CURRENT ASPECTS OF EUROPEAN
ENDOPHYTE RESEARCH

COST Action FA1103:
Endophytes in biotechnology and agriculture

www.endophytes.eu

Workshop 28-30 March 2012
University of Reims, France

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Plants are associated with micro-and nano-organisms: endophytic bacteria and fungi, which live inter- and intra-cellularly in plants without inducing pathogenic symptoms, while interacting with the host biochemically and genetically. Endophytic microorganisms may function as plant growth and defense promoters by synthesising phytohormones, producing biosurfactants, enzymes or precursors for secondary plant metabolites, fixing atmospheric nitrogen and CO$_2$, or controlling plant diseases, as well as providing a source for new bioactive natural products with utility in pharmaceutical, agrochemical and other LifeScience applications. The use of these endophytic microorganisms to control plant-pathogenic bacteria and fungi is receiving increased attention as a sustainable alternative to synthetic pesticides and antibiotics. Furthermore, endophytes may be adapted to the presence and metabolism of complex organic molecules and therefore can show useful biodegradation properties. In order to reduce inputs of pesticides and fertilizers and add value to eco-friendly agriculture in Europe, it will be important to develop inocula of biofertilizers, stress protection and biocontrol agents. But there are currently bottlenecks limiting the development of endophytes for use in biotechnology and agriculture.

To increase understanding about these hidden associations between plants, bacteria and fungi, and to identify bottlenecks in the development and implementation of technologies using endophytes, a network of scientists was recently formed. The present COST Action FA1103: ‘Endophytes in biotechnology and agriculture’ will operate all over Europe during the next four years and will provide a forum for the identification of bottlenecks limiting the use of endophytes in biotechnology and agriculture and ultimately provide solutions for the economically and ecologically compatible exploitation of these organisms within Europe and beyond.

Four working group will be held during the meeting as follow:

- **WG1. Ecology of endophytes**
- **WG2. Identification of new competent endophytes**
- **WG3. Development of new microbial inocula**
- **WG4. New industrial products in life sciences**

Enjoy your meeting and welcome in Reims,

Essaid and Carolin
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<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
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<tr>
<td>13:30-13:45</td>
<td>Welcome, Action Chair Carolin Schneider, Local organizer Essaid Ait Barka</td>
<td>Welcome, Action Chair Carolin Schneider, Local organizer Essaid Ait Barka</td>
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<tr>
<td></td>
<td><strong>Oral presentations WG1, Ecology of endophytes</strong></td>
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<td><strong>Session Chairs: Leo van Overbeek, Andrea Campisano</strong></td>
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<td>Leo van Overbeek, Netherlands</td>
<td>Ecology of bacterial endophytic communities</td>
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<td>13:45-14:00</td>
<td>Iñigo Zabalgogeazcoa, Spain</td>
<td>Ecology and applications of fungal endophytes from wild grasses</td>
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<td>14:00-14:15</td>
<td>Riitta Nissinen, Netherlands</td>
<td>Endophytic bacteria in the Arctic</td>
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<td>14:15-14:30</td>
<td>Marjo Helander, Finland</td>
<td>Endophyte infected tall fescue in Europe and North America</td>
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<td>14:30-14:45</td>
<td>Evelyn Klocke, Germany</td>
<td>The „hidden“ endophytes in protoplast cultures – a critical thinking about</td>
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<td>14:45-15:00</td>
<td>Kari Saikkonen, Finland</td>
<td>Endophytic fungi – Plant mutualists?</td>
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<td><strong>Break</strong></td>
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<tr>
<td>15:30-15:45</td>
<td>Sylvie Pazoutova, Czech Republic</td>
<td>Endophytes travelling, endophytes hiding</td>
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<tr>
<td>15:45-16:00</td>
<td>Nadia Efremova, Germany</td>
<td>Plant protoplast cultures as a source to determine the spectrum of endophytes</td>
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<tr>
<td>16:00-16:15</td>
<td>Andrea Campisano, Italy</td>
<td>Profiling of grapevine fungal endophytic community using Automated Ribosomal Intergenic Spacer Analysis (ARISA)</td>
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<tr>
<td>16:15-16:30</td>
<td>Derek Persoh, Germany</td>
<td>Diversity of endophyllous fungi and their role of in litter decomposition – modern methods to address composition and function of complex microbial communities</td>
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<tr>
<td>16:30-16:45</td>
<td>Soledad Sacristan, Spain</td>
<td>Arabisopsis thaliana and its natural endophytes: A model system for the study of the ecological and molecular factors involved in endophytic interactons</td>
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<tr>
<td>16:45-17:00</td>
<td>Anna-Maria Pirtillä, Finland</td>
<td>Peeking into the endophytic life style: Mechanisms behind induction of plant development and defens</td>
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<td><strong>Break</strong></td>
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<tr>
<td>17:15-18:15</td>
<td>Guided tour through labs</td>
<td>Guided tour through labs</td>
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**Dinner**
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tr>
<td>8:30-8:45</td>
<td><strong>Oral presentations WG2, Identification of new competent endophytes</strong></td>
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<td><strong>Session Chairs: Angela Sessitsch, Heinz-Martin Schumacher</strong></td>
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<td><em>Angela Sessitsch, Austria</em></td>
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<td></td>
<td>Ecology and functional potential of bacterial endophyte communities</td>
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<td>revealed by metagenomics and cultivation-based analysis.</td>
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<td>8:45-9:00</td>
<td><strong>Stéphane Compant, France</strong></td>
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<td>Current research on new competent endophytic actinobacteria in</td>
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<td>Toulouse</td>
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<td>9:00-9:15</td>
<td><strong>Heinz-Martin Schumacher, Germany</strong></td>
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<td>Endophytic bacteria in plant cell lines and in-vitro plants</td>
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<td>9:15-9:30</td>
<td><strong>Philipp Franken, Germany</strong></td>
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<td>Isolation of fungal root endophytes and analysis of their impact on</td>
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<td>plant performance</td>
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<td>9:30-9:45</td>
<td><strong>Olivier Fernandez, France</strong></td>
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<td><em>Burkholderia phytofirmans</em> strain PsJN and grapevine: several routes to</td>
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<td>chilling tolerance</td>
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<td><strong>Break</strong></td>
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<tr>
<td>10:15-10:30</td>
<td><strong>Oral presentations: Biocontrol</strong></td>
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<td><strong>Session Chairs: Pablo Hardoim, Corina Junker</strong></td>
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<td><em>Daniela Bulgari, Italy</em></td>
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<td>Endophytic bacterial community is restructured in grapevine yellows-</td>
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<td>diseased and recovered <em>Vitis vinifera</em> L. plants: outcome of plant</td>
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<td>response to phytoplasma infection and starting point for recovery?</td>
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<td>10:30-10:45</td>
<td><strong>David Ezra, Israel</strong></td>
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<td>Finding the right endophyte for biocontrol application in agricultural</td>
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<td>plants – a study case</td>
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<td>10:45-11:00</td>
<td><strong>Kalliope Papadopoulou, Greece</strong></td>
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<td>A novel tomato-<em>Fusarium solani</em> system for the molecular dissection of</td>
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<td>beneficial plant-fungal interaction</td>
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<td>11:00-11:15</td>
<td><strong>Emilio Stefani, Italy</strong></td>
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<td>Endophytes from <em>Actinidia spp.</em> and their activity as antagonists of</td>
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<td>phytopathogenic bacteria</td>
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<td>11:15-11:30</td>
<td><strong>Piippa Wäli, Finland</strong></td>
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<td>Effects of systemic grass endophytes on resistance and tolerance for</td>
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<td>host grasses to fungal pathogens</td>
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<td>11:30-11:45</td>
<td><strong>Willem Ravensberg, Netherlands</strong></td>
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<td>Endophytes for commercial use in biocontrol and plant growth promotion</td>
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<td>11:45-11:50</td>
<td><strong>Poster oral presentations</strong></td>
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<td><strong>Session Chairs:</strong> Francesca Gaggia, Kieran Germaine</td>
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<td>11:50-11:55</td>
<td><strong>Hans-Josef Schroers, Slovenia</strong></td>
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<td>11:55-12:00</td>
<td><strong>Vittorio Venturi, Italy</strong></td>
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<td>12:00-12:05</td>
<td><strong>Hatice Özaktan, Turkey</strong></td>
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<td>12:05-12:10</td>
<td><strong>Anja Schüffler, Germany</strong></td>
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<td>12:10-12:15</td>
<td><strong>Andrey Shcherbakov, Russia</strong></td>
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<td><strong>Lunch break</strong></td>
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<td>13:15-13:30</td>
<td><strong>Oral presentations WG 3, Development of new microbial inocula</strong></td>
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<td><strong>Session Chairs:</strong> Matthias Döring, NN, Francesca Gaggia</td>
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<td>13:30-13:45</td>
<td><strong>Kieran Germaine, Ireland</strong></td>
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<td>13:45-14:00</td>
<td><strong>Francesca Gaggia, Italy</strong></td>
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<td>14:00-14:15</td>
<td><strong>Carlo Leifert, United Kingdom</strong></td>
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<td>14:15-14:30</td>
<td><strong>Muhammad Naveed, Austria</strong></td>
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<td>14:30-14:45</td>
<td><strong>Nele Weyens, Belgium</strong></td>
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<td><strong>Break</strong></td>
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Continued Thursday, 29.3.2012

