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Interactions bactéries-champignons : un domaine de recherche fédérateur pour la microbiologie fondamentale et appliquée

Bacterial-fungal interactions: a federative field for fundamental and applied microbiology

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Rapport sur la conférence
Conference report
General description of the meeting

Historically, the classical separation of microbiological research between bacteriologists and mycologists has led to the study of bacteria and fungi in axenic settings. This compartmentalization has overlooked the fact that in many environments bacteria and fungi coexist and interact. Furthermore, these bacterial-fungal interactions (BFIs) often have important ramifications for the biology of the interacting partners. In recent years, research in this area has developed significantly both in breadth and depth. Contemporary studies have revealed that fungi and bacteria often form physically and metabolically interdependent consortia that harbour distinct properties from their single component. These reports have also highlighted the multiple practical relevancies of these interactions (FIG 1) to an exceptionally diverse variety of fields including agriculture, forestry, environmental protection, food processing, biotechnology, medicine and dentistry.

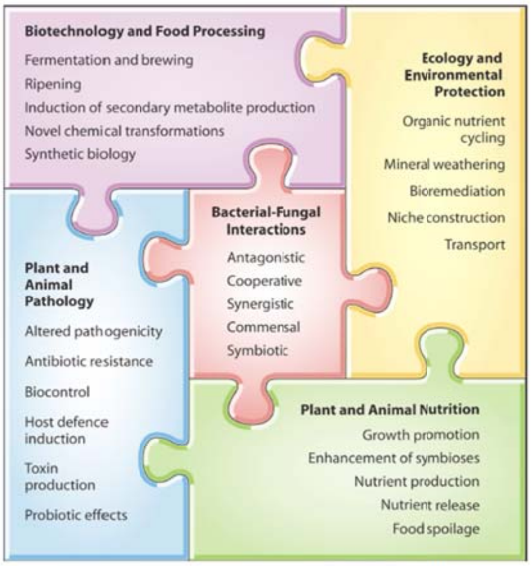

FIG. 1. Relevance of BFIs to different areas of scientific study.

In each of the disciplines to which BFIs are important research has progressed somewhat differently. This is likely a reflection of their distinct contexts but also a reflection of a lack of interaction between researchers working in these different areas. However, many commonalities exist between the BFIs in these different settings and a greater appreciation of them would help scientists to identify potentially relevant studies outside of their normal speciality, a step that is often important when searching for new ideas, hypotheses, methods or collaborators.

In the Jacques Monod Conference, we attempted to bridge this gap by encouraging microbiologists from all these fields to meet and exchange their knowledge and competencies, and to stimulate them to consider other systems in which parallels to their own field may exist.

By not focusing exclusively on one area of application, we seeked to achieve a novel unifying perspective on BFIs that enables the identification of fundamental themes, mechanisms and areas of mutual interest. Given the burgeoning research field, it was an ideal time to attempt such conference, and to stimulate the creation an international scientific community on this research field.

The Jacques Monod Conference was organized around four major themes: (i) the applications of fungal-bacterial interactions including the effects of bacterial-fungal consortia on plant/animal hosts and the environment, (ii) the physical complexes between bacteria and fungi, (iii) the ecology of the interactions between bacteria and fungi, and (iv) the molecular mechanisms of the interactions and communication between bacteria and fungi. To stimulate discussions and reveal commonalities and differences that exist in different BFI contexts, each session gathered scientists coming from distant fields (from soil to human). Today, mankind faces many practical challenges relevant to BFIs that are important for health, food security and sustainable ecosystem management. At the same time technological developments look set to transform our ability to address these problems through science. So far soil, plant, food and animal bacteriologists and mycologists have neglected each other's research field; we think that this conference has contributed to closer collaboration between them.

BFI is settings of economic and cultural importance

Many beneficial and detrimental interactions between bacteria and fungi drive the success and/or failure of products of agricultural, economic or cultural importance. In the following section, we describe the scope of BFIs in these different areas.

