Structure and Activity of Bacterial and Archaeal Community
Inhabiting Rice Roots and Rhizosphere: Review

Yahai Lu
College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China
email: yhlu@cau.edu.cn

Abstract: Rice is cultivated worldwide on approximately 155 million hectares, accounting for 14% of world arable lands. The great challenge in rice agriculture is to increase rice production using limiting planting area and to minimize methane emissions from soil. In this review, I report the recent progresses in microbiological studies which are dedicated to the understanding of organic matter transformation in paddy fields. The cutting-edge technologies have been rapidly developed and applied to environmental microbiology. One of such technologies is carbon stable isotope labeling in combination with molecular ecology tools, which has been proved very powerful in linking microbial community identities to specific biogeochemical processes in situ. This review will be focused on the application of stable isotope probing in the investigations of active microbes responsible for organic C transformation in the soil surrounding rice roots.

Keywords: Rice rhizosphere; bacteria and archaea; stable isotope probing; carbon cycling

1. Introduction

The dynamics of soil organic matter plays a crucial role in soil functioning. The development of sustainable rice agriculture will rely on the understanding of carbon dynamics in soil. Plant photosynthesis serves as the major source for soil organic matter. The deposits of plant-derived material in rice field soils ends up in three fractions: the first is utilized by soil microbes that promote C and N cycling; the second is fixed into soil organic matter that help sustain soil fertility; and the third is emitted back into the atmosphere in the form of greenhouse gas CH4 that enhances global warming. The objective of the present review is to summarize recent progresses in the determination and molecular fingerprinting of bacterial and archaeal communities which are actively involved in organic matter transformation in the rice rhizosphere. The related information on CH4 emission, C cycling and methanogenesis is referred to several previous reviews [3,6,19].

2. Structure and Activity of Bacterial Community

Culture-dependent and independent methods have been used to determine microbial diversity in environments. Due to the difficulty in isolation of most microbes by traditional cultivation techniques, the development and application of multiple molecular ecology tools has obtained a great attention in the recent two decades. One of such advances is the development of stable isotope probing (SIP), which has proved to be very powerful in linking microbial community identities to specific biogeochemical processes in situ [5]. The principle of stable isotope probing is that the supply of an isotopically enriched substrate to soil microbes could produce labeled microbial biomarkers, which can be analyzed using different chemical and molecular techniques. Several studies have been carried out using SIP technology in rice-soil system, which have largely improved our understanding of structure and function of microbial community in rice soil.

The first study using 13C pulse-chase labeling was performed in a Japanese rice soil [10]. In that experiment, rice plants at varying ages were pulse labeled with 13CO2 and the distribution of the assimilated 13C to soil
microorganisms was estimated by analyzing $^{13}$C profile of microbial phospholipid fatty acids (PLFAs). The results revealed that total PLFA increased with plant growth. But the mono-unsaturated PLFAs increased faster than the branched chain fatty acids. The $^{13}$C was incorporated into PLFAs immediately after plant $^{13}$CO$_2$ assimilation, suggesting tight coupling of microbial activity to plant photosynthesis. In consistence with the changes in total PLFAs, more of $^{13}$C was distributed to straight chain fatty acids (16:0; 16:1$\omega$7, 18:1$\omega$7 and 18:1$\omega$9) which represented Gram negative organisms than to the branched chain fatty acids indicating the Gram positives. The total plant C incorporation estimated from $^{13}$C labeling roughly corresponded to the increase in total PLFAs. These results suggest that the organic substances released from rice roots promote microbial growth in the rice rhizosphere, but microbial assimilation of root-derived C differs among different groups. In a further study, Lu and colleagues [7] used PLFA-based SIP to detect the spatial variation of active microorganisms in rice rhizosphere. In the microcosm experiment, rice plants were labeled similarly with $^{13}$CO$_2$. The soil samples, however, were taken from both rhizosphere and bulk soil, and the bulk soil samples were further partitioned vertically (up layer and down layer) and horizontally with increasing distance to root surface. It was found that incorporation of $^{13}$C into PLFAs sharply decreased along the distance from roots. The labeling of 16:1$\omega$9, 18:1$\omega$7, 18:1$\omega$9, 18:2$\omega$6,9 and i14:0 PLFAs was stronger in the rhizosphere while that of i15:0 and i17:0 increased in the bulk soil. The microorganisms represented by i14:0, 18:1$\omega$7 and 18:2$\omega$6,9 exhibited a relatively higher activity in upper layer soil, whereas those represented by i15:0 and i17:0 were more active in down layer soil. These results suggest that in the rhizosphere Gram-negative and eukaryotic microorganisms were most active in assimilating root-derived C, whereas Gram-positive microorganisms became more important in the bulk soil. The active populations also differed between upper- and down-layer soils and changed with distance to roots.

