

Rice Root-Associated Microbiome

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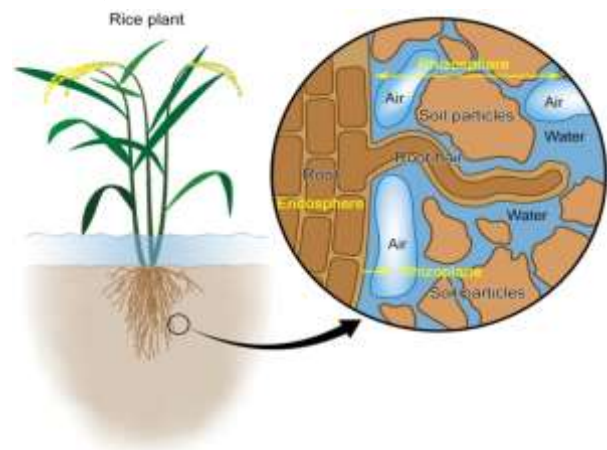
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The microbiome is defined as both the composition of a microbial community in a specific environmental niche (microbiota) and its collective genome. The whole system consisting of plants, their environments and all organisms in that environment is called as phytobiome (Ding *et al.*, 2019). Metagenomic and proteomic approaches have been used to identify different microbial genes present in the rice microbiome. HTS-based studies have revealed that plant-associated microbial communities are more extensively involved in promoting the health and fitness of their hosts than those of human and animal microbial communities. As an example, the root bacterial community is essential for maintaining multi kingdom interactions and for maximal plant growth and health of *Arabidopsis thaliana*. Plants depend upon beneficial interactions between roots and microbes for nutrient availability, growth promotion, and disease suppression. The three root-associated compartments, the endosphere (root interior), rhizoplane (root surface), and rhizosphere (soil close to the root surface), each of which was found to harbour a distinct microbiome. Under controlled greenhouse conditions, microbiome composition varied with soil source and genotype.

In field conditions, geo-graphical location and cultivation practice, such as organic vs. conventional, and their factors contributing to microbiome variation. Rice cultivation is a major source of global methane emissions, and methanogenic archaea could be detected in all spatial compartments of field-grown rice. Dynamic changes observed during microbiome acquisition, as they all

as steady-state compositions of spatial compartments, support a multistep model for root microbiome assembly from soil wherein the rhizoplane plays a selective gating role. Plants and microbes have both adapted to use their close association for their mutual benefit. Critical nutrients are converted to more usable forms by microbes before assimilation by plants. In turn, bacteria in the rhizosphere receive carbon metabolites from the plant through root exudates. Beneficial soil microbes also contribute to pathogen resistance, water retention, and synthesis of growth promoting hormones (Edwards *et al.*, 2015).

The below ground microbiome can be studied in different compartments of rice root as follows,



1. Rhizosphere (soil surrounding the root surface)
2. Rhizoplane (root surface)
3. Endosphere (inside root)

Fig.1 Rice root is composed of three different compartments based on spatial resolution

Rhizosphere

The rhizosphere is defined as the soil adjacent to plant roots. Plant roots alter the chemical and nutrient composition of soil by secreting organic and inorganic substances, which also affects its physical and biochemical properties. Such secreted substances, also referred to as root exudates, are important for maintenance of root-soil contact and contribute to root growth and plant survival (Walker *et al.* 2003). Root exudates facilitate the physicochemical adaptation of plants to the soil environment and provide nutrients for early colonization of soil microbial communities. Consequently, root exudates contribute to the biological adaptation of plants in a soil environment by recruiting microbes that functionally enhance the rhizosphere. Rhizospheric microbes benefit plants by providing nutrients or plant hormones, suppressing pathogens, and increasing resilience to abiotic stresses such as drought. The compositions of communities of rhizosphere bacteria and fungi of rice at various developmental stages are investigated using DGGE and found that both communities varied according to growth stage. Improvements in meta-omics technologies have enabled investigations of the composition and diversity of rhizosphere microbiotas. For example, communities of bacteria and archaea have been found to be more abundant in the rhizosphere than in bulk soil, but the two types of soil have similar diversities of these microorganisms. The compositions of bacterial communities in bulk soil and the rhizosphere are similar to those of the rhizoplane and rhizosphere; only 169 of 27,147 OTUs (152 enriched and 17 depleted OTUs) differed between the soil types (Edwards *et al.* 2015).

Considering that oxic and anoxic zones coexist within the rhizosphere of rice cultivated in flooded condition, strict and facultative anaerobes as aerobes have to be taken into account in terms of

biological and ecological functions. Although some studies identified strictly or facultative anaerobic bacteria and fungi from bulk and rhizosphere soils of rice ecosystems, given that metabolic versatility of anaerobic microbial communities in rice fields and the possibility that aerobic and anaerobic microbes can coexist in the aerobic state, anaerobic microbes might be key modulators or players that maintain the structure of overall rhizosphere microbial communities.

Endosphere

The internal regions of the plant's leaves, stems, roots, fruits, and seeds are termed the endosphere, and the resident microorganisms are endophytes. Commensal endophytes generally show neutral effects on plant performance, whereas beneficial endophytes confer beneficial effects on plant development or protection from pathogens and herbivores. Another group is opportunistic or latent pathogen showing detrimental effects in certain conditions (Hardoim *et al.* 2015; Garcia-Vidal *et al.* 2009). Among these endophytes, beneficial endophytes are considered a new source of biocontrol agents and biofertilizers for increasing crop productivity.

