Summary of Doctoral Thesis

Title: Study on the Community Structure and Spatial Distribution of Soil Protists in a Rice Rhizosphere

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Wetland rice field soil has distinct biogeochemical cycles and microbial communities from upland soils, and rice roots growing in a submerged soil give a specific habitat for microorganisms through supply of oxygen and organic matter to the rhizosphere depending on the growth stage. The rice roots in the early growth stage release oxygen the surrounding soil making the rhizosphere oxic. The rice rhizosphere becomes anoxic when roots grow older due to the enhanced microbial activities on the released organic substrates and sloughed-off cells over the oxygen supply. Besides the oxygen, the rice roots secrete mucilage and exudates; contain both high-molecular weight substances, mainly mucilage and ecto-enzymes, and low-molecular weight substances consisting of organic acids, phenols and amino acids. The amount of organic matter in the of a paddy field is larger than those in the bulk soil.

Since the rhizosphere is a hotspot for microorganisms, a lot of studies have focused on microorganisms, especially on the bacterial and archaeal communities in the rice rhizosphere and the rice roots. Microeukaryotes may also play important roles in the rice rhizosphere as either plant parasites, decomposers of root tissues, or bacterial predators. However, less is still known about the community structure of microeukaryotes and its shaping forces in the rice rhizosphere.

In this study, we first aimed to characterize the protistan community structure of a rice rhizosphere. The universal eukaryotic specific primer was modified to detect a

wide range of soil microeukaryotes, including heterotrophic protists. The microeukaryotic community structures of the rice roots and the rhizosphere soil were explored through polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) targeting the 18S rRNA gene with a field experiment. To characterize the rice rhizosphere specific community, the bulk soil of rice field and the wheat rhizosphere were also examined. DGGE fingerprints showed that the microeukaryotic community of rice roots were distinct from the community of the bulk soil and showed a temporal shift with the growth stage. The rhizosphere soil community was distinct from the root and bulk soil communities, but this could be explained by that the root and bulk soil communities were shared in the rhizosphere.

DNA-based analysis enables us to study the community present in the samples, but they may not be all active. It is generally assumed that RNA-based analysis targets the actively growing microorganisms. A RNA-based approach may be more beneficial to study the effect of the environmental change in the rice rhizosphere on the microeukaryotes in more detail. Thus, we made a pot experiment, in which the potentially active microeukaryotic community structure of a rice rhizosphere was explored through PCR-DGGE targeting the 18S rRNA. The results showed that rice roots and the growth stages shapes the community structure of microeukaryotes. Different microeukaryotes inhabits the rice rhizosphere in each growth stage. In both field and pot experiments, heterotrophic protists were found as one of the main microeukaryotes in the rice rhizosphere and affected by environmental changes in the rice rhizosphere.

Macro- and micro-scale distribution of heterotrophic protists are controlled by their morphological and physiological characteristics such as body size, motility, and

diet, and by environmental factors such as availability of water and oxygen. Studies have been shown that nutrient and water uptake rates and radial oxygen loss are not homogenous along a rice root and highest at the root tip part. The tip part of a root is the most active part and supports microorganisms by the regulated delivery of biologically active root exudates, mucilages, and border cells into the rhizosphere.

A “mini-rhizobox” experiment was conducted to reveal the effects of the physiological differences along a root axis on protistan distribution. Micro-scale distribution of protists in the rhizosphere was observed and protists were enumerated in the early growth period of rice plants. Protistan community was explored through PCR-DGGE targeting 18S rRNA gene. Protistan species inhabiting the rice rhizosphere were affiliated to flagellates, amoeba, and ciliates. Our results clearly showed that the rice roots provide a favorable habitat for protists with extended rhizosphere effect at the tip parts and that the micro-scale distribution among protistan types in the rice rhizosphere is distinct from each other.

As conclusion, growth stages of the rice plants, sections of a rice root, and environmental factors such as availability of oxygen and water affect microeukaryotic community structure in the rhizosphere. This study first demonstrates that the tip part of a rice root is characterized with the expanded population of flagellates and exclusive population of ciliates. So far, our knowledge on protistan roles in rhizosphere mostly depending on controlled laboratory studies, in which mostly a few amoeba species were used. Here, we showed that flagellates and ciliates are closely associated with the rice roots instead of amoeba. Since each protistan species may have different impact on bacterial community structure, we suggest that future studies should focus on the interaction between the rice roots and flagellates and ciliates, and their potential roles on

plant growth.