The BFIs that were the most studied so far are those that occur in the rhizosphere. Bacterial-fungal associations are a fundamental part of plant ecology as they impact multiple crucial processes such as plant nutrition, symbiosis development, plant defense against pathogens or detoxification of soils. For example, Tarkka and collaborators demonstrated how ectomycorrhizal fungi select for specific bacterial communities which protect mycorrhizas and mushrooms from parasites through the production of secondary metabolites that only target parasites. When colonizing the roots of oaks, these bacteria also stimulate the plant defenses which lead to a reduction of the infection of leaves by pathogens. Potential for biocontrol of phytopathogenic fungi by specific bacterial strains (Zachow) and insight into the roles of BFIs in plant nutrient acquisition (Trap) directly highlight the immediate relevance of this work. In the search for new sustainable approaches to manage plant crop production and protection, these research areas offer perspectives on new ways to manage agricultural and forest ecosystems.

BFIs also impact the production and stability of agricultural products in different ways. Alexandre discussed the role of bacteria in malolactic fermentation, a secondary fermentation that occurs after the yeast-driven primary fermentation. The malolactic fermentation usually occurs either spontaneously or after inoculation with selected bacteria after alcoholic fermentation. The sequential and concurrent interactions between fungi and bacteria are not yet well understood, but key to success of this process. One approach to understanding these interactions is through the characterization of the metabolic profiles of stimulatory strains were compared to those from less stimulatory ones using unbiased mass spectrometry techniques. As discussed by Chaucheyras-Durand, metabolic interactions between fungi and bacteria are also key mechanisms for optimal rumen function, and an understanding of these processes may lead to improved feed additives for ruminents.

In contrast to the beneficial BFIs in mycorrhizal interactions, Preston described *Pseudomonas tolaasii* pathogenesis towards mushrooms. *P. tolaasii* produces a pore-forming lipopeptide toxin, tolaasin, which plays a central role in mushroom pathogenesis. Interestingly, the genes encoding this toxin are non-phyletic in their distribution within the *P. fluorescens* complex. Using functional and genomic characterisation of two mushroom pathogens within the pseudomonads, efforts are geared towards being able to detecting and controlling virulence towards mushrooms by *Pseudomonas* species.

Moving beyond antagonistic interactions between a bacterium and a fungus (discussed further below), the concerted action of BFIs can lead to harm. This is demonstrated in opportunitistic infections in the human body, where interactions between two species makes one species more harmful to the host (discussed further below). A negative role for BFIs may also be evident in the challenges associated with the maintenance of historical documents on parchment. It is common for parchment to become “infected” by microbial species that lead to dark pigmentation and destruction of the parchment itself. Work by Pinzari and colleagues clearly show the presence of diverse bacterial and fungal species in these damaged regions of parchment using both community profiling techniques and scanning electron microscopy.

Future studies are aimed determining how the BFIs contribute to the damage, and discovering new ways to prevent or limit infection.

The physical interactions between bacteria and fungi

One of the important themes of the conference was the physical interactions between fungal hyphae and bacteria, and this topic was prominant in talks focused on the environment and in the medical realm. In humans, *Candida albicans*, one of the most commonly encountered fungi within the microbiota, is both a benign commensal and an important and potentially lethal opportunistic pathogen. A number of presentations described the interactions between *Candida albicans* and bacterial pathogens. For example, Bastiaan Krom presented the characterization of the interactions between *C. albicans* and *Staphylococcus aureus*, a Gram positive bacterium, using variety of methods including genetic analyses and atomic force microscopy. These studies revealed differential interactions with yeast and hyphal forms of the fungus. Importantly, the physical interactions between *Staphylococcus aureus* and *C. albicans* contributed to the virulence of *S. aureus* by promoting the migration of the bacteria through tissues and promoted disseminated *S. aureus* infections. This group also demonstrated that the macrophage-mediated clearance of *S. aureus* may be facilitated upon bacterial colonization of *C. albicans* hyphae.

The physical interactions between *C. albicans* and Gram-negative bacteria were also presented. The groups of Hogan and Mylonakis described physical interactions between *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and these studies were performed in *in vitro* liquid and plate associated cultures, and in the context of invertebrate host models for virulence such as *Caenerhabditis elegans*. As discussed below, the physical interactions are central to the antagonistic interactions between *P. aeruginosa* and *C. albicans*. Physical interactions between another medically relevant Gram-negative bacterium, *Salmonella typhimurium*, with the fungus *Aspergillus niger* were also described by Roberto Balbontin. The physical interactions between these species only takes place if the fungus is alive and the interaction was stable for weeks. While there is no evidence for growth promotion or inhibition in the context of the interaction, the bacteria conferred protection to the fungus. These interactions occurred *in vitro* and in the context of plant roots, perhaps providing some insight into where these interactions naturally occur and where they are biologically relevant.

