Geophysical Research Abstracts
Vol. 15, EGU2013-7810-1, 2013
EGU General Assembly 2013

Understanding Aquatic Rhizosphere Processes Through Metabolomics and Metagenomics Approach

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The aquatic rhizosphere is a region around the roots of aquatic plants. Many studies focusing on terrestrial rhizosphere have led to a good understanding of the interactions between the roots, its exudates and its associated rhizobacteria. The rhizosphere of free-floating roots, however, is a different habitat that poses several additional challenges, including rapid diffusion rates of signals and nutrient molecules, which are further influenced by the hydrodynamic forces. These can lead to rapid diffusion and complicates the studying of diffusible factors from both plant and/or rhizobacterial origins. These plant systems are being increasingly used for self purification of water bodies to provide sustainable solution. A better understanding of these processes will help in improving their performance for ecological engineering of freshwater systems. The same principles can also be used to improve the yield of hydroponic cultures.

Novel toolsets and approaches are needed to investigate the processes occurring in the aquatic rhizosphere. We are interested in understanding the interaction between root exudates and the complex microbial communities that are associated with the roots, using a systems biology approach involving metabolomics and metagenomics. With this aim, we have developed a RhizoFlowCell (RFC) system that provides a controlled study of aquatic plants, observed the root biofilms, collect root exudates and subject the rhizosphere system to changes in various chemical or physical perturbations. As proof of concept, we have used RFC to test the response of root exudation patterns of *Pandanus amaryllifolius* after exposure to the pollutant naphthalene. Complexity of root exudates in the aquatic rhizosphere was captured using this device and analysed using LC-qTOF-MS. The highly complex metabolomic profile allowed us to study the dynamics of the response of roots to varying levels of naphthalene. The metabolic profile changed within 5mins after spiking with 20mg/L of naphthalene and reached a new steady state within 72 hours. An active microbial biofilm was formed during this process, which was imaged by light microscopy and confocal laser scanning microscopy and showed active changes in the biofilm.

We have begun to unravel the complexity of rhizobacterial communities associated with aquatic plants. Using fluorescence *in-situ* hybridization (FISH) and Illumina Miseq Next Generation Sequencing of metagenomic DNA, we investigated the root-associated microbial community of *P. amaryllifolius* grown in two different water sources. The community structure of rhizobacteria from plants grown in freshwater lake or rainwater stored in tanks are highly similar. The top three phyla in both setups belonged to *Proteobacteria, Bacteriocedes* and *Actinobacteria*, as validated by FISH analyses. This suggests that the rhizosphere have an innate ability to attract and recruit rhizobacterial communities, possibly through the metabolic compounds secreted through root exudation. The selection pressure through plant host is higher compared to environmental pressures that are different between the two water sources. In comparison with the terrestrial rhizosphere, the aquatic rhizosphere microbiome seems more specialised and has a high influence by the host. We are using these findings to further understand the role of microbes in the performance of freshwater aquatic plants.