



## Rice Root Exudation: Signalling and Behavior of Shaping the DNRA Microbiome

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### Abstract

In natural ecosystems, plant health is mainly dependent on interactions with diverse and dynamic soil microbial communities. The microbial communities particularly those associated with nitrogen (N) cycling pathway inhabiting in rice field ecosystem have been described previously. Although, little is known about the taxonomic microbial players involved with dissimilatory nitrate reduction to ammonium (DNRA), a short-circuit N retention pathway in terrestrial N cycle. Therefore, we have to focus on how rice plants shape DNRA bacteria and how they alter over the course of several rice growth stages. Besides, we also shed lights on rice root physiology and the role of root exudates to address plant physiological aspects that may influence plant-microbe interactions.

**Keywords:** Dissimilatory Nitrate Reduction to Ammonium Pathway (DNRA) Microbiome, Rice, Root exudates, Signalling

### Introduction

The rapidly increasing use of chemical fertilizers in agriculture causes high nitrogen (N) mainly nitrate ( $\text{NO}_3^-$ ) loss, which has become an important issue and received great attention from both environmental and agronomic perspectives. Denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (anammox) are the key processes related to  $\text{NO}_3^-$  reduction. DNRA, which mainly transforms the loss-prone  $\text{NO}_3^-$  to soil-retainable  $\text{NH}_4^+$  (Pandey *et al.*, 2020; Kumar *et al.*, 2021). Both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  forms are important N sources for plant growth. Compared to  $\text{NO}_3^-$ , plants generally have a higher uptake capacity ( $V_{\text{max}}$ ) (*e.g.*, about 16%) and affinity (low  $K_t$ ) for  $\text{NH}_4^+$  (Tylova-Munzarova *et al.*, 2005; Fang *et al.*, 2007). Moreover, DNRA has advantage on increasing N usage efficiency and reducing N loss, which makes great sense to increase food production, especially in the situation of global food shortage.

In contrast to most agricultural crops, rice is often grown in flooded soil, creating oxic and anoxic zones inside the rice rhizosphere that favour particular physiological groups of

bacteria with facultative, aerobic, or anaerobic metabolism (Brune *et al.*, 2000). The microbial populations dwelling in the rice field ecosystem have already been characterised, and the interior, rhizoplane, and rhizosphere of rice roots have all undergone investigation (Edwards *et al.*, 2015). In addition, the microbial communities in various zones, such as rhizosphere, anoxic bulk soil, and oxic surface soil have been reported (Breidenbach and Conrad, 2015; Lee *et al.*, 2015). Soil management practices may affect the diversity, composition, and functioning of N-cycling microbial communities in soil, either directly or indirectly due to altered soil abiotic factors (Xue *et al.*, 2013). The microbial activity of the soil, such as respiration, ammonia oxidation, denitrification, N mineralization, and  $\text{N}_2\text{O}$  emissions, is impacted by soil fertility amendments such as manure, composted organic waste, and synthetic fertilisers (Niboyet *et al.*, 2010).

Root exudates are essential for solubilizing and mobilising nutrients in the soil, which improves plants' ability to absorb nutrients (Figure 1). Additionally, it can supply substrates for microbial activity in the rhizosphere and immobilise toxic elements to protect plants from toxicity. Root exudates are

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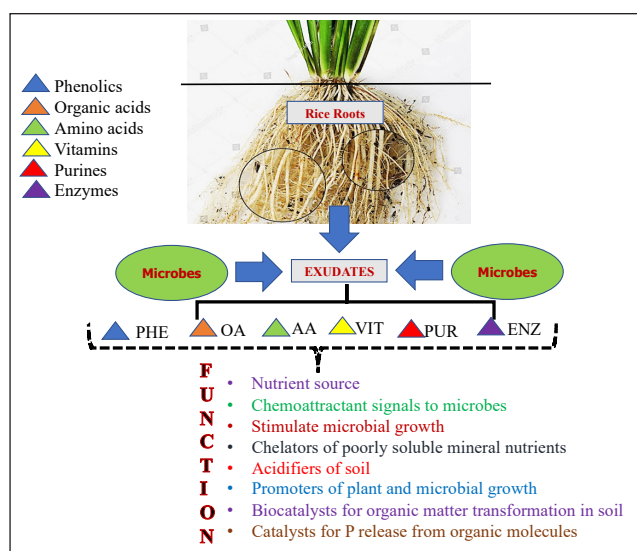


Figure 1: Overview graphical representation on functional aspects of secreted rice root exudates in plant rhizosphere to shape the overall microbiome

significant C sources for the production of methane ( $\text{CH}_4$ ) in flooded rice soils (Dannenberg and Conrad, 1999; Lu *et al.*, 2000). Organic acids in root exudates supply energy to soil microbial communities, including methanogens, and also mobilize soil phosphorus and micronutrients. Several greenhouse and field measurements showed a large  $\text{CH}_4$  flux at the late development stage of rice plants, which has been attributed to the supply of organic C from rice root exudation. However, none of these studies focused on the impact of the rice root exudates on DNRA microbial community and its status at several growth stages of rice. Therefore, in this present article we summarized the impacts of rice root exudation on unnotified short circuit DNRA pathway in terrestrial ecosystem.