Physical interactions were also described between environmental fungi and bacteria. One of the important developments in the field was the mounting evidence for the roles that physical associations between bacteria and fungi play in the movement and dispersal of bacteria and chemicals in soils. Evidence, such as that presented by Junier, suggests that these networks of fungal hyphae are particularly important in unsaturated soils where there are air spaces that bacteria would not traverse in the absence of fungal « highways ». Using both laboratory mesocosm studies and computer modeling, Berthold and Wick showed the emergent properties that arise through BFIs. For example, the increased frequency of contact between bacteria moving across fungal hyphae led to increased conjugation events providing insight into factors that are important in the facilitation of horizontal gene transfer. Studies also demonstrated that fungal highways are relevant dispersal routes for oxalotrophic bacteria, thus stimulating the degradation of calcium oxalate in insoluble. Importantly, it highlights the importance of the often underestimated role of fungi-bacteria interactions in biogeochemical processes.

Last, the potential presence of bacteria living inside fungi has been often underestimated and was addressed in this session. Bonfante *et al.* described a high genetic diversity among bacteria colonizing arbuscular fungi and showed that these bacteria can alter the physiology of the fungi including central metabolic pathways involved in phosphate and nitrogen metabolism. These intimate associations appeared early in the evolution as they were retrieved in fungi colonizing liverworts and have led to coevolution processes including genome size reduction. As developed by Pawlowska, these endosymbiotic interactions can be used as models to understand mechanisms of co-evolution.

The molecular mechanisms of the interactions and communication between bacteria and fungi.

Many different molecular mechanisms were shown to participate in different BFIs in different settings. One key environment represented at this conference in which molecular mechanisms of BFIs were deeply studied was the environment within the lungs of individuals with cystic fibrosis (CF). CF is a genetic disease that most often leads to chronic lung infections that are incurable and cause progressive lung damage and ultimately respiratory failure. Ramage and Robertson both presented chemical interactions between *Pseudomonas aeruginosa* and *Aspergillus fumigatus*. Data from human subjects was considered in light of results from *in vitro* studies and experiments performed in an invertebrate model for virulence indicated changes in *P. aeruginosa* in the presence of *A. fumigatus* and inhibition of the fungus by the bacterium. O'Gara reported that the bile aspiration is capable for modulation of microbial virulence in the context of chronic CF lung infections. Biofilm formation in *Candida albicans* and *Aspergillus fumigatus* was modulated by bile, while cell- cell communication with *P. aeruginosa* was also perturbed, apparently in favour of the bacterial pathogen. In *P. aeruginosa*-*C. albicans* interactions, the action of a bacterial phenazine, 5-methylphenazine-1-caboxylic acid (5-MPCA), and its accumulation within fungal cells led to a change in metabolism in favour of ethanol production. The fungally-produced ethanol promoted an increased in bacterial biofilm formation through activation of the *P. aeruginosa* WspR pathway. Collectively, these interactions may describe why the presence of fungi in the CF lung has been associated with worse lung function in several publications.

The conference also highlighted many other types of chemical signals, exchanges, and communication. For example, Prado highlighted interactions between the endophytic fungus *Paraconiothyrium* and the bacterium *Bacillus subtilis,* as well as the high diversity of secondary metabolites which are involved in these interactions. One particularly interesting interaction is that in which the toxin rhizoxin is produced by a bacterium within a fungus, but is modified by its fungal host (Partida-Martinez). Han and colleagues described compounds produced by *Rhizobium radiobacter*, including AHL-signaling compounds and related derivatives that could be involved in synergistic interactions with *Piriformospora indica* and the host plant. AHL-deficient *Burkholderia* spp. mutants showed a strong reduction in chitinolytic, proteolytic and glucanolytic activities and a highly reduced virulence on *Agaricus bisporus*. The action of extracellular enzymes was a theme across multiple BFIs. Loper, through genetics, biochemistry and genomics, characterized the role of compounds such as glucinol in *Pseudomonas protegens* interactions with fungi. BFIs between *Laccaria* and *Burkholderia terrae* cause changes in exudates in ways that are very likely ecologically important (Van Elsas). *P. fluorescens* suppression of *Gaeumannomyces graminis,* the "take-all" fungus is also likely in part through the effects of metabolic products (Sarniguet). In addition to the water soluble compounds that mediate many microbe-microbe signaling interactions, volatile compounds were also discussed. De Boer and Iacobellis presented work on the roles of VOCs in the interactions between microbes in the context of wood decomposition and rhizosphere interactions.

