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Development of a simple root model to study the effects of single exudates on the development of bacterial community structure

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ABSTRACT

The plant root interface is a hot spot for microbial activities. Root exudates are the key compounds that drive microbial performance. However quality and amount of root exudates are highly dynamic in time and space, thus a direct influence of a single compound on a microbial community composition is fairly impossible to study in nature. Therefore it was the aim of this project to develop an artificial root model (ARM), and investigate the influence of three compounds which have often been described as root exudates acting as model compounds for carbohydrates, organic acids and amino acids (glucose, malic acid and serine) on the development of bacterial communities and time on the ARM based on 16S rRNA derived TRFLP pattern. The ARM consisted of a slide covered with low melting agarose, where 8 different compounds which have been described as typical root exudates were embedded. The ARMs were incubated in soil for 2, 5, 9 and 20 days, before the analysis of the developed bacterial community structure was done. The bacterial community composition was in good agreement after 9 days of incubation of the ARM in soil with the root associated microflora of *Arabidopsis thaliana* shortly before flowering. The single compounds of the exudates mix had different effects on the development of ARM derived bacterial communities. Whereas the experiments where glucose was omitted gave no significant differences in the development of bacterial communities over time compared to the ARM where the standard mixture of exudates had been applied, there was a pronounced effect visible mainly after two days of incubation of the ARM in the experiments where no malic acid was added to the exudate mixture. At later time points ARMs with standard exudates' mixture and those where malic acid had been omitted, the bacterial community composition did not differ. The experiments where serine was omitted mainly induced shifts in the bacterial community composition compared to the ARM with standard exudates' mixture at the latest sampling time point (20 days of incubation).

1. Introduction

The rhizosphere has been defined as the soil compartment which is highly influenced by the plant root system (Hiltner, 1904). Compared to bulk soil, the rhizosphere is rich in compounds such as amino acids, organic acids, sugars, phenolic acids and other secondary metabolites as well as high molecular weight compounds such as mucilage (polysaccharides) and proteins (Bais et al., 2006), which are released by the plant as root exudates (Nguyen, 2009). Up to 40% of the carbon fixed by photosynthesis can be released by the plant root depending on the plant species, the plant development stage and the environmental conditions into the surrounding soil (Grayston et al., 1997; Lynch and Whipps, 1990).

Many heterotrophic soil microorganisms make use of these carbon rich compounds as a nutrient source leading to an increase in microbial biomass and activity in the plant soil interface, inducing the so-called rhizosphere effect (Sørensen, 1997). However several studies indicate that plants are able to shape the microflora in the rhizosphere due to root exudates by selective feeding of plant growth promoting microorganisms or by blocking soil born plant pathogens or unwanted competitors for nutrients like ammonia oxidizers by excreting substances with antimicrobial potential (Badri et al., 2009; Bais et al., 2006; Hartmann et al., 2009). The exudation pattern of plants therefore highly influences plant performance as well as plant health.

To study plant-associated microbial communities *in planta* based on directly extracted nucleic acids from the plant root interface often contain huge amounts of plant derived DNA. Especially plant mitochondrial DNA interferes with PCR analysis targeting microbial 16S rRNA genes. Hence, a strong PCR bias occurs towards plant-derived DNA where less abundant microbial DNA will not be amplified and minor variations in bacterial communities may not be detected. Therefore it is still a major challenge in microbial ecology to develop strategies to reduce the bias of plant derived DNA if microbial communities from the rhizosphere should be analyzed as most “universal” primers for 16S rRNA gene amplification also amplify mitochondrial 16S rRNA genes from plants (Chelius and Triplett, 2001; Edwards et al., 2007; Rastogi et al., 2010; Sakai et al., 2004).

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Furthermore the composition of plant-released compounds in the rhizosphere is highly dynamic in time and space and can vary even during day and night or between lateral roots and root hairs (Berg and Smalla, 2009). Therefore studies addressing a direct link between certain substrates and microbial community structure and function are only possible if well defined plant mutants are used. However even then, abiotic factors like light, water, soil type etc. may influence the plant phenotype in a quite unpredictable way due to the complexity of the plant genome and the different regulatory networks and consequently also influence root exudation.

Therefore several attempts have been performed in the past to set up more defined and reproducible models in order to study the effects of root exudates as mixtures or single compounds on the soil microflora. These approaches include microcosm studies where root exudates were directly applied to soil either as a single dose (Eilers et al., 2010) or repeatedly (Shi et al., 2011), as well as artificial root models delivering a continuously root exudate flow to the surrounding soil (Griffiths et al., 1998; Odham et al., 1986; Paterson et al., 2007).

