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ABSTRACT

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Species interations within the microbiome mediate potential for disease suppression

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Microbes and plants exist within complex networks of interacting plant and microbial species. Our work explores the roles of plant-microbe and microbe-microbe interactions in determining the pathogen-suppressive potential and composition of rhizosphere and endophytic foliar microbiomes, and the potential for managing these interactions to suppress bacterial and fungal plant pathogens. Of particular interest is the role that soil nutrients play in mediating microbial interactions within endophytic and rhizosphere populations. Among foliar endophytes, we found that nitrogen, phosphorous, and potassium (NPK) amendments significantly altered fungal, but not bacterial microbiome composition. Fungi, but not bacteria, had significantly smaller niche widths and were generally poorer competitors for resources in NPK-treated than control leaves. Endophytic fungi that were poor competitors for nutrients were significantly better at inhibiting plant pathogenic bacteria than strong nutrient competitors. Among rhizosphere populations, we found similarly that *Fusarium* populations from high-nutrient soils were more antagonistic than populations from low-nutrient soils. In contrast, *Streptomyces* populations from low-nutrient soils were more pathogen-inhibitory than populations from high-nutrient soils. Moreover, data from both endophytic and rhizosphere populations show that microbial species interactions, including bacterial-bacterial, fungal-fungal, and bacterial-fungal interactions, are critical to determining the pathogen-suppressive capacity of microbiome populations. Our data show that widely-used agricultural inputs significantly alter the composition and functional capacities of soil and endophytic microbiomes, and that these changes are driven by nutrient-related shifts in microbial species interactions. Enhanced understanding of microbial species interaction networks within plant-associated microbiomes will provide novel insights into microbiome management for bacterial (and fungal!) disease suppression.

Plants attract biocontrol agents and consequently modify their metabolism

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Plants live in close association with a large variety of microbes. The resulting interactions play key roles in important biological processes and affect, for instance, host growth and ability to tolerate stress. Actually, plants are able to change both quantitatively and qualitatively the microbial composition especially of the root zone, thus modulating and adapting to their needs microbiota activity. Studies performed on both rhizobacteria and rhizofungi demonstrate the function of bioactive molecules exuded from roots and acting as chemical signals for beneficial and/or pathogenic microbes. We have recently identified at least two classes of compounds whose secretion by tomato roots is modulated by biotic and/or abiotic stresses and that function as chemoattractants for *Trichoderma harzianum* and *Fusarium oxysporum* f. sp. *lycopersici*. Interestingly, the various patterns of secreted molecules affected the biocontrol and the pathogenic fungi in a different, and sometime opposite, manner. *In vivo* experiments indicate that some of the identified compounds could be used to treat tomato in order to potentiate the effect of applied biocontrol strains. The relevant molecular mechanism could be involved in many plant-microbe chemical cross-talks, including those regulating root-rhizobacteria interactions.

Sustainable control of Pierce's disease of grapevine and citrus greening with a benign strain of *Xylella fastidiosa*

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Pierce's disease (PD) of grapevine and HLB of citrus, caused by *Xylella fastidiosa* subsp. *fastidiosa* and *Candidatus* Liberibacter spp., respectively, cause large economic losses in their hosts. When injected into the xylem with a pin-pricking technique, a benign strain of *X. fastidiosa* (EB92-1) has provided control of PD in field trials with various *Vitis* spp. In Chardonnay treated at planting in Florida, EB92-1 provided control throughout an 8-year-trial, reducing the incidence of PD from 50% to 17% and the plant loss to PD from 33% to 8%. In a trial established in the UC Riverside vineyard in 2011, EB92-1 provided control of PD for 6 years in Pinot Noir and Cabernet Sauvignon grapevines, reducing the vine loss from 40% in the untreated to 10% in treated vines. In trials established in 2014/2015, a power drill and syringe were used to inject the biocontrol strain into mature grapefruit trees in a Florida grove with a high incidence of citrus greening. Three years after treatment, none of the

Isolation and characterization of bacteriophages specific to *Acidovorax citrulli*