In addition to small molecules, there are other types of molecules involved in BFIs. For example, the type III secretion system is involved or suspected to be involved in translocating proteins from bacterium to fungus in addition to well characterized roles for this system in effector delivery to plants and mammalian cells as highlighted in several presentations (Génin, Pivato, Van Elsas, Loper, Preston). Other secretion systems, such as type six secretion systems, may also play a role (Saniguet). Ipcho and colleagues also introduced the interesting concept of MAMPs in which fungi respond to bacterial products such as lipopolysaccharides in addition to the more specific interactions described above. The role of IncP1 plasmids in mediating horizontal gene transfer between bacteria in the rhizosphere was discussed as a way for bacteria to rapidly adapt to different environments (Zhang). Community structure may also be shaped by fungus-fungus interactions, such as those shaped by STAND proteins which mediate non-self recognition and can trigger cell death (Saup).

While the physical interactions were often discussed separately from the molecular mechanisms by which fungi and bacteria affect one another, these two processes are intimately linked. For example, for *P. aeruginosa* 5MPCA to have an effect on *C. albicans,* close proximity such as that attained in biofilms of bacteria growing on fungal surfaces, is required. This is because the 5MPCA molecule is highly reactive and thus labile. The spatial relationship between the bacterial producer and the fungal target drives the outcome of this antagonistic interaction. Similarly, quorum sensing regulated processes, such as those described above, are likely strongly promoted by biofilm formation by virtue of the high local densities of bacterial cells.

The ecology of the interactions between bacteria and fungi

In the past 20 years, technologies for profiling both bacteria and fungi in soils have been developed and the advent of relatively inexpensive sequencing has allowed for unprecedented insight into microbial community composition and how it changes over time or in response to environmental stimuli. Cébron reported on the bacteria and fungi in PAH- and heavy metal-contaminated soil coming from a coking plant wasteland. In *in situ* experiments, different bacterial and fungal communities were observed to change with time. These community profiling approaches were complemented by the assessment of the diversity among the PAH-ring hydroxylating dioxygenase genes. As the introduction of foreign strains for the purposes of bioremediation has been met with challenges, studies such as these are likely to lead to insights into how a natural community can be modulated to improve biotransformations of importance.

Another BFI of ecological importance is that which occurs in the context of lichens. Grube and colleagues presented a metagenomic analysis-derived view of the roles that bacteria play in nutrient acquisition and vitamin or cofactor production. Other potential roles for bacteria were revealed through these types of analyses. One of the exciting outcomes of these studies was the revelation of the functional diversification strategy within lichens in multi-player networks. This strategy may be important in the long term survival of these multispecies communities.

Impact of high-throughput sequencing technologies on the study of bacterial-fungal interactions

The study of bacterial-fungal interactions has been profoundly impacted by the arrival of high throughput sequencing technology. The availability of genome sequences for different microbial species, the potential for RNA Seq analyses of single species and mixed species cultures, and the ability to profile communities has provided great insight into which species are likely important in specific environments. This is the key step in determining the molecular mechanisms that drive functioning of different ecosystems.

In terms of community profiling, these new generation sequencing techniques have been applied to probe BFIs in different communities and ecosystems such as the degrading wood microbiome (Hervé), contaminated soils (Giebler), the Black truffle ecosystem (Deveau) and snow pack (Larose) ecosystems, and in association with grapevine (Pinto) and desert plants such as cacti and agave (Partida-Martinez). These descriptive approaches highlighted the complexity and the diversity of bacterial and fungal communities, notably their temporal dynamic and the existence of multiple biotic and abiotic factors which can influence them, emphasizing the importance of studying bacterial-fungal interactions in mixed microbial communities. The metatranscriptomics of bacteria-fungi interactions in a naturally disease suppressive soil of Chapelle and colleagues provided a look towards the future in which the community analyses can be used to understand the transition between suppressive and non-suppressive soils.