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<th>Time</th>
<th>Event</th>
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<tr>
<td>15:00-15:15</td>
<td><strong>Oral presentations WG4 'New industrial products in life sciences’</strong></td>
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<td><strong>Session Chairs: Marc Stadler, Gerald Bills</strong></td>
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<td><em>Gerald Bills, Spain</em></td>
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<td>Phylogenetic mapping of the distribution and diversity of antibiotic</td>
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<td>metabolites in plant-inhabiting fungi</td>
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<td>15:15-15:30</td>
<td><strong>Tytti Sarjala, Finland</strong></td>
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<td>Endophytic fungi from scots pine roots – potential source of</td>
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<td>antioxidative compounds</td>
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<td>15:30-15:45</td>
<td><strong>Peter Schröder, Germany</strong></td>
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<td>Uptake and transport of bacterial N-acyl-homoserine lactones in barley</td>
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<td>(<em>Hordeum vulgare</em>)</td>
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<td>15:45-16:00</td>
<td><strong>Ioannis Zabetakis, Greece</strong></td>
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<td>The biosynthesis of strawberry flavour and the role of the endophyte</td>
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<td><em>Methylobacterium extorquens</em></td>
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<td>16:00-16:15</td>
<td><strong>Marc Stadler, Germany</strong></td>
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<td>The “model endophyte” family Xylariaceae - polythetic taxonomy,</td>
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<td>functional biodiversity and bioprospecting</td>
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<td>Break</td>
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<td>16:30-17:30</td>
<td>Parallel Workplan workshops for WG1-4 *[rooms 101, 102, 103 and</td>
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<td>amphitheater]*</td>
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**Dinner**

Friday, 30.3.2012

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<tr>
<td>8:30-10:30</td>
<td>Parallel Workplan workshops for WG1-4 *[rooms 101, 102, 103 and</td>
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<td>amphitheater]*</td>
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<td>Break</td>
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<tr>
<td>11:00-12:00</td>
<td>Plenary meeting: Presentation of workplans WG 1-4</td>
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<td>12:00</td>
<td>Closure and departure</td>
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Our lab has developed a suite of integrated tools for high-throughput and species-specific culturing of plant-associated filamentous fungi [1] and complementary fermentation methods to manipulate fungal secondary metabolism [2, 3]. Growth of fungi in nutritional arrays, the parallel growth of many strains across multiple growth parameters in microplate fermentations, increases the probability of detecting antibiotic signals against *Candida albicans* and human pathogenic bacteria and more thoroughly exploits the metabolic potential of individual strains. Complementing nutritional arrays with other methods, e.g. fungal growth in chemically inert solid matrices [2], likewise enables a more thorough search for antibiosis across the filamentous Ascomycetes and the Basidiomycetes. Known and potentially unknown fungal-produced antibiotics are detected by LC-MS database matching so that chemical isolation work is focused on the most novel chemistry. Results are mapped onto a DNA barcode phylogeny [4]. Phylogenetic mapping has corroborated hypotheses about fungal secondary metabolism gleaned from genomic data and has directly confirmed that antibiosis is a universal and essential traits of the filamentous fungal life cycle. Plant-associated fungi exhibiting significant antibiosis towards model pathogens, *C. albicans* and *Staphylococcus aureus*, have been scaled up to produce enough antibiotic extract to generate hypothetical mechanism of action profiles in genome-wide fitness tests in *C. albicans* and *S. aureus*, while often yielding quite novel metabolites [5]. The strategies have revealed new patterns of secondary metabolite distribution among plant-associated fungi while contributing to the discovery of several new antibiotics with new mechanisms of action. The platform will find utility in genomic mining for products of cryptic metabolite pathways.

References:

BIOCONTROL OF SOIL-BORNE PATHOGENS BY STREPTOMYCETES

Maria Bonaldi¹, Andrea Kunova¹, Marco Saracchi¹ and Paolo Cortesi¹
¹DiPSA, Milan State University, Italy, maria.bonaldi@unimi.it

Soil-borne pathogens such as Pythium, Fusarium, Rhizoctonia or Sclerotinia spp. cause severe epidemics and extensive crop losses. Disease management employs cultivation of resistant varieties and use of fungicides. However, these strategies meet serious problems, such as evolution of virulent pathogen strains, environmental pollution or decreased diversity of non-target organisms. Furthermore, recent legislation restricts the use of synthetic chemicals and favors the use of biological control products. Until now, only a small number of microorganisms have been formulated as biocontrol agents.

Streptomycetes are common inhabitants of rhizosphere. They have been exploited in pharmaceutical industry for the production of antibiotics, but very little is known about their potential in agrobiology applications as plant growth promoters or biocontrol agents.

In our study, Streptomyces spp. strains isolated from diverse plant species were studied for their plant growth promoting and biocontrol potential against selected fungal pathogens. Strains were selected based on their in vitro antagonism against several pathogenic agents of root rot. These strains were successively tested for their plant growth promoting activity in vitro and in vivo, with particular reference to tomato, lettuce, corn salad, rocket and onion. Five strains were identified in in vitro antagonistic assay, that showed inhibitory effects against at least five diverse pathogens. The ability of selected strains to colonize seed coats was confirmed by SEM. They were further studied in vitro for their effects on seed germination and radicle and hypocotyl growth. The activity of individual strains depended on plant species used in the test and according to their performance the best combinations of strain-plant species were identified. 3 Streptomyces strains were investigated also in vivo to evaluate their promoting activity on seedling emergence and plant growth. All three strains improved substantially seed germination of three selected plant species. The strains were re-isolated from surface-sterilized plant roots to confirm their ability to colonize root tissues. The ability of these strains to control plant diseases in vivo will be determined successively by greenhouse pathogenicity tests.

References:


ENDOPHYTES IN BRYOPHYTES: SPHAGNUM MOSSES HARBOR DIVERSE BACTERIA
INTERESTING FOR BIOTECHNOLOGY

Anastasia Bragina¹, Christian Berg², Massimiliano Cardinale¹, Henry Müller¹, Andrej Shcherbakov³, Vladimir Chebotar³ and Gabriele Berg¹

¹Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria
²Institute of Plant Sciences, Karl-Franzens-University, Graz, Austria
³All-Russia Research Institute for Agricultural Microbiology, St. Petersburg, Russia
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Sphagnum mosses as main component of peatland ecosystems are important for our world climate. Using a multiphasic approach, we studied bacterial biodiversity of cosmopolitan Sphagnum species (S. magellanicum, S. fallax and S. angustifolium) with a special focus on their ecology. Application of the fluorescent in situ hybridization (FISH) combined with confocal laser scanning microscopy (CLSM) showed that leaves and stems of the mosses are densely colonized by bacterial colonies especially inside plant cells. Molecular fingerprinting, clone libraries and deep-sequencing of the 16S rRNA genes revealed great differences between the microbial communities associated with Sphagnum species. Multivariate statistical analysis defined pH and nutrient richness as the main ecological drivers for the microbial diversity. Interestingly, analyses of the FISH-CLSM and deep-sequencing datasets demonstrated that Sphagnum sp. maintain their bacterial diversity within the whole lifecycle; a core microbiome is transferred inside the sporophytes. The functional role investigated by the deep-sequencing, quantification of the functional genes as well as cultivation dependent methods showed that the bacteria are important for nutrient supply and pathogen defense. Both functions are interesting for biotechnological applications. Therefore, a new method via bait plants was developed to use moss-associated bacteria as microbial inoculants for agriculture.

References:

The plant root defines the interface between a multicellular eukaryote and soil, one of the richest microbial ecosystems on earth. Soil bacteria are able to multiply inside roots as benign endophytes and modulate plant growth and development, with implications ranging from enhanced crop productivity to phytoremediation. Here we describe a methodology to characterize and compare soil and root-inhabiting bacterial communities, which reveals not only a function for metabolically active plant cells but also for inert cell wall features in the selection of soil bacteria for host colonization. We show that roots of *Arabidopsis thaliana*, grown in different natural soils under controlled environmental conditions, are preferentially colonized by Proteobacteria, Bacteroidetes, Chloroflexi and Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. The identification of soil type-specific members within the root-inhabiting assemblies supports our conclusion that these represent soil-derived root endophytes. Surprisingly, plant cell wall features of other tested plant species appear to provide a sufficient cue for the assembly of ~30% of the *Arabidopsis* bacterial root-inhabiting microbiota, with a bias for Betaproteobacteria. Thus, this root sub-community may not be *Arabidopsis*-specific but saprophytic bacteria that would naturally be found on any plant root or plant debris in the tested soils. In contrast, colonization of *Arabidopsis* roots by members of the Chloroflexi and Actinobacteria depends on additional cues from metabolically active host cells.
Flavescence doree (FD) and Bois noir (BN), grapevine yellows (GY) diseases associated with phytoplasmas, induce severe crop losses. Until now, none grapevine varieties have been found resistant to phytoplasmas infection (Laimer et al., 2009). FD control is based on chemical treatments against the insect vector, Scaphoideus titanus Ball; on the other hand, this strategy is not efficient for BN containment since biological complexity of this disease. In recent years, there has been an increasing interest about the recovery from GY diseases and in the role of endophytic bacteria as biocontrol agents (Bulgari et al., 2009, 2011). Composition and structure of endophytic bacterial community were examined in healthy, phytoplasma-diseased and recovered grapevine plants. Length heterogeneity-polymerase chain reaction (LH-PCR) of total DNA from grapevine leaves was used to generate amplicon profiles that were analyzed with univariate and multivariate statistical methods. Jaccard analyses highlighted that microbial diversity and structure are different in healthy, diseased and recovered grapevine plants. Multivariate analyses confirmed this trend and showed which LH-PCR peaks determined the variation in microbial composition. Furthermore, LH-PCR electrophoretic peaks, assigned to isolated cultivable single bacterial strains, were used to monitor their distribution in total DNAs from analyzed plants. Bacterial community associated with healthy plants was characterized by a greater richness (higher number of LH-PCR peaks) than that present in diseased and recovered plants. Observed low bacterial richness and different microbial composition in diseased and recovered plants suggest that phytoplasma infection can directly and/or indirectly restructure bacterial community selecting endophytic strains that are able to elicit plant defense response.