### Root Physiological Features Shape Rhizobiosomes and Exudation

The spatial orientation of rhizobiosomes towards roots affects them in two different ways. First, as documented in multiple recent studies, the complexity and variety of microbial communities depend on their radial closeness to roots (Lundberg *et al.*, 2012; Edwards *et al.*, 2015). Second, according to early investigations, the community is shaped by the lateral positioning of bacteria along a root. The former is important, but the latter is not taken into account in contemporary microbiome investigations. This section covers the role of regionally distinct root exudation as well as particular microbial interactions with diverse root areas. Root tips are the first tissues that make contact with bulk soil: root tips are associated with the highest numbers of active bacteria compared to other root tissues, and likely select microbes in an active manner (DeAngelis *et al.*, 2005). Mature root zones featured a microbial community distinct from root tips (Massalha *et al.*, 2017). Their community included decomposers (Cardinale *et al.*, 2015), which could be involved in degradation of dead cells shedding from old

root parts (Jones *et al.*, 2009). Similarly, lateral roots were associated with distinct microbial communities, differing between tips and bases, as well as between different types of lateral roots (Kawasaki *et al.*, 2016).

The varying exudation characteristics of the various root sections may be a factor in the variable microbial colonisation of root tissues. The following illustration exemplifies this: Some plants that grow in highly nutrient-poor soils produce cluster roots, which are tightly packed lateral roots that release large amounts of organic acids (Neumann and Martinoia, 2002). Cluster roots have a specific rhizobiome that is characterised by low pH and a carboxylate-rich rhizosphere that is dominated by *Burkholderia* species that metabolise citrate and oxalate (Weisskopf *et al.*, 2011). In addition to organic acids, mature cluster roots also release isoflavonoids and enzymes that break down fungal cell walls, which reduce the number of bacteria and promote fungal sporulation (Weisskopf *et al.*, 2006). Together, cluster root exudates solubilize phosphate and control microorganisms so that they do not obstruct phosphate uptake. Beyond this illustration, preferred N transformational forms' geographical patterns of metabolite exudation are mainly unknown. Such patterns, according to our hypothesis, are present in all root systems. Overall, the structure of the rhizobiome is probably influenced by the spatially specified metabolite exudation by various root parts.

### Exudates are Diverse and Dynamic

Plant exudates shape the entire rhizospheric microbial communities. Overall, plants exude up to 20% of fixed carbon and 15% of nitrogen (Zahar Haichar *et al.*, 2016; Venturi and Keel, 2016), which includes an array of simple and complex polymer molecules such as sugars, organic acids, secondary metabolites and mucilage, respectively. Every plant produces exudates, however the quantity and nature of exudates from the roots vary. First, exudation is defined by the hosts' genotype. Second, exudation changed with plant developmental stage. Third, exudation is modulated by abiotic stresses. The amounts of exuded amino acids, sugars and organic acids changed in maize grown in phosphate, iron, nitrogen, or potassium deficient conditions. Therefore, there is a need to study on mechanism by which plants could modulate their interaction with microbes, and can be exemplified by the correlation between exudation patterns and rhizobiome variation.

### Characterized and Putative Plant Transporters for Exudation

A significant portion of exudates contain organic acids, which are nutrition for microorganisms. Although no importers have been identified to yet, the MATE (multidrug and toxic compound extrusion) and ALMT (Aluminum activated malate transporters) families' release of malate and citrate is one of the few known examples of a transporter involved in exudation. Metal ions and microorganisms frequently regulate the activity of members of both families. Uncharacterized members of the ALMT and MATE families

are the leading candidates for exporters of other organic acids because of their resemblance to described members, their location to the plasma membrane, and their role as proton antiporters.