While PLFA-based SIP studies demonstrated the differential assimilation of root-derived C among soil microbes, the resolution of microbial community is limited by PLFA analysis. DNA- and RNA-based SIP, on the other hand, could provide much better resolution. The DNA-based SIP was first introduced by Radajewski and colleagues [17] and then extended to RNA-based SIP by Manefield and colleagues [14]. These techniques take the advantage of molecular ecology tools that are highly sensitive in resolving the community structure even at the genus level. Lu and colleagues [11] for the first time applied RNA-SIP to rice-soil system to determine the active microorganisms responsible for decomposition of plant-derived material in rice rhizosphere. In the greenhouse experiment, they labeled rice plants with $^{13}$CO$_2$ (99% of atom $^{13}$C) for successive 7 days. The bacterial RNA was isolated from rhizosphere soil and subjected to density gradient centrifugation. RNA samples from density fractions were analyzed using terminal restriction fragment length polymorphism (T-RFLP) fingerprinting, cloning and sequencing of bacterial 16S rRNA genes. The results revealed that the most active bacteria assimilating C from the labeled rice consisted mainly of *Azospirillum* spp. (Alphaproteobacteria) and members of *Burkholderiaceae* (Betaproteobacteria). It has been reported that inoculation of rice roots with *Azospirillum* could enhance plant N uptake and rice yield [2]. Thus, a positive feedback interaction might exist between rice plant and *Azospirillum* by exchange of carbon and nitrogen. The members of *Burkholderiaceae* are metabolically diverse and frequently detected in the rhizosphere of plants. Apparently, this group of organisms was active in assimilating $^{13}$C released from rice roots.

3. Identification of Active Methanotrophs

Methane emission from rice field soil is the result of CH$_4$ production, oxidation and transport from soil into the atmosphere. While most of CH$_4$ is emitted via rice gas vascular system into the atmosphere, oxygen is diffused to the living roots in the reverse direction, and hence, the rhizosphere and roots of rice serve as an important habitat for CH$_4$ oxidation which reduces CH$_4$ emission from flooded rice fields. In order to identify active methanotrophs in rice soil system, Qiu and colleagues [16] carried out an in situ SIP experiment in a Chinese paddy field. They labeled rice plants with CH$_4$ (99% $^{13}$C) for 7 days under the field condition. The rate of $^{13}$CH$_4$ loss during $^{13}$C-application was
comparable to CH₄ oxidation rate measured by the difluoromethane-inhibition technique. The methanotrophic communities in the rhizosphere were analyzed by T-RFLP, cloning and sequencing of the particulate methane monoxygenase (pmoA) genes. It was found that populations of type I methanotrophs were more abundant than those of type II. Furthermore, both PLFA- and RNA- based SIP analyses demonstrated that type I methanotrophs were more active in assimilating ¹³C compared to type II group. In a further study with excised rice root material [15], however, the same authors found that type II methanotrophs related to Methylocystaceae were predominant and remained relatively stable over 20-days incubation. Moreover, 16S rDNA-based SIP analysis revealed that not only methanotrophic Methylocystaceae but also Sphingomonadales were strongly labeled with CH₄-derived ¹³C on young-nodal roots, while Methylotrophic Methylophilales most actively assimilated ¹³C on old-nodal roots. Since Sphingomonadales and Methylophilales are not known to utilize CH₄ directly, these observations suggest the occurring of a potentially important CH₄-connected food web on rice roots. It, however, remains to be elucidated on the striking discrepancy between in situ rhizosphere study and laboratory incubation of the excised root material.