References
Plants demonstrate superimposed complexity levels in the response to environmental stressors. Here, recent data on providing fitness for the plant partner by endophytic bacterium in the stress environment is summarized. The fluorescence parameters of photosystem II (PSII) (effective quantum yield ΦPSII, nonphotochemical quenching NPQ, photochemical quenching coefficient qP) can be interpreted as an early sign of stress impact in plants. Photosynthetic efficiency of PSII complexes of Crassulacean Acid Metabolism (CAM) plant Kalanchoë daigremontiana Hamet & Perr. inoculated with endophytic bacterium Methylobacterium sp. IMBG290 and exposed under ambient and stressful conditions (low atmospheric pressure, high CO₂ concentration, and UVB) was assessed in a short-duration experiment. The maximum and effective quantum yield of PSII (Fv/Fm and ΦPSII respectively) and other parameters were measured in situ fluorometrically under simulated adverse conditions in the simulation chamber (DLR, Berlin). The ΦPSII and Fv/Fm -values of kalanchoe plants grown in mineral substrate or earth soil under simulated conditions were lower than under normal conditions. The ΦPSII and Fv/Fm -values in inoculated plants were significantly (12 and 20%, respectively) higher than in control plants within the chamber, implying a better energy transfer within PSII. These results were supported by increase in malate content in inoculated plants compared to non-inoculated control plants. The processes, reflected by NPQ and qP, help to minimize production of reactive oxygen species in the chloroplast. In this experiment, NPQ was a 3-fold increased in non-inoculated plants within chamber as compared to plants outside the facility, exhibiting enlarged de-exciting by thermal dissipation processes. The nonphotochemical dissipation in PSII antenna complexes was reduced by Methylobacterium sp. IMBG290, indicating improved electron transport downstream from PSII compared to non-inoculated plants. Under stressfull conditions qP decreased in impaired nonprotected plants while in inoculated ones it practically was not changed. The positive effect of bacterial inoculation on plant accommodation to simulated adverse conditions was more pronounced in kalanchoe grown on mineral substrate, especially, under rigorous conditions of cooperative action of low pressure, high content of CO₂, and UV irradiation. Comparative studies on changes in stress-responsive epigenomes and transcriptomes will enhance our understanding of stress adaptation of kalanchoe plants mediated by Methylobacterium sp. IMBG290.

References:
STUDY OF INTERACTIONS BETWEEN TOXIGENIC FUNGI AND ENDOPHYTIC BACTERIA IN THE WHEAT GRAIN FOR DEVELOPMENT OF METHODS FOR CONTROL OF TOXIGENIC FUSARIUM INFECTIONS AND PRODUCTION OF HIGH QUALITY FOOD STUFFS

Vladimir K. Chebotar¹, Andrey V. Shcherbakov¹, Natalia V. Malfanova¹,³, Tatyana Yu. Gagkaeva², Olga P. Gavrilova²

¹- All-Russian Research Institute for Agricultural Microbiology (ARRIAM), Saint-Petersburg-Pushkin, Russia, e-mail: vladchebotar@rambler.ru
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Collection including 44 samples of winter wheat collected from main region of its cultivation in Russia has been created. Analysis of microbial communities in the wheat grain and study of spatial localization of endophytic bacteria in the grain originated from various ecological and geographical places have been performed. Totally 87 morphotypes of endophytic bacteria were isolated which were studied on their fungicide, bactericide and plant growth promotion activity. Among 44 studied cultivars of wheat from 15 it was impossible to isolate endophytic bacteria on any of tested growing media and only from 5 wheat cultivars endophytic bacteria were isolated on all tested growing media. Most number of endophytes was isolated on nutrient agar. According data of luminescent microscopy were identified 2 special regions in cover structures of wheat embryo, where were observed enhanced fluorescence in intercellular spaces. Phytopathological analysis of collected wheat samples was performed. Only one sample (cv. Dar Zernograda) didn’t contain toxigenic fungi. Contamination of two other samples (cv. Esaul, cv. Krasnodarskaya 99) from Stavropol’skiy krai was low 4-5%. High contamination rate with Fusarium fungi was observed on the samples of winter wheat from Republic of Kabardino-Balkariya: 16% – cv. Arfa and 46% – cv. Moskvich. Identification of isolated 87 morphotypes of endophytic bacteria on the basis of PCR amplification and sequencing of gene 16sRNA was performed. Molecular analysis showed that most number of endophytic isolates belong to the genera Bacillus, Pseudomonas, Agrobacterium, Serratia. Most perspective isolates of endophytic bacteria were selected on the basis of study of their fungicide, bactericide and plant growth promotion properties. Efficiency of experimental batches of microbial inoculants was shown in greenhouse and field experiments with wheat. In model gnotobiotic system it was shown that introduced gfp-marked strains of endophytic bacteria are able actively colonize roots of wheat as well as roots of radish in high cells density. So, selected strains have high colonization potential which is very important during competition for nutrient sources and sites for colonization on the wheat roots in natural conditions. Unique collection of strains of endophytic bacteria inhabiting grain of wheat originated from various ecological regions in Russia and possessing various beneficial properties such as fungicide, bactericide and plant growth promotion has been created.

References:


CURRENT RESEARCHES ON NEW COMPETENT ENDOPHYTIC ACTINOBACTERIA IN TOULOUSE

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Since works of Galippe, Jorissen and others pioneers, knowledge on endophytic bacteria colonizing various hosts, helping plant growth as well as reducing phytopathogen diseases has increased (1; 2). However there is still a gap of knowledge on researches of new competent endophytes as well as on their beneficial properties. In this way, various strains are currently characterized in our laboratory, LGC UMR 5503, for their biocontrol properties, secondary metabolites secretion, endophytic colonization in plants as well as for phytopathogen reduction. This corresponds to several actinobacteria isolated from harsh environments. Results show that some strains can secrete various metabolites having biocontrol activities towards different fungi, could colonize various plants, as well as reduce, on plants, some diseases caused by phytopathogens. Mechanisms of metabolites secretion, local and systemic colonization as well as diseases reduction induced by these actinobacteria is additionally currently studied to get more information on such strains as well as to increase knowledge on endophytes.

References:


Among bacteria, the actinomycetes are well known as important producers of bioactive compounds and constitute thus promising biocontrol agents. The Moroccan actinomycete microflora has been poorly explored to search new means of biocontrol, suggesting that a careful exploration of new habitats might be useful. In this way, 142 actinomycete strains were isolated from rhizosphere of healthy *Vitis vinifera* L. in four Moroccan sites and tested against five pathogenic fungi (*Pythium ultimum*, *Fusarium oxysporum* f. sp. *albedinis*, *Sclerotium rolfsii*, *Verticillium dahliae* and *Botrytis cinerea*). Results showed that 24 isolates have an *in vitro* inhibitory effect toward at least 4 of the indicator fungi, but only 9 inhibited all these pathogens. One of these strains, identified as *Streptomyces anulatus* S37 was further studied and patented (Patent EP 09 290 240.2).

Endophytic colonisation pattern was monitored by the presence of actinomycete in grapevine plantlets. Results showed that strain S37 (i) was present inside the tissues of the fifth leaf of plantlets and (ii) was transmitted through the next plantlets generation without any further inoculation. Grapevine plantlets thus colonised by the endophytic strain S37 have shown an improved growth by comparison with non-inoculated control. We also reported the synthesis of cell wall-degrading endoglucanase explaining how strain S37 could migrate through plantlets internal tissues.

Regarding antagonistic properties of *S. anulatus* S37 towards *B. cinerea*, this strain has also a great potential to produce antifungal metabolites. The extraction of fermentation broth followed by various separation and purification steps using centrifugal partition chromatography led to isolation and the characterization of several active molecules. The biological assay achieved with these products confirmed their inhibitory activities against *Botrytis cinerea* both *in vitro* and *in vivo* conditions. Further studies are ongoing to better understand the activity of each of the purified molecules.

References:
COMMERCIAL MICROBIAL INOCULANTS WITH ENDOPHYTES – AN OVERVIEW

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One major challenge of many others for the 21st century will be to have an environmental friendly and sustainable agriculture especially on the field of crop production. This is an important base for the nutrition of a very increasing human population on our earth. Otherwise there are some negative situations accompanying this – limited natural resources of P and N and other elements in soil which are important for plant growth and yield. Furthermore chemical pesticides and fertilizers are also used improper and strong in current cultivation methods of plants which induce many environmental and health problems. In this case microbial inoculants could be an effective and applicable alternative to preserve environment and healthiness of humans. This microbial products are besides of mycorrhizal fungi and soil microorganisms (nematodes, non-mycorrhizal fungi, protozoa, rhizobacteria) also endophytic bacteria and fungi living in roots, leaves and seeds. Inocula of endophytes can be work as biocontrol agents against pathogens and to improve the yield and development of plants without any damage of environmental components. The used endophytes can produce secondary metabolites, extracellular enzymes and plant hormone similar substances which are active biochemical components in the positive interactions with plants.

Actually a big worldwide market is available with different microbial products with tendency to rise. In year 2004 the ratio of world biopesticide market was 20% for Europe, 13% for Asia and 44% for NAFTA countries. The globale microbial biopesticide market has an value of ca. 160 MM US-Dollar (Cherry, 2005). This oral presentation want to give an actually overview about range of products especially of Endophytes with their characteristics in function and application in Agriculture and Biotechnology of Europe and oversea. Also to show innovations in development of new products and the importance of this business.