However most studies have not taken into account that not only exudates drive the composition of microbial communities in the plant soil interface. Besides differences in water supply and physicochemical properties like pH or redox conditions compared to bulk soil, the presence of the plant root itself acts as a surface that facilitates the colonization of microbes and the establishment of microbial consortia (Morris and Monier, 2003; Rudrappa et al., 2008a, 2008b). In this study we have developed an artificial root model (ARM) using typical root exudates together with a surface for microbial colonization. The model which consists of glass slides was covered with agarose. The agarose was amended with different mixtures of various carbon rich compounds simulating root exudates. The slides were incubated in soil for different periods in order to monitor the development of bacterial communities by analyzing the diversity of the 16S rRNA gene using amplified DNA and T-RFLP fingerprinting. To calibrate our model system we compared the development of the bacterial community pattern in the artificial system with the rhizosphere microflora of the well studied model plant *Arabidopsis thaliana*. To test which exudates are key drivers for the microbial community development, we performed experiments where glucose (a representative of carbohydrates), malic acid (organic acid) and serine (amino acid) were omitted from the artificial exudate mix of the ARM. We hypothesized that subtracting glucose as an easy degradable carbon source would have the strongest effect on the microbial community structure.

2. Material and methods

2.1. The artificial root model

The artificial root model (ARM) consisted of glass slides (7,5 cm × 2,5 cm; Elka, Germany) covered with an agarose layer (1% low melting agarose; Serva, Germany) of 0.01 cm thickness containing a standard exudate mix with the carbohydrates glucose, fructose and sucrose (each 300 mM), the organic acids: malic acid and succinate (each 150 mM) and the amino acids: serine, cysteine and arginine (each 75 mM) to simulate the exudate composition of plants (Griffiths et al., 1998). In a second series of experiments, the influence of different substances on the development of microbial communities was studied; for this purpose glass slides were covered with an exudate mixture, but selected compounds were omitted (Glu(−)ARM, Mal(−)ARM, Ser(−)ARM). Slides covered only with agarose (without additional compounds) served as controls.

The solution simulating the root exudates had a concentration of 1 mg/ml of carbon and 900 μg/ml of nitrogen. To enhance the attachment of the agarose/exudate mix to the glass slides, the surface was roughened by sandblasting before the exudates were applied. Glass slides which were only covered with agarose served as control. In order to prepare the slides the low melting agarose was boiled to 60 °C and the exudates' mix was added; finally the slides were dipped in the solution and stored at 4 °C not longer than one week after preparation before they were used.

2.2. Incubation procedure of the ARM in soil

The prepared glass slides were incubated in pots (9 × 9 × 9.5 cm) with 300 g of sieved (2 mm mesh) and homogenized soil (loamy Cambisol; pH 6,0; 1,3% total organic carbon; 0,1% total nitrogen) collected from the surface layer (0–20 cm) of an arable field at the agro-ecological research center of the Helmholtz Zentrum München in Scheyern (Bavaria, Germany; latitude 48°29′51″N; longitude 11°26′39″E; elevation 455 m). Each pot contained five glass slides which were equally distributed in the pots and at least 1 cm away from the edge of the pot. The soil water content was kept at 50% of the maximum water holding capacity by irrigation with tap water throughout all experiments. ARMs were incubated at 18 °C and samples were taken after 2, 5 and 9 days of incubation. For some experiments the incubation period was prolonged and the last sampling was done 20 days after the start of the experiment. In addition pots without ARM slides (bulk soil) were prepared.

For each experiment and time point, 5 independent replicates (different pots) were analyzed. Agarose obtained from the 5 slides of the same pot were homogenized to reduce variability in each individual pot.

2.3. Extraction of the microflora from the ARM

In order to analyze the bacterial communities attached to ARM, the glass slides were gently pulled out of the soil and dipped in 1× PBS Buffer to remove adhering soil. Agarose from glass slide surface was harvested with a sterile cell scraper and stored at −20 °C until further usage. For each sample, 200 mg of the harvested agarose-matrix was melted completely for 10 min at 70 °C. After equilibration for 5 min to 42 °C, 2 U of Agarase enzyme (Fermentas, Germany) were added to each reaction mix and incubated for 30 min at 42 °C. Bacterial cells were harvested by centrifugation for 10 min at 2000 ×*g* and washed with 1× PBS Buffer. The samples were stored at - 20 °C until further use.

2.4. Cultivation of A. thaliana

To compare the bacterial community of the ARM with the root associated microflora of real plants, *A. thaliana* wild-type (Columbia 0) was used as a model plant. Pots (9 × 9 × 9.5 cm) with 300 g sieved soil from Scheyern (described above) were grown under the same conditions as the ARM. Approximately 30 seeds of *A. thaliana* were planted per pot. After two weeks of incubation under comparable conditions as described above, the number of germinated seedlings was adjusted to 5 plants per pot. After approximately four weeks of growth (prior to flowering) *A. thaliana* plants were harvested by using sterile forceps. The root samples were obtained by cutting the above-ground plant and washing roots in 1× PBS buffer to remove attached soil particles. Again samples from one pot were pooled. 5 pots served as independent replicates. The samples were stored at −20 °C until further use.

2.5. Bacterial community analysis

DNA was extracted from harvested cells from the ARM and 0.3 g of the rhizosphere samples of *A. thaliana* respectively. The FastDNA Spin Kit for soil (MP Biomedicals, Germany) was used according to manufacturer's protocol for extraction of genomic DNA. DNA extracts were quantified with a Nanodrop 1000 Spectrophotometer (Peqlab, Germany).