Complete genome sequence of a thermophilic methanogen, *Methanocella conradii* HZ254, isolated from Chinese rice field soil. J Bacteriol 194:2398–2399

Lu Z, Lu Y (2012b) *Methanocella conradii* sp. nov., a thermophilic, obligate hydrogenotrophic methanogen, isolated from Chinese rice field soil. PLoS One 7:e35279

Lu Y, Wassmann R, Neue HU, Huang C (1999) Impact of phosphorus supply on root exudation, aerenchyma formation and methane emission of rice plants. Biogeochemistry 47:6

Lu Y, Wassmann R, Neue HU, Huang C (2000a) Dissolved organic carbon and methane emissions from a rice paddy fertilized with ammonium and nitrate. J Environ Qual 29:8

Lu Y, Wassmann R, Neue HU, Huang C (2000b) Dynamics of dissolved organic carbon and methane emissions in a flooded rice soil. Soil Sci Soc Am J 64:7

Lu Y, Wassmann R, Neue HU, Huang C, Bueno CS (2000c) Methanogenic responses to exogenous substrates in anaerobic rice soils. Soil Biol Biochem 32:8

Lu Y, Watanabe A, Kimura M (2002) Contribution of plant-derived carbon to soil microbial biomass dynamics in a paddy rice microcosm. Biol Fertil Soils 36:136–142

Lu Y, Lueders T, Friedrich MW, Conrad R (2005) Detecting active methanogenic populations on rice roots using stable isotope probing. Environ Microbiol 7:11

Lueders T, Friedrich M (2000) Archaeal population dynamics during sequential reduction processes in rice field soil. Appl Environ Microbiol 66:2732–2742

Lueders T, Chin KJ, Conrad R, Friedrich M (2001) Molecular analyses of methyl-coenzyme M reductase alpha-subunit (mcrA) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. Environ Microbiol 3:194–204

Lueders T, Pommerenke B, Friedrich MW (2004) Stable-isotope probing of microorganisms thriving at thermodynamic limits: syntrophic propionate oxidation in flooded soil. Appl Environ Microbiol 70:5778–5786

Luo HW, Zhang H, Suzuki T, Hattori S, Kamagata Y (2002) Differential expression of methanogenesis genes of Methanothermobacter thermoautotrophicus (formerly Methanobacterium thermoautotrophicum) in pure culture and in cocultures with fatty acid-oxidizing syntrophs. Appl Environ Microbiol 68:1173–1179

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10

Lyu Z, Lu Y (2015) Comparative genomics of three Methanocellales strains reveal novel taxonomic and metabolic features. Environ Microbiol Rep 7:526–537

Lyu Z, Lu YH (2018) Metabolic shift at the class level sheds light on adaptation of methanogens to oxidative environments. ISME J 12:411–423

Makarova KS, Koonin EV (2003) Filling a gap in the central metabolism of archaea: prediction of a novel aconitase by comparative-genomic analysis. FEMS Microbiol Lett 227:17–23

Manevich Y, Sweitzer T, Pak JH, Feinstein SI, Muzykantov V, Fisher AB (2002) 1-Cys peroxiredoxin overexpression protects cells against phospholipid peroxidation-mediated membrane damage. Proc Natl Acad Sci USA 99:11599–11604

Minoda T, Kimura M (1994) Contribution of photosynthesized carbon to the methane emitted from paddy fields. Geophys Res Lett 21:2007–2010

Neubauer SC, Emerson D, Megonigal JP (2002) Life at the energetic edge: kinetics of circumneutral iron oxidation by lithotrophic iron-oxidizing bacteria isolated from the wetland-plant rhizosphere. Appl Environ Microbiol 68:3988–3995

Neue HU, Wassmann R, Kludze HK, Bujun W, Lantin RS (1997) Factors and processes controlling methane emissions from rice fields. Nutr Cycl Agroecosyst 49:111–117

Peng J, Lu Z, Rui J, Lu Y (2008) Dynamics of the methanogenic archaeal community during plant residue decomposition in an anoxic rice field soil. Appl Environ Microbiol 74:2894–2901

Rossmann R, Sawers G, Bock A (1991) Mechanism of regulation of the formate-hydrogenlyase pathway by oxygen, nitrate, and pH – definition of the formate regulon. Mol Microbiol 5:2807–2814