Research on endosphere microbiome is technically constrained when compared to other compartments because of the enormous quantity of genomic DNA present in a host plant, which might mask the endophyte's very little contribution. Specifically, during the creation of the bacterial amplicon, the mitochondrial and plastid DNAs are co-amplified. In the endophyte's raw sequence data, the high ratio of host to endophyte DNA leads to low sequencing depth and quality.

Rhizoplane

The rhizoplane is the root surface where the host plants are in direct contact with the rhizosphere soils (Fig.1) (Ding *et al.* 2019). The separation distance between the rhizoplane and rhizosphere is

extremely close and thus both zones are commonly regarded as a continuum. At the rhizoplane level, roots actively interact with the rhizosphere soils through releasing substrates and competing for nutrients, and hence, the rhizoplane is anticipated to be a compartment that drives nutrient exchange and transformation in the soil-plant systems. Moreover, the rhizoplane might act as a selective barrier in the establishment of plant root microbiome, as only a proportion of microbes in the rhizosphere could attach the rhizoplane, and then a subset of them could invade and thrive in the endosphere (Edwards *et al.* 2015).

Generally, bacteria are the major population colonizing the rice rhizoplane, and can exert positive, negative or neutral influences on plant growth. *Bacillus* (e.g., *Bacillus velezensis*) and *Pseudomonas* genera (e.g., *Pseudomonas fluorescens*) have been reported to be typical rhizoplane inhabitants for rice plants on the basis of culture-dependent studies. *B. velezensis* is capable of phosphate solubilization as it possesses genes encoding phosphorus-solubilizing proteins. *P. fluorescens* has high antifungal activity due to the production of cyanide or siderophore, and is a putative antagonistic bacterium for the biocontrol of fungal pathogens of roots.

Therefore, colonization of these bacterial populations on the rice rhizoplane could favor the growth of rice plants through improving P availability and antagonism of soil-borne phytopathogens. Additionally, microbes involved in the N₂ fixation (e.g., *Azospirillum* spp.) and nitrification (e.g., *Nitrobacter* and *Nitrosospira* spp.) has also been isolated from the rice rhizoplane. Proteins of α -, β - and δ -proteobacteria phyla have been found to be predominant groups on the rice rhizoplane using metaproteomic analyses (Knief *et al.* 2012). These proteins are assigned to multiple genera responsible for the biogeochemical cycles, like *Azospirillum* and *Bradyrhizobium* for N₂ fixation,

Methylosinus for CH₄ oxidation, and Anaeromyxobacter and Geobacter for Fe (III) reduction (Knief *et al.* 2012). The rhizoplane and endosphere they are the most similar rhizocompartments, sharing 271 enriched OTUs. Most of the OTUs enriched between the rhizoplane and endosphere compartments belonged to Alpha-, Beta-, and Deltaproteobacterial classes, Chloroflexi, and Bacteroidetes. Not surprisingly, a subset of the OTUs enriched in the endosphere and rhizoplane belong to Fibrobacteres and Spirochaetes, phyla that are associated with cellulose degradation (24, 25) In comparison, the rhizoplane enriches for many OTUs while simultaneously depleting a larger proportion of OTUs (422 vs. 730). The endosphere is the most exclusive compartment, enriching for 394 OTUs while depleting 1,961 OTUs.

The separation distance between the rhizoplane and rhizosphere is extremely close and thus both zones are commonly regarded as a continuum as only a proportion of microbes in the rhizosphere could attach the rhizoplane, and then a subset of them could invade and thrive in the endosphere AT this compartment roots actually interact with rhizosphere soil through releasing substrates and competing. Proteins of Alpha-, Beta- and Delta proteobacteria phyla have been found to be predominant groups on the rice rhizoplane using metaproteomic analyses. Ex: Bacterial community *Bacillus* and *Pseudomonas*

Composition and structure of rice microbiome

Before the emergence of HTS platforms, other molecular techniques such as 16S rRNA gene cloning, polymerase chain reaction-denaturing gel gradient electrophoresis (PCR-DGGE), and terminal restriction fragment length polymorphism analyses is used to evaluate the composition of microbial communities in soil and plant tissues. The emergence of HTS has accelerated research on the composition and structure of rice-associated

microbial communities. Microbiome is distributed ubiquitously in or adjacent to rice plants. Rice microbiome can be dissected into the compartments where microbial communities reside. Among the compartments, bulk soil and root system (rhizosphere and root endosphere) are more widely investigated than other compartments. Community analyses with respect to aboveground parts (phyllosphere and seed) are also employed with both culture-dependent and -independent approaches. Before the introduction of omics technologies, PCR-denaturing gel gradient electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), and clone libraries of 16S rRNA genes are used for investigating the compositions of microbiome. However, these techniques are not enough to unveil the nature of microbiome because of their low resolutions. With the application of omics technologies, the composition and functions of microbiome are widely investigated. Another is a culture-dependent approach. Despite the low recovery of natural communities, this approach is inevitable in order to prove predicted functions of microbiome. For this, isolation of microbes from samples and characterization of isolates, using both *in vitro* and *in-vivo* assays are conducted.

This parallel application of both culture-independent and -dependent approaches can give us the chance to construct synthetic communities or combinations of metabolites as probiotics and prebiotics for improving sustainability and productivity of rice. ACC, aminocyclopropane-1-carboxylate; IAA, indole-3-acetic acid; M-, not inoculated with beneficial microbes; and M+, inoculated with beneficial microbes.

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