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**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1529744> since 2015-12-18T11:28:31Z

*Published version:*

DOI:10.1111/1758-2229.12220

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# UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on:***

*Questa è la versione dell'autore dell'opera:*

*[[Environmental Microbiology Reports](#), 7(1): 15-17, 2015, DOI: 10.1111/1758-2229.12220]*

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:*

*[<http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12220/full>]*

Crystal Ball

## **From environmental microbiology to ecogenomics: spotting the emerging field of fungal–bacterial interactions**

Paola Bonfante

When I attended the meeting on ‘Molecular Microbe–Plant Interaction’ in Rhodes (6–10 July 2014) this summer, I had the feeling that a relevant change was taking place. The meeting is traditionally devoted to investigations of plant–microbe communication and gives the most attention to the molecular relationships between plants and pathogenic or symbiotic microbes, to signal transduction and to the characterization of virulence factors in bacteria and fungi. This year, however, the 2014 Rhodes meeting opened its doors to a plenary lecture entitled ‘The Phyllosphere Microbiome’ presented by Julia Vorholt (ETH Zurich) and to sessions like ‘The Plant Microbiome’ and ‘Evolution and Ecology’, which were well received by large audiences. Just a couple of examples from these sessions: starting from their previous characterization of the microbiota associated with *Arabidopsis* (Bulgarelli et al., 2012), Paul Schulze-Lefert demonstrated how *Arabidopsis thaliana* shares a largely conserved microbiota with its relatives, revealing quantitative species-specific footprints (Schlaeppi et al., 2014). Moving from characterization to function, the Schulze-Lefert group has started a systematic analysis of root microbiota functions under laboratory conditions. Interestingly, the first step required the isolation of 70% of the members of the *A. thaliana* root-enriched microbiota as pure cultures and the further generation of annotated whole-genome sequence drafts for all the cultivated rhizobacteria. Moving above ground, Vorholt pointed out in her lecture that leaves represent one of the largest terrestrial habitats for microorganisms. Also, in this case, after the identification of the main bacterial groups, the researchers aimed to understand the general strategies by which bacteria adapt to the phyllosphere, a very special habitat involving exposure to drastic, always-fluctuating conditions. Most of the presentations related to the microbiota field led to similar conclusions: as observed for the human microbiota, the bacterial root microbiota appears to have a major role in promoting plant growth and providing indirect protection against pathogens. Interestingly, to verify such hypotheses, traditional, cultivation-dependent approaches have to be examined, in addition to data from the much more recent cultivation-independent analyses.

What does this mean? I reasoned that the use of similar omics approaches in environmental microbiology and in molecular biology-based researches has led to the merger (or at least to a closer approach of) these fields, which only some years ago were very far apart. Questions like ‘which host genetic factors shape microbial populations in a given environment’ and ‘how does a plant interact with a plethora of microbes, activating or not activating its immune system’, may have more convergent responses when expertise from molecular ecology, genomics transcriptomics and metabolomics converges. It seems that the concept of systems biology has also incorporated knowledge of microbial ecology.

In this environmental-genomics context, the emerging field of fungal–bacterial interactions is assuming a new dimension. On the one hand, studies of the plant microbiota have mostly focused on bacteria, with only a few recent papers demonstrating that fungi play equally important and widespread roles (Orgiazzi et al., 2013; Smith and Peay, 2014). The obvious next step with such new data is to understand whether these two crucial microbial components interact, and, if the reply is positive, how they interact. Indeed, the novel question of fungal–bacterial relationships is not exclusive to the plant microbiota: Frey-Klett and colleagues (2011) produced a very accurate summary demonstrating that bacterial–fungal interactions are fundamental to agriculture, clinical and natural environments, as well as in food technology. Their novel results also demonstrated how fungi and bacteria coexist in the human body. What is our microbiome doing and how does it interact with bacteria? In their Nature paper, Findley and colleagues (2013) elegantly demonstrated that physiological attributes and topography of human skin differentially shape the pathogenic and commensal communities of fungi and bacteria. They conclude that the interaction between these microbes is crucial to the maintenance of human health and to disease pathogenesis. A Jacques Monod conference ([http://www.cnrs.fr/insb/cjm/2013/Frey-Klett\\_e.html](http://www.cnrs.fr/insb/cjm/2013/Frey-Klett_e.html)) devoted to the topic revealed an unexpectedly broad and interdisciplinary audience: soil microbiologists like Wietse de Boer and Dirk Van Elsland in The Netherlands alternated with speakers from hospitals and medical schools (Deborah Hogan and Gordon Ramage, United Kingdom) as well as speakers from food and wine departments, shedding new light on the relevance of interactions between prokaryotes and eukaryotes.

Of course, the crucial open questions involve the mechanisms behind these inter-domain interactions, as well as whether specificity exists and the identities of the involved molecular determinants. Results from the A. Brackage and C. Hertwech laboratories in Jena offer strong evidence on the communication between soil bacteria and fungi. The plant-pathogenic fungus *Rhizopus microsporus* hosts a bacterium, *Burkholderia rizoxini*, which is involved in the production of the pathogenicity factor rhizoxin, exclusive of the interaction (Partida-Martinez and Hertweck, 2005). The intimate contact between a soil *Streptomyces* and the human-pathogenic *Aspergillus fumigatus* leads to the activation of novel metabolic pathways, regulated by activator genes as well as by epigenetic factors (Nützmann et al., 2011; König et al., 2013). Such discoveries suggest that close fungal–bacterial encounters may have unpredictable consequences for diverse aspects of fundamental and applied biology.

Understanding fungal–bacterial interactions may also offer clues to new fields: for example, we are used to thinking that symbiosis is the result of two organisms that live together. However, an increasing body of knowledge demonstrates that bacterial communities often form crucial components of a symbiosis. Lichens offer a good example of this claim: algae and fungi strictly interact with many diverse bacterial communities, which probably also exert physiological functions (Grube et al., 2014). Systems biology approaches will be instrumental in dissecting the specific roles of all the partners. Similarly, mycorrhizas can be described as tripartite interactions where bacteria colonize the hyphal surface and act as helper bacteria (Frey-Klett et al., 2007), or may live inside the cytoplasm of arbuscular mycorrhizal fungi (Bonfante and Anca, 2009; Bonfante, 2014). The discovery that a very ancient and still enigmatic group of endobacteria described as Mollicutes-related endobacteria (Mres) occurs inside the spores of most Arbuscular Mycorrhizal (AM) fungi (Naumann et al., 2010; Desirò et al., 2014) opened the door for many studies on the biodiversity of these endobacteria, which probably represent the top of a still-hidden iceberg. The discovery that Mres are present not only inside AM fungi but also inside the hyphae of other basal fungi,

like the Mucoromycotina *Endogone*, opens new scenarios on the evolution of plant-associated fungi and bacteria (Desirò et al., 2015).

In conclusion, a combination of omics approaches, advanced phylogenetic studies and genetics experiments, all inserted in an evolutionary biology framework, will allow us, on the one hand, to extend our knowledge on bacterial–fungal biodiversity, and, on the other, to better understand the mechanisms underlying these interactions. Mixing environmental approaches and functional-genetics studies will be instrumental to providing a deeper understanding. Environmental Microbiology is already on this track.