Reference:
Endophytes are defined as fungi and bacteria that colonize the internal tissue of plants, some even reside inside the plant cells. In contrast to pathogens they show no external sign of infection or negative effect on their host. Endophytes have been found in hundreds of plants, including many important agricultural useful plant species. Practically all identified endophytes were frequently isolated from plant explants after surface sterilization or from in vitro shoot cultures. But many endophytes cannot be isolated from explants, because of their low density in plants and their dependence to divide in the presence of host tissue. One of Phytowelt’s basic technologies is the application of protoplast fusion for increasing the biodiversity of plant cultivars by transfer of useful traits from wild species. We already started in vitro cultures of more than 150 different plant species including ornamentals, medicinal plants, trees and various crop plants (Solanceae, Gramineae, Fabaceae) and their wild relatives. Almost in all of them we have identified several endophytes. Usually the endophytes did not harm the in vitro shoot cultures or calli induced from explants. But in cell suspensions and protoplast cultures most endophytes show negative effects like overgrowing the plant cells and production of necrosis inducing metabolites. We observed that endophyte growth was often correlated with the plant cell division rate. Most endophytes could only be detected in highly dividing protoplast cultures. We found that protoplast cultures are optimal source to determine the spectrum of endophytes. For example in some ornamental species it was not possible to isolate the endophytes from explants. Only from protoplast cultures enriched fractions of endophytes start to grow after plating on fungi and bacteria specific media. The isolated endophytes vary in growth rates, some of them start to grow directly, other 7 or 14 days after plating. Additionally, they showed different antibiotic sensitivity. Phytowelt developed this isolation method to select optimal antibiotics for reduction of the endophyte level in cell suspensions and protoplast cultures. Additionally, this method offers an ideal approach for enrichment and separation of the various endophytes colonizing a plant. This is an important prerequisite for testing the benefits of the species-specific endophytes in agriculture and biotechnology.
Isolation and implementation of endophytes into agricultural crops for the control of pathogens is a multi-stage process. It usually begins in the laboratory with strategy determination step, continues in the field by locating a potential source for endophytes isolation, followed by the isolation, testing, manipulation, and introduction of chosen endophyte into desired plants. The next step will, in most cases be: introducing the endophyte inoculated plant to pathogens of interest, evaluating disease development in them compared to control, endophyte free, plants. Assuming the procedure went as desired, the next step in the process is formulation of the endophyte for commercialization. In real life, finding the right, most suitable, effective endophyte is not as easy. In this presentation I will try to describe, step by step, the process of finding a promising, potential endophyte for use as a biological control agent of plant diseases addressing possible obstacles we have faced in our efforts to find such an endophyte.
Burkholderia phytofirmans strain PsJN is an endophytic Plant Growth Promoting Rhizobacteria (PGPR) that enhances chilling tolerance of grapevine plants (Ait Barka et al. 2006). Molecular and biochemical approaches were investigated to explore several mechanisms by which this endophyte could improve cold tolerance by analyzing: (i) effect on expression of defence and cold related genes, (ii) modulation of H\textsubscript{2}O\textsubscript{2} production and (iii) stimulation of plant primary metabolism.

Before chilling exposure, bacterized plants displayed higher concentrations of carbohydrates known to be involved in cryoprotection (starch, sucrose and raffinose). When exposed to chilling, bacterized plants exhibited priming of several defence related genes (\textit{VvPAL}, \textit{VvStSy}, \textit{VvLOX}…) as well as priming of the key cold regulator \textit{VvCBF4} (Theocharis et al. 2012). Furthermore, bacterized plants were characterized by a faster and stronger production of H\textsubscript{2}O\textsubscript{2} and subsequent faster detoxication of this oxidative species.

Overall, our results suggest that \textit{B. phytofirmans} strain PsJN promotes grapevine chilling tolerance using several distinct pathways, whether existing or cold-induced mechanisms. Future prospects include testing whether these variations also occurred in inflorescence of bacterized inflorescence, since this organ is especially sensitive to cold damages.

Keywords: \textit{Burkholderia phytofirmans} strain PsJN, chilling, grapevine, priming, \textit{VvCBF}, H\textsubscript{2}O\textsubscript{2}, carbohydrates.

References:

Endophytic bacteria have considerable potential as bio-fertilisers, biocontrol agents and for improving the phytoremediation capacity of plants. This study investigated the culturable-aerobic bacterial diversity associated with two plants species (Miscanthus giganteus and Iris pseudacorus) that were being used in a biosolids amendment and in a wastewater treatment system respectively. A collection of 63 isolates from Miscanthus and 100 isolates from Iris were identified through partial 16S rDNA gene sequencing. Ten different genera were identified in Miscanthus originating from the leaf, stem and rhizome tissues, and seventeen different genera were identified from the leaf, rhizome and rhizosphere of Iris. The majority of these isolates were gamma-Proteobacteria. In Miscanthus, Pseudomonas and Acinetobacter species dominated the isolate collection while in Iris it was Microbacterium, Enterobacter, Pseudomonas and Rahnella species. Many of the isolates were found to possess resistance to heavy metals and possessed inherent organic xenobiotic degradation abilities. A number of isolates have been characterised in more detail with respect to plant growth promotion properties and some have been labeled with gfp marker genes through a mini-Tn5 vector system. Inoculation experiments using alginate encapsulation indicate that some of these isolates can colonize other plant species e.g. oilseed rape and rye grass in addition to Miscanthus. These isolates may prove to be useful inoculants for improving plant biomass and biodegradation efficiency within a range of plants suitable for phytoremediation and bioenergy production.

References:
ISOLATION OF FUNGAL ROOT ENDOPHYTES AND ANALYSIS OF THEIR IMPACT ON PLANT PERFORMANCE

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Root of plants in natural or man-made ecosystems harbour many different microorganisms, but their impact on plant performance is largely unknown. Root systems without any disease symptoms are used as starting material to obtain fungal isolates. After a first rough screen to exclude potential pathogens, fungi are taxonomically characterised by their morphology and by molecular methods. Those which show reasonable in vitro growth are analysed for their root colonisation patterns and for their impact on plant biomasses. If it could be confirmed that they are root endophytic fungi without negative activities they are analysed in more detail. This comprises their impact on plant development and nutrition, on plant-pathogen interaction, on yield and on plant product quality.

References:
EFFECT OF ENDOPHYTES ON PLANT GROWTH AND METABOLISM: IN VIVO AND IN VITRO STUDIES


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A new emerging approach for the development of sustainable agriculture system is the exploitation of endophytic bacteria and fungi and natural compounds that can benefit plant growth and control diseases by different mechanisms. Highlights of the research, within these topics performed in our laboratory, are reported below:

a) Microbial inoculants can improve root development in plants: the inoculation effect of a commercial product (EM), containing a wide variety of “Effective Microorganisms”, has been investigated on perennial ryegrass (L. perenne L.). The evaluation is based on greenhouse pot trials. In all experimental conditions no significant effect of different treatments was observed at the leaf level, while EM induced significant effects on root development, with an average increase of total root length equal to 35% and 70%, respectively. Preliminary data by PCR-DGGE techniques, allow to evidence different microbial profile on root samples among treatments (sequencing of excised bands is in progress). Identification, by DNA sequence of the 16S rDNA gene and 28S rDNA, of the isolated bacteria (lactobacilli, aerobic bacteria, yeasts) from the commercial product is in progress. Freeze dried cultures will be prepared from the isolates to have new potential inocula available (data submitted for oral presentation at Environmental Microbiology and Biotechnology, April 10-12, 2012).

b) Use of in vitro micropropagation technique allows fast evaluation of endophytes effects on plants growth: in vitro controlled culture media and environmental conditions allow fast evaluation of plant responses (e.g. growth, rooting, enzymes activity, mineral nutrition etc) to several treatments, including bacterial contaminations. Previous studies showed variable effects on growth of plum plants previously inoculated with different bacterial strains. In particular, shoot weight (40%) and proliferation (20%) reduction was caused by B. circulans; S. paucimobilis also inhibited root development (Marino et al., 2009).

c) Treatments with natural compounds induce positive effects on in vitro growth and metabolism of plant cultures, previously experimentally inoculated with bacterial contaminants: extracts from plants of the Meliaceae family has shown antimicrobial activity. Leaf, seed and fruit extracts of Melia azedarach showed bacteriostatic/bactericidal activity against a wide range of bacterial isolates. Some extracts were also effective in reducing/eliminating bacterial contaminants from in vitro-grown plum shoot cultures (Gaggia et al., 2008; Marino et al., 2009, 2011).

d) Scansion Electron Microscope (SEM) analysis allow time course studies of microbial colonization in plants: research by SEM analysis evidenced variable time courses of colonization of xilematic tissues by different microorganisms under repeated sub-cultures of shoots under controlled growth conditions (e.g medium pH, temperature etc.). In particular specific interactions were found between plant and bacteria genotypes (ref. in Marino et al., 2009).

References:
ARABIDOPSIS THALIANA AND ITS NATURAL ENDOPHYTES: A MODEL SYSTEM FOR THE STUDY OF THE ECOLOGICAL AND MOLECULAR FACTORS INVOLVED IN ENDOPHYTIC INTERACTIONS

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Arabidopsis thaliana L. (Brassicaceae) is the laboratory plant model par excellence. The genome of several genotypes has been sequenced and there is a large amount of available information about its functional genetics and genomics and a variety of tools to deepen in the knowledge of plant biology. For over 20 years, A. thaliana has been developed as the model organism for molecular plant genetics, including the analysis of the mechanisms of resistance to parasites.

However, A. thaliana is not just a lab model plant: it is an annual species with a worldwide distribution which can be found in a variety of anthropic and wild habitats. The knowledge generated in the lab is being applied to the study of its wild populations, so it has become a model, as well, for the study of plant ecology, adaptation and evolution. More recently, the ecological and evolutive aspects of plant-microbe interactions are also being studied using. However, the study of interactions other than highly pathogenic with A. thaliana is now just sprouting, with only few published examples.

We have surveyed different populations of A. thaliana from Central Spain, which is onsidered an origin and diversification centre of the species. The prospected A. thaliana populations are situated at different ecological environments at the Central Plateau of Spain, and have been very well characterized for their genetic variation and geographic structure. We have isolated a high diverse number of fungal species from above ground organs of asymptomatic plants. OTUs abundance distribution shows the typical pattern of microbial communities, with a long right tail: 59% of the species are represented by just one isolate and just eight species account for 50% of the isolates. The species accumulation curve is non-asymptotic, so the sampling effort has been insufficient to approach the total number of different OTUs. These preliminary results indicate that the diversity, structure and composition of the fungal endophytic assemblages of natural occurring A. thaliana populations is similar to those found in surveys of other hosts in temperate regions. Isolates of the most abundant genera were chosen in order to set into a point their inoculation to A. thaliana plants under controlled conditions and investigate the outcome of the interactions. This is, to our knowledge, the first work that investigates the endophytic mycobiota of A. thaliana, and one of the few in which the outcome of the interactions has been assayed under controlled conditions, validating the description of the isolates as endophytes. The availability of endophytic fungal isolates naturally infecting A. thaliana offers a great opportunity for the molecular approach of the factors affecting the interactions between endophytes and plants.