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Bacterial fruit blotch and seedling blight, caused by *Acidovorax citrulli*, is one of the most destructive diseases of melon and watermelon worldwide. Biological approach in the disease control might be a potential solution and substitute for available bactericides of poor efficacy. Therefore, we isolated twelve bacteriophage strains specific to *A. citrulli* from rhizosphere of watermelon plants showing symptoms of the disease. The strains were characterized based on host range, plaque and virion morphology, thermal point of inactivation, adsorption rate, one step growth curve, and RFLP analysis. All phages lysed 30 out of 32 tested *A. citrulli* strains isolated in Serbia, and did not lyse other less related species. They produced clear plaques on bacterial lawn of different *A. citrulli* strains after 24 h of incubation. Examination by transmission electron microscopy of three phage strains indicated that they belong to the order *Caudovirales*, family *Siphoviridae.* The thermal inactivation point of phages was 66 or 67 °C. They were sensitive to chloroform, stable in pH 5-9, but inactivated after 5-10 min exposure to UV. RFLP analysis using *Eco*RI, *Bsm*I and *Bam*HI enzymes did not show genetic differences among the tested phages.

In vitro evaluation of the antibacterial activity of some essential oils and the antagonistic activity of Algerian *Trichoderma asperellum* isolates against three gram negative phytopathogenic bacteria

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The biocontrol of the three gram-negative phytopathogenic bacteria: *Erwinia amylovora*, *Pectobacterium carotovorum* and *Pseudomonas* sp. isolated from potato is based on the search for biomolecules with bactericidal potentialities and antagonistic isolates. The essential oils of five medicinal plants of *Pelargonium graveolens, Mentha pulegium, Mentha rotundifolia, Origanum glandulosum* and *Eugenia caryophyllus* extracted by hydrodistillation were tested *in vitro* using the aromatogram technique at concentrations of 5%, 2.5%, 1.25% and 1%. The antagonism focused on the direct confrontation of 13 Algerian isolates of *Trichoderma asperellum* against the three studied phytopathogenic bacteria, but the antibacterial activity of the methanolic extracts of their culture filtrates was carried out according to the same study technique as that for essential oils. The results showed a variability in bacterial growth inhibition rates according to plant essential oils, their concentrations, antagonist isolates and extracts of their culture filtrates. This study confirmed the effectiveness of certain essential oils of plants and certain antagonists isolates of *Trichoderma asperellum* to integrate them in the biocontrol of the studied phytopathogenic bacteria.

Study of *Erwinia* phage-host interactions through quantitative real-time PCR

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The goal of many biocontrol programs is the use of bacteriophage to target and repress the population of a specific bacterial pathogen. Regardless of the environment in which the treatment will be applied, the study of the interactions between the phage and its host is primarily performed using plaque assays, spot tests, and optical density measurements: all techniques which have remained vastly unchanged over the last 70 years. Using a combination of chloroform-based sampling, centrifugation, DNase treatment, and quantitative real-time PCR, the stage of the lytic phage lifecycle an individual *Erwinia* phage genome can be determined. Monitoring the rate of transition between these stages in a population then allows one to calculate adsorption rate, burst size, and the latent period of a phage-host combination within a single experiment. The characteristics of four different genera of *Erwinia* phage on their ideal hosts were determined using this technique. The *Ea214virus* and *Sp6virus* genera are able to adsorb to their hosts at a rate up to 6.6 times faster than *Ea92virus* and *Agrican357virus* while producing a comparable phage output over time suggesting they may make more effective phage-mediated biocontrol agents.

Antibacterial *in vitro* activity of experimental and formulated essential oils towards *Erwinia amylovora*

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The control of *Erwinia amylovora* (*Ea*), causal agent of bacterial fire blight, is one of the major challenges in pome fruit production. Nowadays, the main control strategy of this disease is based on the use of integrated methods: the infections are mainly controlled through the use of chemical treatments, mainly based on heavy metals/antibiotic compounds and resistance inducers, and by biological control agents (BCA). Among BCA, antagonistic bacteria and natural compounds as essential oils/plant extracts were studied to find more eco-friendly control tools targeted to the control of *Ea* infections. In the present work, experimental and formulated essential oils were tested for their antimicrobial activity against *Ea* in *in vitro* experiments. The synergistic effect of the experimental oils was also evaluated by checkerboard assay, and the Fractional Inhibitory Concentration index was calculated; the synergistic effect of oil combinations was assayed by macro-dilution assay as well. Among the experimental oils tested, those of *Origanum compactum* and *Satureja montana*, carvacrol chemotyped (48.13% and 54.86%, respectively),