### Root Exudates Signals and Their Molecular Mechanisms

Plant-plant interactions are mediated by signalling molecules found in root exudates. However, methodological constraints and the intricacy of root-soil interactions make it very challenging to clearly extract and identify signalling molecules within root exudates. With an increased understanding of root exudate roles in plant-plant signalling and developments in methodological strategies, several root-secreted signalling chemicals have been identified including ethylene, strigolactones (SLs), jasmonic acid (JA), (-)-loliolide and allantoin (Figure 2). Below, we briefly outline

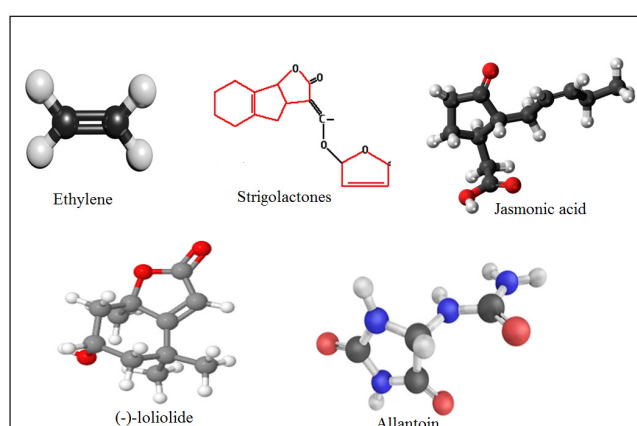


Figure 2: Molecular structure of rice root-secreted signalling chemicals

the roles of each chemical in plant root signalling.

Ethylene is a classical phytohormone and emitted from rice roots acts as a signal molecule with a dual role in neighbour detection and rhizosphere microbial assembly ((Baldwin *et al.*, 2006; Chen *et al.*, 2020). Besides ethylene, other volatile organic compounds emitted by plant roots may participate in belowground signalling interactions but they may not directly mediate root-soil interactions (Huang *et al.*, 2019). SLs are an essential class of newly identified plant signalling chemicals that reported with both external rhizospheric and internal hormonal functions in plants (Machin *et al.*, 2020). SLs are also important for rice tillering and have further biological functions as yet undetermined (Sang *et al.*, 2014). JA is a very common signalling chemical that elicits the production of defensive metabolites in plants against attacking microbes, feeding herbivores or plant competitors (Kong *et al.*, 2018). The role and mechanism of JA in aerial signalling interactions are well established (Baldwin *et al.*, 2006), but less information is available on JA as a root-secreted signalling chemical. A few studies have indicated that plants release JA from roots, inducing allelochemical production of neighbouring plants (Kong *et al.*, 2018). Therefore, JA may be an integral part of a general signal transduction system that regulates belowground plant-plant interactions. Besides, (-)-Loliolide, a carotenoid

metabolite, is the most ubiquitous monoterpenoid lactone in plant families (Grabarczyk *et al.*, 2015). Recent studies have shown that (-)-loliolide may act as a signalling chemical in plant defences against weeds (Li *et al.*, 2020), pathogens (Pan *et al.*, 2009) and herbivores (Murata *et al.*, 2019). Plants can detect (-)-loliolide released from the roots of interacting plant species and then respond by increased production of allelochemicals to benefit their own growth. Importantly, (-)-loliolide and its occurrence with JA shows a joint action in belowground signalling (Kong *et al.*, 2018). Therefore, (-)-loliolide may be a ubiquitous signalling chemical in plant-plant belowground interactions. Allantoin, a nitrogen-rich compound derived from a purine, is found in many plant species (Baral and Izaguirre-Mayoral, 2017; Nourimand and Todd, 2016). Allantoin not only participates in species interaction but has also been suggested to protect plants from abiotic stresses (Nourimand and Todd, 2016). In particular, allantoin can activate JA signalling pathway in an abscisic acid-dependent manner (Takagi *et al.*, 2016). Root-secreted allantoin alters root growth and shifts biomass allocation from roots to reproduction (Yang *et al.*, 2018), suggesting allantoin's potential role as a root signal of kin recognition (Figure 2).

### Conclusion and Future Directions

The formation of the rhizobiome and the role of the rice plants are still not well-known so far. Here, we addressed about a range of aspects, such as root morphology and root exudates, that influence the rice rhizobiome. The dynamic process of root exudation is probably reliant on numerous, largely unidentified transporters. The unique microbial communities that were discovered to be connected to specific root portions are most likely the result of exudation that is spatially specified. Chemotaxis, substrate specificity, competitiveness, and cooperativeness are a few factors that may affect how successfully DNRA microbial colonisation and rhizosphere stabilisation occur. In order to determine how the various substrates affect the rhizobiome and, ultimately, the health of the rice plant, innovative technologies for high-throughput screening of putative transporters against potential substrates are necessarily required. The customized design of unique N-transforming microbial communities would be made possible by a comprehensive understanding of the functions of a healthy rice plant rhizobiome.

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