References:
This is a newly developped research line and a new, young, research group. Hence, we do not have publications yet in this area although I have two publications in preparation as result of the present work.
Selected publications in other areas:

Many endophytic bacteria (especially those associated with plant roots) possess plant growth promoting properties. We are investigating the possibility of harnessing these plant growth promotion properties for improving agricultural sustainability of bioenergy crop production. Our focus is on two bioenergy crops: Miscanthus (used for biomass production) and Oilseed rape (used for biodiesel production). A collection of over 450 individual endophytic strains were isolated and characterised from these plant species. An experiment was designed to screen this collection of strains for growth promotion in Oilseed rape which involved inoculating seeds with mixed inocula and a number of treatments were found to have resulted in a significant increase in plant biomass. The strains from these mixed inocula were inoculated individually onto Oilseed rape to assess their potential contributions to plant growth promotion and they were characterised in more detail for phosphate solubilisation, indole-3 acetic acid production, ACC deaminase activity, production of volatile compounds, biocontrol ability, resistance to heavy metals and metabolism of organic xenobiotics. The data indicated that there was a correlation between the growth promotion observed through inoculation of multiple strains and the presence of various plant growth promotion traits carried by these strains. A field trial is planned this year in collaboration with Teagasc (the Irish Food and Agricultural research institute) on Oilseed Rape to evaluate survival of selected strains within the crop under field conditions and to determine their effect on plant growth and seed yield. Two other themes of our research focus on the combination of endophytic bacteria and bioenergy crops for use in phytoremediation of heavy metals and organic pollutants and on the interaction of endophytic bacteria with plant and soil nematodes.

References:


The capability to colonize internal host tissues and contribute to plant growth has made endophytes valuable for agriculture as a tool to improve crop performance, and they are especially relevant in low-input sustainable systems. The aim of this ongoing study is to identify endophytic bacteria in cultivated and wild melons (Cucumis melo, Cucurbitaceae) with potential application in agriculture. The Cucurbitaceae is a large family which includes commercially important crops such as melon, cucumber, squash and watermelon. At Newe Ya’ar we have a large collection of wild and cultivated cucurbit species which serve as a potential source for plant beneficial traits. The specific objectives set for the project are to: a) Identify and characterize the diversity of endophytic bacteria found in association with wild and cultivated melons; b) Analyze the localization and distribution patterns of selected endophytic strains; c) Determine the interaction and beneficial traits of selected endophytes with their host plants, and d) Examine possible transmission routes of selected endophytes.

In order to identify beneficial bacteria, we plan to characterize melon-associated endophytic community by cultivation and cultivation-independent methods. So far five endophyte isolates were cultured from surface-sterilized melon fruits and identified using sequence-based techniques. A GFP-marked plasmid has been inserted to one of them that will be used for following the localization of the bacterium in the plant. The influence of the different isolates on various phytopathogens will be discussed.

References

Plants have evolved from symbioses with microorganisms, where key microbial traits (e.g., photosynthesis) were positively selected by firstly being vertically transmitted and subsequently being fully incorporated into host genome. Today, we know that symbiosis interactions are tightly controlled by the host plants, where they can reward efficient beneficial associations while applying sanctions to associated cheaters (Kiers et al., 2006). Nevertheless, for the vast majority of plant-microbe mutualistic relationships, no conspicuous structures are formed and even then, remarkably, microorganisms might affect almost half of the plant functional traits (Friesen et al., 2011). These ‘unnoticed’ relationships might improve plant growth as much as the host genotype. Therefore, it has been observed that plants, to ensure successive ecological fitness, select and transmit via seeds the beneficial microorganisms (Puente et al. 2009; Hardoim et al., 2012). In our recent studies on bacterial endophytes of rice plants, we assessed the bacterial communities associated with root tissues of 10 rice cultivars. It was observed that the great majority of endophytes was erratically distributed amongst rice replicates, whereas many ‘common’ species were found across cultivars (e.g. *Enterobacter* sp., *Dickeya zeae*, *Azospirillum* sp., *Mycobacterium* sp. and uncultured *Alpha-, Beta-proteobacterium* and *Bacilli*) and only few were cultivar-specific members (e.g. *Escherichia coli*, *Burkholderia kururiensis*) (Hardoim et al., 2011). Rice genotype determine to a large extent the composition of the different bacterial communities across cultivars, with several cultivars belonging to *Oryza sativa* subspecies *indica* tending to select similar communities, whereas those belonging to subspecies *japonica* and *aromatica* selected ones with divergent community structures. A comprehensive clone assessment of the bacterial 16S rRNA gene retrieved from root tissues of a selected cultivar (denoted APO) revealed a great diversity of bacteria with 16 phyla/classes being identified (Hardoim, 2011). Members of *Gammaproteobacteria* were the most abundant endophytes followed by *Alphaproteobacteria*. In addition to clone library, 82 distinct bacterial genotypes were isolated, indicating high genetic diversity within the culturable fraction of rice endophytes. In both approaches, *Enterobacter*-like species was the most assessed bacterium. *Enterobacter* sp. showed many plant growth-promoting properties such as reduction of acetylene, phosphatase and ACC deaminase activity *in vitro* (Hardoim, 2011). We also addressed the bacterial community from the endosphere of rice seeds in two consecutive generations and observed that 45% of the bacterial community from the first seed generation was recovered in the second generation (Hardoim et al., 2012). Furthermore, half of the seed endosphere bacterial community was also detected in rice plants growing in field conditions. The dynamic of rice seed-borne endophytes was further evaluated up to tillage vegetative stage from plants growing in gamma-irradiated soils. The endophytic bacterial community was largely influenced by soil type (i.e. low and neutral pH). Surprisingly, several members of the seed-borne community were found in the rhizosphere and surrounding root-free soils. Our results indicate that soil-originated bacteria might invade the host internal tissues as well as seed-borne endophytes might leak out from the host tissues to colonize surrounding soils (Hardoim et al., 2012), thus improving the host fitness both inside and outside of the plant as being observed in field conditions (van Overbeek et al., 2011).
References:


Systemic, asymptomatic fungal endophyte – grass symbioses form a continuum from mutualistic to antagonistic relationships in natural and manmade environments. The endophyte is always dependent on the resources and shelter provided by the host plant, and transmitted via seeds to the next grass generation. However, the endophytic fungus may increase the growth, reproduction, drought tolerance and herbivory resistance of the host in some environments and endophyte-grass combinations, while in some e.g. in low nutrient conditions the endophyte may be costly to the host plant. In some tall fescue (*Schedonorus phoenix*), the most widely grown and economically important forage grass in US, has been used as a model species when examining the relationship between endophytic fungus and its host grass. Generalizations considering endophyte-plant symbiosis are based on experiments performed with few cultivars in resource-rich agricultural conditions. However, little is known about the effects of endophytes on natural tall fescue populations and performance of tall fescue in varying environmental conditions outside the agricultural arena. We performed two common garden experiments, one in Europe (Finland) and one in USA (Kentucky) between years 2005-2007. Both experiments were conducted with five different tall fescue origins (three wild populations from northern Europe and two cultivars, ‘Kentucky-31’ and ‘Retu’) applying nutrients (N), water (W) or their combination (WN). In both experiments, we used naturally endophyte-infected (E+) and endophyte free (E-) plants, and plants from where the endophyte was manipulatively removed (ME-). Our results show that endophyte infection increases tall fescue performance in general, but there is high variation in effects of endophytes on plant growth and reproduction among tall fescue origins under different environmental conditions.

References:
THE POTENTIAL OF RHIZOSPHERE MICROORGANISMS IN PHYTOEXTRACTION OF HEAVY METALS BY WILLOWS

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Willows (Salix spp.) can be used successfully for phytoextraction of heavy metals from contaminated soils. Inoculation of willows with suitable microorganisms seems to be a promising strategy to promote this process. Mycorrhiza formation can efficiently ameliorate abiotic stress of the host plant e.g., by absorption of metals in the hyphal sheath or secretion of chelating exudates. Symbiosis between host plants and mycorrhizal fungi can be enhanced by associated bacteria that can support mycorrhiza formation and function. Fundamental understanding how mycorrhizas and associated bacteria influence the vegetation development and on their effects at polluted sites is still a big challenge. On the basis of numerous experiments we have developed strategies both for selection criteria of microorganisms for inoculation of plants at heavy metal contaminated sites and for evaluating the potential of microorganisms for accumulation of toxic cations in their cells.

Plants have developed several strategies for high heavy metal tolerance, like the expression of metallothioneins (MTs) or the control of reactive oxygen species (ROS) level. We have revealed that the expression of MTs and level of ROS in willows can be significantly affected by ectomycorrhiza formation and/or associated bacteria. Our results suggest that microorganisms have influence on stress related proteins which can affect the metal concentrations in the plant biomass, and hence, the efficiency of metal extraction.

References:
ENDOPHYTIC INFECTION OF OLIVE PLANTS BY BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE ISOLATES

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Verticillium wilt (VW) caused by the soil-borne fungus *Verticillium dahliae* limits severely olive and olive oil production worldwide (1,2). Control measures currently available are not efficient enough for the management of the disease when used individually making thus unavoidable for this aim the implementation of integrated disease management strategies (3). Nowadays, the use of fungal endophytes as biological control agents (BCAs) may open novel avenues for the development effective disease management strategies of VW in olive (4,5,6). In this work we aim to ascertain the potential of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to endophytically establish in olive tissues that eventually may be colonized by *V. dahliae* during the development of VW. Olive plants in pots were inoculated *B. bassiana* or *M. anisopliae* by spraying above-ground tissues with conidial suspensions of each isolate at an inoculum density of 10\textsuperscript{8} conidia/ml. Stem and leaf tissues were sampled on a time course up to 30 days after inoculation and used for isolations of those fungi. Stems pieces were deprived of bark and 1-cm-long pieces of stem and leaves were heavily surface-desinfested with NaClO, plated onto Sabouraud Dextrose Agar and incubated at 25 ºC and a 14-h photoperiod of fluorescent light at 36 \(\mu\)E m\(^{-2}\) s\(^{-1}\). Fungal growth from plated stems and leave pieces occurred by 7 to 21 days, showing a polar growth pattern out of the tissues. The average incidence of internal colonization was 91.6\% of stems for each of the potential endophytes, and 50\% and 75\% of leaves for *B. bassiana* and *M. anisopliae*, respectively. Overall, the colonization frequencies by *B. bassiana* and *M. anisopliae* averaged, respectively, 58.3\% and 72.2\% for stems, and 13.8\% and 29.2\% for leaves. No symptoms were observed in both inoculated and non-inoculated plants. These results suggest that the two entomopathogenic fungi of the study are able to penetrate, colonize and persist in the inner olive tissues and encourage pursuing further studies to demonstrate their potential as BCAs for the leaf infection phase of VW in olive.