4. Structure and Activity of Archaeal Community

Methanogens are generally thought to inhabit the anaerobic bulk soil. However, they are also detected in the rhizosphere and on rice roots where O₂ is possibly released during plant growth [1,20]. Furthermore, CH₄ production was found to be more significant in the rhizosphere than in the bulk soil [12,13]. Two hypotheses may be proposed to explain the existence of methanogenesis on rice roots: (i) methanogens on rice roots are probably resistant to O₂ toxicity; (ii) they may adapt a spatial strategy, residing where O₂ does not exist, e.g. old root segments. In order to identify the active methanogenic populations inhabiting rice roots responsible for CH₄ production, Lu and colleagues [9] carried out a laboratory incubation experiment using soil-free root material. Rice roots were incubated anaerobically under an atmosphere of H₂/¹³CO₂ or N₂/¹³CO₂ with phosphate or carbonate (marble) as buffer medium. The Methanosarcinaceae and Rice Cluster I (RC-I) were predominant in the root incubations when carbonate buffer and N₂ headspace were used. The DNA-SIP analysis showed that the relative abundance of RC-I increased whereas that of the Methanosarcinaceae decreased with increasing DNA buoyant density, indicating that members of RC-I were more active than Methanosarcinaceae. However, RC-I was unexpectedly suppressed in the presence of high H₂ concentrations (80%, v/v). Phosphate buffer appeared to inhibit the activity of the Methanosarcinaceae, resulting in lower CH₄ production as compared to carbonate buffer. These results suggest that the active methanogenic populations on rice roots change in correspondence to H₂ availability and the type of buffer medium used in the system.

Since the distinct ecophysiology of methanogens may prevail on root surface compared with soil, Lu and Conrad [8] performed a RNA-SIP experiment in an intact rice-soil system. In this study, rice plants were labeled with ¹³CO₂ and microbial RNA was extracted directly from the rhizosphere soil. ¹³C-labeled methanogens were analyzed following standard RNA-SIP procedure (Whiteley et al., 2007). The study demonstrates that of the archaea detectable in rice rhizosphere, the RC-I methanogens became specifically labeled. A pure culture of RC-I methanogens has been recently isolated from a Japanese rice soil which indicates that these methanogens utilize H₂/CO₂ as energy and carbon sources. Hence, it appears that RC-I methanogens play a key role in CH₄ production from the root-derived C. These results are consistent with earlier findings that in the anoxic incubations of excised rice roots CH₄ is mainly produced from H₂/CO₂ [4].

5. Conclusive Remarks

The application of stable isotope probing has revealed the most active bacteria and archaea which are involved in C dynamics that is initiated by rice plant photosynthesis. The findings suggest that the proteobacterial organisms
such as *Azospirillum* and *Burkholderiaceae* and euryarchaeotal RC-I methanogens probably plays the key role in the decomposition of plant-derived organic matter and production of CH$_4$ in the rhizosphere soil. For CH$_4$ oxidation, however, controversial findings on the active methanotrophs have been observed from the rhizosphere and excised root studies. Several key issues can be identified that needs to be addressed in the further studies:

i. While the active microorganisms in rice rhizosphere have been determined using SIP technology, the active organisms involved in plant residue decomposition in the anoxic bulk soil remain to be identified,

ii. Elucidation of the mechanisms for the prevalence of RC-I methanogens in the soil close to rice roots and their functioning in the partially oxic environments,

iii. Identification of the driving forces for the tempo-spatial variation of active microorganisms in rice rhizosphere,

iv. Identification of the controlling factors influencing the functioning of active microorganisms in the rhizosphere and bulk soil.

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**References**


