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***Rice microbiome and rice plant health***

**Summary:**

Crops are constantly exposed to biotic and abiotic stresses related to drought, climate change and pests. The microbial community that lives in association with the plant is called microbiome and is considered as a second plant genome (Berendsen et al 2012; Schlaeppi et al, 2015); it plays a crucial role in plant health, nutrient uptake and stress tolerance. Plant microbiomes can be a significant ally for the plant in controlling colonization/infection by plant pathogens. Novel methodologies now allow the analysis of total microbial populations thus opening the avenue on the role of microbiome in plant disease. Rice is the most important cereal crop in the world and it is currently unknown what is the potential effect of the microbiome on two important bacterial diseases of rice: bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* and the bacterial foot rot, caused by *Dickeya zeae*. Bacterial leaf blight is a prevalent and destructive disease which affects millions of hectares throughout Asia, Africa and Americas, while bacterial foot rot and other soft rots diseases caused by the genera *Dickeya* are spread in Europe. Thanks to a collaborative research program between the Bacteriology Laboratory of ICGEB and the Institute of Microbiology and Biotechnology of the National University of Vietnam, this project will investigate the role of the microbiome in rice related to *Xanthomonas oryzae* pv *oryzae* and *D. zeae* resistance and susceptibility. Comparing rice microbiomes of healthy and infected rice from the same location could provide insight on the potential role of microbiome in rice resistance towards bacterial pathogens increasing the opportunity to isolate and characterize natural biocontrol agents. Furthermore the study of the plant microbiome at the site (pathobiome) of infection of these two important rice diseases could reveal potential commensal/resident bacteria that can cooperate with the pathogen: the concept of monostrain/monospecies infections is changing and different studies indicate interactions between pathogens and the residential microbiota (Venturi et al 2012). Results obtained here will allow the understanding of rice microbiome at the global level since the study will be conducted on rice samples from Italy and Vietnam thus providing avenues for the design of microbial solutions for a more sustainable rice agriculture with a decreased use of pesticide and an increased attention to human and environmental health.

**Objectives:**

A major challenge of modern agriculture isto increase yield and reduce the impact on the environment and human/animal health by reducing the use of chemical additives such as fertilizers, herbicides and pesticides. The aim of this proposal is to harness the rice endosphere bacteriome and its interactions with the two rice pathogens (*Xanthomonas oryzae* and *Dickeya zeae*) to enable progress in crop improvement.

The project will have four main objectives (i) determine core endophytic bacterial members of healthy and infected rice (ii) perform endosphere microbiome analysis at the sites of rice infections by *X.oryzae* pv *oryzae* and *D. zeae* to determine if there are bacterial rice commensal partners of the two pathogens, (iii) investigate the genetic mechanisms of this pathogen-commensal interaction and iv) isolate from healthy plants, bacteria able to antagonize the two rice pathogens and test the most promising ones, via the microencapsulation method, for their efficacy in green house experiments and in filed trials. The first 3 objectives have a basic research value being devoted to reveal healthy microbiomes, potential commensal/resident bacteria playing a role in the disease process, and allowing parallelisms and comparisons of rice healthy and diseased microbiomes in two different growing areas. The the third objective, involves the isolation and characterization of natural biocontrol agents against the pathogens considered, follows an idea-to-application research scheme promoting lab-to-market approach

**Methodology:**

The project will involve 1) the rice microbiome analysis, 2) the pathobiome of pathogen-resident bacteria interaction and mechanisms and 3) the screening for natural biocontrol agents against *X. oryzae* and *D. zeae*

1. Microbiome studies of healthy, diseased and infection site samples. For the determination of rice microbiomes, healthy and *D. zeae* infected rice will be harvested and studied in Italy, where *D. zeae* represents an emerging epidemic pathogen, while healthy and *X. oryzae* pv*. oryzae* infected rice will be harvested and studied in Vietnam, where this pathogen is endemic. Bacterial DNA will be extracted and processed for 16S-ITS rDNA amplicon bank construction. OTUs (Operational Taxonomic Units) will be deciphered by NGS (Next Generation Sequencing) technologies and processed with different soft-ware for the enrichment, network and functional analysis. From the diseased samples, the determination of the microbiome at rice infections sites of *D. zeae* and *X. ozyzae* pv. *oryzae* will also be performed. Microbiome analysis of healthy and diseased plants will reveal potential role of the resident microflora in resistance/susceptibility to the two diseases and provide avenues for their possible control. Pathobiome analysis of infection sites on the other hand will identify pathogen-associated bacteria which cooperate with the pathohgen.
2. Study of pathogen-pathogen associated bacteria interaction mechanisms. Putative pathogen–associated bacteria identified by the pathobiome analysis of the infection site will be isolated and collected in a culturable bacterial collection of representative strains which could be involved in disease development with *D. zeae* and *X. oryzae*. *In planta* virulence tests using co-inocula will be performed with these bacterial isolates together with the pathogen in order to establish possible interspecies interactions, synergy and cooperativity in disease development and genetic studies will be conducted in order to elucidate the mechanisms of these interactions.
3. Screening for natural biocontrol agents against *X. oryzae* and *D. zeae*. A collection of culturable bacterial endophyte enriched in healthy plants will be generated and screened *in vitro* for antimicrobial features against *D.zeae* and *X. oryzae*. Several putative biocontrol endophytes will then be tested *in planta* for their ability to control the two bacterial pathogens (*D.zeae* in Italy, *X. oryzae* in Vietnam). Different methodologies of inoculation will be tested: seed and/or root submersion, spray and microencapsulation (coating of bacteria with a biodegradable polymer to ensure a slow release of the bacteria in the soil by pore-assisted diffusion or degradation of capsules). This last method in particular is very promising since encapsulated bacteria will be released in the plant-soil microenvironment slowly and continuously and they are expected to possess an extended shelf life at room temperatures. Furthermore this method reduces the risk of bacterial transport in water and soil as well as minimizes the spread of hazardous residues in various environments.

**Expected results:**

Research on plant microbiome interactions will point towards a deeper understanding of agro-ecological niches. Results from this work will highlight the role of interactions among rice and inhabiting bacteria providing information on unexplored diversity. Core and variable endosphere microbiota (core: observed across all environments; variable: either environment- or genotype- specific) of the roots and aerial parts of healthy and diseased rice will be analysed and differences between diseased and healthy rice will be evidenced. These OTUs could be responsible for making the plant either more resistant or susceptible to the bacterial pathogens. This project will provide insights about the influence of the endosphere microbiome on two important rice diseases: more specifically on the role of the bacterial endosphere community in controlling *Dickeya zeae* and *Xanthomonas oryzae* pv. *oryzae* infections. Bacterial endophytes, which could play a role in anthagonize this process, will be isolated and tested for being potential biocontrol via the microencapsulation methodology. Similarly the impact of the microbiome in positively influencing the two diseases will also be studied. This will provide evidence of possible harmless bacterial which team up with the incoming pathogen playing a role in the disease process. In future, microbiome inocula in cereal crops will be considered as management practices to harness microbiological soil functions as a whole.

**References**

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