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ENDOPHYTIC COLONISATION OF OPPIUM POPPY TISSUES BY BEAUVERIA BASSIANA REVEALED USING CONFOCAL LASER MICROSCOPY

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Beauveria bassiana (Balsamo) Vuillemin is well-known as an entomopathogenic fungus, with worldwide distribution (1). We have previously reported that Beauveria bassiana strain EABb 04/01-Tip obtained from larvae of the opium poppy borer Iraella luteipes (Hymenoptera; Cynipidae) could be established endophytically in opium poppy (Papaver somniferum L.) plants by microbiological, electron microscopy and molecular tools (2,3). However, we have used confocal laser scanning microscopy in order to elucidate the patterns of plant colonization and growth within the plant tissues with GFP-expressing B. bassiana EABb 04/01-Tip. Five-week-old opium poppy plants were inoculated with GFP-expressing strain by spraying above-ground tissues with a conidial suspension at an inoculum density of 3 x 10^8 conidia/ml. Stem and leaf tissues were sampled on a time course up to 15 days after inoculation (ddi) without any further handling prior to microscopic observation. Fungal occurrence was detected in outer and inner plant tissues by 1 to 15 ddi, although it was noticed a relevant decrease of colonization level by time. Intercellular spaces constituted the preferential penetration sites of entry of B. bassiana into leaf tissues. Also, it is worthwhile to point out B. bassiana conidia and hyphae were frequently observed on leaf trichomes; therefore these structures could play a main role in the access of fungus to inner leaf tissues. Fungal colonization of inner tissues was scarce and not uniform, and higher in leaf than stem. Fungal hyphae were confined to apoplast, which involves this space is the main avenue of this fungus to endophytically colonize the host plant. No symptoms were observed in both inoculated and non-inoculated plants. These results will contribute to improve the knowledge of B. bassiana as endophyte on opium poppy in order to deploy it as a potential biological control agent of I. luteipes.

References:
Desert agriculture is a strongly growing field of land use. However, emerging problems with soil-borne pathogens limit the yield. Endophytic biological control agents (BCAs), who are able to suppress soil-borne pathogens, are promising candidates for plant protection but for desert application specific, drought-resistant strains are required. Here we report a stepwise selection procedure of BCAs from one of the most prominent organic desert farms SEKEM in Egypt. In a first step, we characterized the bacterial and fungal communities of the target habitat – the endorhiza of medical plants (Matricaria chamomilla L., Calendula officinalis L. and Solanum distichum Schumach. & Thonn.) in comparison to the surrounding field and desert soil. The bacterial communities were highly different for the plant species. The fungal community was less discriminative but characterized mainly by phytopathogens. In a cultivation-dependent approach, isolates from all parts were obtained and characterized by their anti-phytopathogenic potential against fungi (Fusarium culmorum, Rhizoctonia solani, Verticillium dahliae), bacteria (Ralstonia solanacearum) and nematodes. In parallel, genotypic diversity was analysed by ARDRA and BOX-PCR. Both procedures resulted in the selection of 46 unique, broad-spectrum antagonists. However, their diversity was low: 89% of the selected strains belonged to the Bacillus/Paenibacillus cluster. Bacillus subtilis (subsp. subtilis and spizizenii) was the main species identified in cultures and also a dominant band in soil, rhizosphere and endorhiza in microbial fingerprints. Furthermore, using a metagenomic approach, it was shown that Firmicutes and especially Bacillus was enriched in SEKEM soil in comparison with the surrounding desert. In contrast, from the original desert soil, diverse antagonistic Streptomyces strains were selected. The most interesting endophytic strains were evaluated in a field trial: they were not only able to improve the yield also the secondary metabolites of chamomile were found to be enhanced by bacterial inoculants.

Recent reference:
EFFECT OF ENDOPHYTE FUNGUS FUSARIUM SOLANI (STRAIN FSK) ON ECOPHYSIOLOGICAL BEHAVIOR OF TOMATO UNDER WATER STRESS

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It is widely known that plants in natural ecosystems often grow in association with a number of soil microorganisms that can reduce abiotic stress symptoms. The non pathogenic *Fusarium solani* strain *FsK* is able to colonize the roots of tomato plants conferring induced systemic resistance against plant pathogens. In the present study, we elaborated on the behavior of tomato plants colonized by the endophyte *FsK* under water deficient conditions and show an ability of the endophyte *FsK* to confer stress tolerance, which is related to morphological, physiological and biochemical adaptations of the plant.

Thus, colonization of plants by *FsK* under water deficient conditions can alleviate stress symptoms as revealed by increased photosynthesis and growth rate (increased shoot and root fresh weights) compared to non inoculated plants under the same irrigation. Also the F variable/F maximum (Fv/Fm) value decreased in the non inoculated plants under water deficient conditions while no significant change was observed for the *FsK* inoculated plants under water stress.

Interestingly, the endophyte seems to be entirely dependent upon the growth and productivity of the host plant, consuming a large proportion of its photosynthetic output. As a result, colonization of plant by the fungus under well watered conditions decelerates plant growth, although the photosynthetic rate is increased. Therefore the benefit of host by the colonization of the fungus is based on a fine-tuned balance between the demand of the invader and the plant response, and we are interested in characterizing genetic factors and environmental conditions that determine this beneficial balance.

We subsequently focused on the effects conferred by the endophyte on biochemical pathways involved in minimizing oxidative damage under drought stress. Enzymatic activities of SOD, GPX, APX and CAT were determined on leaves and roots of plants inoculated and non inoculated with *FsK* and a enzyme as well as a tissue-specific effect was recorded. For example, a higher SOD activity and CAT activity was observed on colonized roots, regardless of the water regime applied or only under stress, respectively. GPX enzyme activity on roots was not considerably affect but it was lower on leaves of colonized plants compared to non colonized under drought. Furthermore, there were cases where the colonization process itself led to alterations in enzymatic activities, i.e. CAT activity exhibited lower values in the colonized compared to non colonized plants.

The impact of this mutualistic system on drought tolerance under field conditions is currently a main research target.

References:


Almost 10 years of *Piriformospora* research has eventually revealed an exciting novel symbiosis of higher plants with root colonizing fungal endophytes of the order Sebacinales (Agaricomycotina, Basidiomycota). Along with conceivable potentials for agronomic application, such as *systemic induced resistance and growth promotion activity* (1), this research discovered a novel fungal lifestyle characterized by earlier biotrophic growth and subsequent cell-death associated colonization (2, 3).

*P. indica* is a model fungus of a large number of beneficial root endophytes which form a symbiosis with a thus far unlimited number of mono- and dicotyledonous plants. This seemingly boundless host range prompted us to investigate the fungus’ molecular strategies to overcome host defence. By plant mutant analysis, we found that *P. indica* exploits the antagonism between defence pathways as it strongly reduces salicylate-based defence responses by up-regulating the jasmonate pathway (Plant Physiology DOI: 10.1104/pp.111.176446). Jasmonates are also involved in the Induced Systemic Resistance (ISR) response elicited by the root fungus in plant leaves against both, the powdery mildew fungus (*Plant Cell Physiol* 49:1747-1751) and the bacterium *Pseudomonas syringae*. Because all Sebacina species, so far investigated, form a tripartite symbiosis with plants and specific ß-proteobacteria, such as *Rhizobium radiobacter* (*Cell Microbiol* 10:2235-2246), it is yet unexplored if bacteria contribute to beneficial activities including ISR.

We also assessed the mechanism by which the fungus induces plant cell death during its late colonization phase. To this end, the fungus induces ER stress while inhibiting the resulting Unfolded Protein Response (UPR) which eventually leads to a vacuolar-driven cell death mediated by caspase-like activities of the vacuolar processing enzymes (VPEs, 4).

Elucidation of the complete genome of *P. indica* (5) discovered a huge battery of approximately 250 small secreted cysteine-rich proteins that are candidates for fungal suppressors. This opens a new avenue for translational research into most efficient factors that could improve crop plants field performance in the future.

References:

POTATO ENDOPHYTIC MICROORGANISMS AND THEIR RELATION TO THE HOST’S PHYSIOLOGICAL STATE

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At the National institute of biology, Department of biotechnology and systems biology, we are specialised in qualitative and quantitative detection of nucleic acids of different organisms, using different molecular approaches like qPCR, LAMP, on site detection. Moreover we develop and optimise different procedures of DNA and RNA extraction from various materials. Up till now we were mainly concentrated on phytopathogenic microorganisms (bacteria, viruses and phytoplasma) and development of methods for new emerging pathogens. On the other hand, we are also exploring the plant response to pathogen attack, mostly working with potato plants and grapevine as a model plants. Systems biology methods, such as microarrays, quantitative real-time PCR, mass-spectrometry, bioinformatics and biostatistics are used to analyse physiological changes in plants infected with pathogens.

One of the questions, we addressed, is, whether changes in plant metabolism, observed as a consequence of virus infection, affect endophytic population in potato plants. Apart from this task, we can contribute within this COST FA1103 action with our expertise in developing methods for detection and quantification of microorganisms in various types of samples. Methods used for plant-pathogen studies could be implemented in exploring plant-endophyte interaction which would contribute to better understanding of the interaction.
Rice (*Oryza sativa* L.) is one of the most important grain crops in the world and half of the world’s population survives wholly or partially on rice. Recent metagenomic analyses of the endophytic bacterial community colonizing rice roots suggests a high potential for plant growth promotion, improvement of plant stress resistance, biocontrol and bioremediation (Sessitsch et al., 2011). In the present study we analyze the general characteristics of the rice endophyte community and relate it to the distribution and abundance of bacteria with beneficial functional genes in different rice varieties during different plant development stages.

To study the impact of plant genotype and plant growth stage on the microbial community composition and function, four different rice varieties belonging to *Oryza sativa* L. were harvested at four separate time points of the rice life cycle. Community analyses are performed based on 16S rRNA gene using DNA and RNA extracted from root as well as from the stem part of the plant. Furthermore, to identify important functions of rice endophytes relevant for nutrient cycling (nitrogen cycle, sulfur cycle), screening of the key functional genes is going to be performed.