There is much excitement about the potential for combining community-wide profiling technology with techniques that provide spatial resolution such as fluorescence *in situ* hybridization (FISH) analysis using phylogenetic probes. The use of the technology was demonstrated in the analysis of *Sebacinales* interactions with mycorrhizal ecto- and endo-fungal bacteria. Another technology that provides spatial information is mass spectrometric imaging of small molecules, such as the antifungal compound tolaasin, *in situ* as demonstrated by Scherlach and colleagues. Zachow also demonstrated the power of combining ‘omic’ technologies with genetics, microscopy, and laboratory models. The “omics” approaches will continue to lead to the development of more powerful laboratory models through a cycle of analysis of naturally occurring communities and model systems. The data from both of these approaches will also be key in developing computational models that provide insight into how these communities respond to different perturbations.

Future Directions

The conference organization was excellent thanks to the hard work of the staff at the CNRS. Many stimulating discussions occurred during the sessions as well as over the delicious meals.

- The possibility to write a collective opinion paper has been discussed at end of the conference.
- Inclusion of insect related systems that were missing because Cameron Currie (USA) who was contacted as an invited speaker was not available to attend the conference, but should perhaps be included to the opinion paper.
- Inclusion of some of the exciting studies in cheese microbiology
- Inclusion of speakers that can highlight cutting-edge methods of interest to the BFI community to contribute to the next meeting.

- Attendees discussed the potential for moving the meeting to the summer time to avoid travel delays due to weather, and seasonal epidemics.
- Create a ResearchGate profile for the community who attended the conference, to exchange information, job proposal, publications and to promote interfield collaborations.

Summary of the conference

A tribute to bacteria-fungal interactions

The first International Jacques Monod conference took place in Roscoff (Bretagne) from 7 to 11 December. This conference co-organized by CNRS, INRA and Labex ARBRE was titled « Bacterial-fungal interactions: a federative field for fundamental and applied microbiology ». No fewer than 77 scientists of 15 different nationalities (France, Europe, United States, Mexico, Sri Lanka) participated in learning more about the wide cross-sectoral nature of this area of research which is rapidly gaining momentum. Representing a wide cross-section of research areas (agronomy-forestry, environment, agri-food, medicine, biotechnology, synthetic biology and cultural heritage), these researchers in turn discussed their most recent results relevant to ecology and the molecular mechanisms of bacteria-fungius interactions and their applications and implications to all areas combined. The dynamic momentum created by this conference led participants to a program a similar event for 2016-2017 and to formalize the development of an international network of laboratories working on this theme. This network is open to any and all laboratories interested (contact A. Deveau; deveau@nancy.inra.fr).

Les interactions Bactéries-Champignons à l'honneur.

Du 7 au 11 décembre 2013 s'est tenue à Roscoff (Bretagne) la première conférence internationale Jacques Monod sur les interactions bactéries-champignons, co-organisée par le CNRS, l'INRA et le Labex ARBRE. Cette conférence s'intitulait « Interactions bactéries-champignons : un domaine de recherche fédérateur pour la microbiologie fondamentale et appliquée ». Ce ne sont pas moins de 77 scientifiques de 15 nationalités différentes (France, Europe, Etats-Unis, Mexique, Sri Lanka) qui ont pu appréhender la grande transversalité de cette thématique de recherche qui vit actuellement une importante montée en puissance. Issus de différents domaines (agronomie-sylviculture, environnement, agroalimentaire, médecine, biotechnologie, biologie synthétique, patrimoine culturel), ces chercheurs ont tour à tour discuté des derniers résultats concernant l'écologie et les mécanismes des interactions entre bactéries-champignons, leurs applications et implications tous domaines confondus. La dynamique créée par cette conférence a conduit les participants à programmer un événement similaire en 2016-2017 et à formaliser la construction d'un réseau international de laboratoires travaillant sur cette thématique. Ce réseau est ouvert à tout nouveau laboratoire intéressé (contact A. Deveau ; deveau@nancy.inra.fr)

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