Rui J, Qiu Q, Lu Y (2011) Syntrophic acetate oxidation under thermophilic methanogenic condition in Chinese paddy field soil. FEMS Microbiol Ecol 77:264–273

Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H, Kamagata Y (2007) Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate affiliated with the clone cluster rice cluster I. Appl Environ Microbiol 73:4326–4331

Sakai S, Imachi H, Hanada S, Ohashi A, Harada H, Kamagata Y (2008) *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order Methanocellales ord. nov. Int J Syst Evol Microbiol 58:929–936

Sakai S, Conrad R, Liesack W, Imachi H (2010) *Methanocella arvoryzae* sp. nov., a hydrogenotrophic methanogen isolated from rice field soil. Int J Syst Evol Microbiol 60:2918–2923

Sakai S, Takaki Y, Shimamura S, Sekine M, Tajima T, Kosugi H, Ichikawa N, Tasumi E, Hiraki AT, Shimizu A et al (2011) Genome sequence of a mesophilic hydrogenotrophic methanogen Methanocella paludicola, the first cultivated representative of the order Methanocellales. PLoS One 6:e22898

Sass RL, Fisher FM, Harcombe PA, Turner FT (1991) Mitigation of methane emissions from rice fields: possible adverse effects of incorporated rice straw. Glob Biogeochem Cycles 5:275–287

Saunois M, Jackson RB, Bousquet P, Poulter B, Canadell JG (2016) The growing role of methane in anthropogenic climate change. Environ Res Lett 11:120207

Schaefer H, Fletcher SEM, Veidt C, Lassey KR, Brailsford GW, Bromley TM, Dlugokencky EJ, Michel SE, Miller JB, Levin I et al (2016) A 21st-century shift from fossil-fuel to biogenic methane emissions indicated by (CH_4)-C-13. Science 352:80–84

Scheid D, Stubner S (2001) Structure and diversity of Gram-negative sulfate-reducing bacteria on rice roots. FEMS Microbiol Ecol 36:175–183

Seedorf H, Dreisbach A, Hedderich R, Shima S, Thauer RK (2004) F420H2 oxidase (FprA) from Methanobrevibacter arboriphilus, a coenzyme F-420-dependent enzyme involved in O-2 detoxification. Arch Microbiol 182:126–137

Silaghi-Dumitrescu R, Ng KY, Viswanathan R, Kurtz DM (2005) A flavo-diiron protein from Desulfovibrio vulgaris with oxidase and nitric oxide reductase activities. Evidence for an in vivo nitric oxide scavenging function. Biochemistry 44:3572–3579

Sizova MV, Panikov NS, Tourova TP, Flanagan PW (2003) Isolation and characterization of oligotrophic acido-tolerant methanogenic consortia from a Sphagnum peat bog. FEMS Microbiol Ecol 45:301–315

Sneppen K, Pedersen S, Krishna S, Dodd I, Semsey S (2010) Economy of operon formation: cotranscription minimizes shortfall in protein complexes. MBio 1:e00177

Susanti D, Wong JH, Vensel WH, Loganathan U, DeSantis R, Schmitz RA, Balsera M, Buchanan BB, Mukhopadhyay B (2014) Thioredoxin targets fundamental processes in a methane-producing archaeon, Methanocaldococcus jannaschii. Proc Natl Acad Sci USA 111:2608–2613

Thauer RK (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Microbiol 144:2377–2406

Thauer RK (2012) The Wolfe cycle comes full circle. Proc Natl Acad Sci USA 109:15084–15085

Thauer RK, Kaster AK, Goenrich M, Schick M, Hiromoto T, Shima S (2010) Hydrogenases from methanogenic archaea, nickel, a novel cofactor, and H-2 storage. Annu Rev Biochem 79 (79):507–536

Vandergon HACD, Neue HU (1995) Influence of organic-matter incorporation on the methane emission from a wetland rice field. Glob Biogeochem Cycles 9:11–22

Wind T, Stubner S, Conrad R (1999) Sulfate-reducing bacteria in rice field soil and on rice roots. Syst Appl Microbiol 22:269–279

Yagi K, Minami K (1990) Effect of organic-matter application on methane emission from some Japanese paddy fields. Soil Sci Plant Nutr 36:599–610

Yu JP, Ladapo J, Whitman WB (1994) Pathway of glycogen-metabolism in Methanococcus-maripaludis. J Bacteriol 176:325–332