In view of the fact that methane (CH$_4$) is a significant greenhouse gas, one part of this study is focused on methane-oxidizing communities. A microbial diagnostic microarray targeting *pmoA* gene (particulate methane monooxygenase) (Bodrossy et al., 2003) as well as sequence analysis is applied for specific detection of methane oxidizing bacteria (MOB) down to the species level. In addition, community analyses based on the 16S rRNA gene are carried out.

Global rice agriculture relies on extensive use of herbicides witch represents environmental hazard. Endophytes have been reported to take part in the degradation of organic pollutants. The endophyte metagenome harbored a high number of genes for ring-cleaving enzymes, witch emphases the potential of endophytes for bioremediation (Sessitsch et al., 2011). For that reason, DNA and RNA extracted from rice plants harvested instantly before and after herbicide treatment are being used to investigate the abundance and activity of genes involved in pollutant degradation (dioxigenases, monooxigenases). Moreover, a community analysis is performed to observe the change in the endophytic community structure.

Little information is available on the functional activities or the effects on plant performance of cultivated bacteria. Metagenomic approaches and other cultivation independent techniques might in the future reveal more information on not yet cultivated microorganisms. A better understanding on how beneficial bacteria colonize different plant niches will not only result in increased knowledge on plant-microbe interactions but will also lead to a more successful and reliable use of bacterial inoculants (Compant et al., 2009).

References:
PHYLOGENETIC STUDY OF ENDOPHYTES HARBORED IN CANNABIS SATIVA

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Cannabis sativa is an annual herbaceous plant of the Cannabaceae family from central Asia. Cannabinoids are one of the major secondary metabolites of this plant, which are known to have important pharmaceutical benefits like analgesic, anti-inflammatory, neuro-protective, appetite-stimulant and many more. Tetrahydrocannabinol (THC), cannabinol, cannabidiol, cannabigerol are the most prevalent ones reported to be effective in treating various diseases across a number of therapeutic areas. Endophytic microorganisms (endophytes) still remain an unexplored group of very promising organisms with diverse potential for exploitation, that are capable of producing bioactive secondary metabolites, sometimes even those natural products considered exclusive to their host plants. Thus, these microorganisms are important not only from a molecular and biochemical standpoint but also from the ecological perspectives. We have isolated a plethora of endophytes, both bacteria and fungi, from various tissues of Cannabis plant sampled from different locations. We are evaluating the ecological significance of these endophytes by taxonomically characterizing these microfloras using several microbiological, molecular and bioinformatics tools and techniques. Such a comprehensive phylogenetic approach will enable us in understanding the spatial distribution and species diversity of endophytes of this important plant. Consequently, the synergy between compounds and potential for interaction within the plant may provide successful and better therapeutic potential of Cannabis as a medicine.

References:
Fungal endophytes constitute a remarkably multifarious group of polyphyletic fungi ubiquitous in plants, and maintain an imperceptible dynamic association with their hosts for at least a part of their life cycle. Endophytes inhabit healthy tissues of all plant species studied till date and occasionally produce associated host plant bioactive secondary metabolites. The potential of novel endophytes capable of biosynthesizing bioactive plant metabolites has undoubtedly been recognized. However, it is disappointing that there is still no known breakthrough in the commercial production of these bioactive secondary metabolites using endophytes. It is important to elucidate the biosynthetic pathways in endophytes correlating to their associated plants on a case-by-case basis to understand how the biogenetic gene clusters are regulated and their expression is affected in planta and ex planta (by environmental changes and axenic culture conditions). Only a deeper understanding of the host-endophyte and endophyte-endophyte relationships at the molecular and genetic levels might help to induce and optimize secondary metabolite production under laboratory conditions to yield plant metabolites in a sustained manner using endophytes. The biosynthesis of plant metabolites in endophytes could further be manipulated to yield new lead structures which could act as pro-drugs. In addition to identifying new natural products, genome mining, metabolic engineering and metagenomics would certainly have an impact on the understanding and manipulation of secondary metabolite production by endophytic microorganisms. We stand at the cross-roads of time when the world’s biodiversity is declining at an alarming rate. Many endemic, endangered and medicinally valuable plants are presently vulnerable. Along with these plants, the endophytes harbored in them are also threatened. Further fundamental research must be addressed to ensure a continuous and sustained supply of bioactive pro-drugs against the current and emerging diseases. The above mentioned issues will be addressed in detail with several examples from our group, such as endophytic fungi producing the anticancer pro-drugs camptothecin, its structural analogues, deoxypodophyllotoxin, and the antidepressants hypericin and emodin.

References:

IMPROVING NUTRIENT USE EFFICIENCY IN CROP PRODUCTION – THE NEED TO UNDERSTAND THE ECOLOGY OF ROOT COLONISING ENDOPHYTES

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Nutrient use efficiency (NUE) in crop production has decreased significantly over the last 40 years (1). This together with the increasing cost and rapid depletion of know deposits of phosphorus, potassium and other mined minerals has raised concerns about the sustainability of current crop production systems and future food security (2,3). Root colonising endophytes (free-living and symbiotic N-fixing bacteria, AM-fungi and antagonistic or crop-resistance eliciting micro-organisms) may significantly increase nutrient use efficiency in crop plants. However, the utilisation of endophytes requires a detailed understanding of their ecological and environmental requirements (4-10).

The importance of improving our understanding of (a) the direct and indirect modes of action(s) of endophytes and (b) plant-endophyte-environment interactions that affect their impact on the NUE of crop-plant will be described using examples of published research results (4-10). Potential strategies to increase the activity of direct (e.g. N-fixation, P and micronutrient-uptake) and indirect (e.g. antagonism against soil and seed borne pathogens, induction of plant resistance) modes of actions linked to NUE will be discussed.

References:

Seed-transmitted endophytic fungi have been recognised as beneficial in many ways for host plants, but our knowledge of their impact on seed quality remains elusive. In a food choice experiment, we examined whether the endophytic fungus *Epichloë typhina*, which grows into the seeds of weeping alkaligrass *Puccinellia distans*, reduces herbivory by the grove snail *Cepaea nemoralis* in developing grass seedlings. Starved naïve snails consumed ~20% less biomass of infected seedlings than uninfected seedlings. When the same snails were tested again shortly after their first contact with the endophyte, they exhibited nearly 40% reduction in seedling consumption, and food discrimination became undetectable. Our results suggest that toxins produced by endophytic fungi can significantly reduce vulnerability of grass seedlings to snail herbivory. We propose that this increase in seed quality caused by endophytic fungi enables mother plants to produce more seeds at the expense of seed supplies for embryos. Such shifts in resource allocation agree with principles of life history theory and may explain why, in nature, weeping alkaligrass produces several times smaller seeds when infected with the *Epichloë* endophyte.

References:

**ENDOPATHOGENIC LYFESTYLE OF THE BACTERIAL PATHOGEN OF WOODY HOSTS**

**PSEUDOMONAS SAVASTANOI: A FUNCTIONAL GENOMICS PERSPECTIVE**

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*Pseudomonas savastanoi* pv. savastanoi (Psv) is a tumour-inducing pathogen of olive plants (*Olea europaea* L.) causing olive-knot disease. Construction of a Gfp-tagged Psv strain, in combination with the use of *in vitro* olive plants, allowed real-time monitoring of Psv colonization of the plant tissues and characterization of the endophytic phase of this pathogen in olive stems. Bioinformatic analysis of the draft genome sequence and the three plasmid complement of the model Psv strain NCPPB 3335, revealed a large degree of conservation with other strains belonging to the *Pseudomonas syringae* complex. However, NCPPB 3335 contains twelve variable genomic regions, which are absent in all previously sequenced *P. syringae* strains. Various features that could contribute to the ability of this strain to survive in a woody host were identified, including broad catabolic and transport capabilities for degrading plant-derived aromatic compounds, the duplication of sequences related to the biosynthesis of the phytohormone indoleacetic acid (*iaaM, iaaH*), and the repertoire of strain-specific putative type III secretion system effectors. Signature-tagged mutagenesis (STM), a powerful functional genomics approach to identify genes in pathogens that are required for growth in a host, was applied to Psv NCPPB 3335 during infection of young micropropagated olive plants. For this study, we screened approximately 5,000 STM mutants and found 78 of them to be attenuated *in vivo*. Molecular characterization of transposon insertion sites for all of these mutants allowed the reconstruction of the metabolic network required for full fitness of Psv during colonization of the olive tissues as well as the identification of several novel virulence factors in this bacterium. The interaction of Psv with other olive endophytes is currently under analysis in collaboration with other members of this COST Action (see communications presented by J. Mercado-Blanco and V. Venturi).

**References :**


In this work, the effect of a rare actinobacterium *Saccharothrix algeriensis* NRRL B-24137 was evaluated to control gray mould caused by *Botrytis cinerea* on vine plants, *Vitis vinifera* L. cv. Cabernet-Sauvignon. The colonization ability of the strain was also evaluated. Results have demonstrated interestingly that up to 75% of protection towards *B. cinerea* can be obtained by using strain NRRL B-24137 on plants. Data have also demonstrated that the beneficial strain can efficiently colonize the rhizosphere and the endorhiza of plants before to induce the systemic resistance enabling reduction of *B. cinerea* infection. Some mechanisms related to resistance have been correlated to this protection, and others are currently under evaluation.

In parallel to the study NRRL B-24137-*Vitis vinifera* L. plants, the beneficial association between NRRL B-24137 and plants was further evaluated with the model plant *Arabidopsis thaliana*. Results have shown parts of mechanisms of protection. Interestingly new components of the state of resistance induced by beneficial rhizo-/endophytic bacteria in general have been also demonstrated.

This work demonstrates therefore the high potential of strain NRRL B-24137 to control *B. cinerea* infection on vine plants, its niches of colonization as well as mechanisms enabling plant resistance; a pre-requisite for a sustainable agriculture as well as for a better knowledge of plant-rhizo/endophytic associations.
The bacterial genus of *Stenotrophomonas* comprises at least eight species of which the most predominant is *Stenotrophomonas maltophilia*. These bacteria are found throughout the environment and in particular in close associations with plants. Strains of *S. maltophilia* exhibit an extraordinary range of capabilities that including detrimental effects as opportunistic multidrug-resistant human pathogens, beneficial effects in biocontrol of plant diseases, breakdown of natural and man-made pollutants that are central to bioremediation and phytoremediation strategies, and production of biomolecules of economic value. *Stenotrophomonas* species are found growing epiphytically on plant leaves, in the rhizosphere and as endophytes. We are interested in understanding and exploiting this versatility of bacteria within this genus. To do this, we are adopting a comparative and functional genomic approach with multiple clinical and endophytic isolates of *S. maltophilia* (and other species) to provide insight into the adaptation of these bacteria, the basis of their capabilities in phytostimulation, plant protection and bioremediation as well as an assessment of the potential risks that the use of some species may pose to human health.

References:

Research efforts in our group have been focused on biological control as one of the measures to be implemented within an integrated management strategy of Verticillium wilt of olive (*Olea europaea* L.)(VWO), a disease caused by the soil-borne fungus *Verticillium dahliae* (1). *Pseudomonas* spp. strains natural colonizers of olive roots were earlier demonstrated as efficient biological control agents (BCAs) against VWO. Endophytic lifestyle of *Pseudomonas fluorescens* PICF7 - one of the best BCAs so far identified - was undoubtedly assessed in roots under non-gnotobiotic conditions by combining fluorescently-tagged bacteria, three dimensional root tissue sectioning and confocal laser scanning microscopy (2). The same strategy enabled us to confirm the simultaneous endophytic colonization of PICF7 and *Pseudomonas putida* PICP2, another indigenous olive roots BCA, although *in vitro*-propagated olive plants and a gnotobiotic study-test system was used in this case. Remarkably, root hairs were demonstrated to play an important role in the endophytic colonization of inner root tissues by both biocontrol bacteria (4). Moreover, PICF7 and PICP2 cells could simultaneously colonize the same root hairs and spots in the intercellular spaces of the root cortex, suggesting a lack of competence between the two strains. Our observations also indicated that surface colonization of intact roots and endophytic establishment by strain PICF7 seem to be required for the effective control of VWO (3). Yet, the mechanism(s) operating in the PICF7-mediated biocontrol of *V. dahliae* remains mostly unknown. We therefore aim now to elucidate the genetic and molecular processes taking place during the interaction between olive roots and strain PICF7. Suppression subtractive hybridization (SSH) methodology and transcriptome analysis has allowed the identification of genes differentially expressed during the early moments (up to 21 days after bacterization) of the olive root colonization process by PICF7 (5). Results indicate that PICF7 triggers a range of resistance responses in olive. Moreover, diverse transcription factors implicated in plant signaling pathways for both biotic and abiotic stimulus response have been identified. These studies represent one of the first attempts to unravel gene functions and regulatory networks involved during a woody host-endophytic BCA interaction. Future matters to be investigated for this tripartite interaction will be presented as well.

Research supported by grants P07-CVI-02624 (Junta de Andalucía, Spain), and AGL2009-07275 (Spanish Ministerio de Ciencia e Innovación), co-financed by EU ERDF.

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THE AIT ENDOPHYTE STRAIN COLLECTION

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During more than ten years of endophyte research at the AIT we collected and characterized more than thousand bacterial endophytes. The strains were isolated from various plant species, including various crops such as potato and maize, vine, mosses, wild flowers and woody tree species and various plant compartments such as roots, leaves, stems, flowers, fruits and seeds (Kuffner et al., 2010; Yousaf et al., 2010; Prischl et al., 2011; Compant et al., 2011). The strains were identified by partial sequencing of the 16S rRNA gene and occasionally alternative phylogenetic markers such as the gyrase B gene. More than half of the strains belong to the Proteobacteria, around 20% are Actinobacteria, 14% Firmicutes and the others belong to the Bacteroidetes/Chlorobi group of bacteria. The majority of our strains were screened for potential plant growth promoting activities such as IAA production, ACC deaminase, siderophore production and phosphate solubilization and/or for potential antagonistic activities towards important plant pathogens. The endophytes are organized in our strain collection and all information is saved in our database for which a web interface is currently designed.

References:
EFFECTS OF ENDOPHYTIC BACTERIAL STRAINS ON YIELD AND GROWTH PARAMETERS OF MAIZE

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Plant-associated microorganisms are important, not only for understanding their ecological role and interaction with plants but also for biotechnological application. Bacterial endophytes originate from the soil and enter plants via the roots followed by further spread through the inner tissues. Plant growth promoting rhizosphere and/or endophytic bacteria play an important role in agriculture by improving plant growth and yield through various direct or indirect mechanisms. (Sessitsch et al., 2004; Compant et al., 2010; Weilharter et al., 2011). In search of efficient endophytic strains with multiple activities, a total of 5 bacterial isolates belonging to Caulobacter sp., Pantoea agglomerans 598, Sphingobium sp., Pseudomonas sp., and Pantoea agglomerans 132 were isolated from maize plant roots. These bacterial strains and Burkholderia phytofirmans strain PsJN were evaluated for improving germination, growth and colonization of different maize cultivars under axenic conditions. Inoculation significantly improved the germination, root/shoot length and biomass up to 59, 112, 98 and 132% of maize seedling compared to uninoculated control. Two strains P. agglomerans 132 and B. phytofirmans strain PsJN showing the highest growth promoting activity under axenic conditions were selected and evaluated for improving maize growth and yield under greenhouse conditions (50 kg soil container trials). Two methods were used for bacterial inoculation (seed inoculation and spray of bacterial suspension e.g. $10^8$-$10^9$ cfu/mL at flowering) besides control. Results showed that inoculation significantly increased the growth and yield of maize cultivars compared to uninoculated control. However, B.phytofirmans strain PsJN inoculation response was more prominent compared to Pantoea sp. It increased the number of leaves per plant, leaf area, leaf weight, root weight, plant biomass and grain yield up to 13, 24, 35, 33, 31, 42%, respectively compared to uninoculated control. Similarly, inoculation also improved the chlorophyll fluorescence (photochemical efficiency of PsII) content of maize plant. The inoculant strains were recovered from the root, rhizosphere, stem, and grains and were identified by 16S rDNA- and IGS region-RFLP analysis. The results suggested that inoculation could be employed for improving maize growth and yield. However, B.phytofirmans strain PsJN may have better prospects and further work is needed to explore the effectiveness of strain PsJN under field conditions.

References

Endophytic bacteria inhabit the internal plant tissues, and have been isolated from a large diversity of plants, where they form non-pathogenic, often mutualistic, relationships with their hosts. Our research focuses on endophytic bacteria inhabiting arctic plants, their diversity, interactions with host plants and their contribution to plant ecophysiology in cold climates.

Our recent study combined molecular and culture-dependent approaches to characterize endophytic bacterial communities of three arcto-alpine plant species (Oxyriadigyna, Diapensialapponica and Juncustrifidus) in the low Arctic in Kilpisjärvi area, northwestern Scandinavia (69°03′N). Analysis of a bacterial endophyte library of over 350 isolates, as well as 6 clone libraries, collected from seven distinct sampling sites revealed a high diversity, representing mainly Actinobacteria, Bacteroides, Firmicutes, Acidobacteria and α-, β- and γ-proteobacteria. Taxonomic distributions of the culturable isolates as well as the clone libraries were dependent on host plant species as well as on sampling site properties. Significantly, several bacterial genera, including Burkholderia and Sphingomonas associated tightly with specific host plant species. The isolated bacteria were well adapted to growth at low temperatures, as the majority of the tested isolates grew well and retained e.g. chitinase and protease activities at +0 - +4°C. This preference for cold temperature habitats was also reflected in the phylogenetic affiliation of the isolates and clone libraries, with the closest relatives in public databases often representing isolates from cold environments.

In follow-up studies, we investigate the basis of host specificity in endophytic Sphingomonasspp. Further, we are following seasonal dynamics of endophytic and rhizospheric bacterial communities in low arctic fell tundra, as well as addressing biogeographical diversity of plant-associated bacteria in the Arctic.

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The objectives of the study were to find new endophytic bacterial antagonists effective against *Verticillium dahliae*. This paper describes i) the isolation of root-associated potential rhizobacteria and ii) evaluation of the *in vitro* effectiveness of isolated *rhizobacteria* and iii) *in planta* screening of the most promising antagonists by using eggplants as test plants. Forty four endophytic bacterial strains isolated from healthy olive roots were screened *in vitro* for their ability to inhibit the hyphal growth of *V. dahliae* and twenty one of them showed inhibitive effect at the rate of 30 to 48 %, compared with hypal growth in control plates with the fungus only. Bacterial strains which were inhibitive more than 30 % against the pathogen *in vitro*, were selected to test their reducing effect on Verticillium wilt in eggplant *in vivo*. Six out of 21 bacterial strains tested significantly suppressed Verticillium wilt of eggplant by giving 30 % to 45 % reduction in disease severity, compared with non-treated control. In the second repetition, the disease was being controlled 26-49 % by those antagonists. The strains identified as *Bacillus cereus*, *B. subtilis*, *B. mycoides* and *Pseudomonas putida* were found more successful to reduce Verticillium wilt with the rate between 30 and 49 %.

**Key Words:** endophytic bacteria, biological control, Verticillium wilt, *Verticillium dahliae*,

**References :**

The use of endophytic bacteria (EB) to control plant-pathogenic bacteria and fungi is receiving increasing attention as a sustainable alternative to synthetic pesticides. In order to reduce the input of pesticides and fertilizers and to make an eco-friendly agriculture, it will be important to develop inocula of biofertilizers, and biopesticides. The aim of this project proposal is to investigate the potential of plant growth-promotion and biological control through inducing systemic resistance against Fusarial wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) and angular leaf spot of cucumber caused by *Pseudomonas syringae* pv. *lachrymans* (PSL) by using endophytic bacteria, would be isolated from surface-disinfected healthy cucumber tissues, and to determine of this by molecular methods. Our research project is composed of 7 steps: (1) isolation and preliminary characterization of EB, and elimination of the EB strains, which would be able to carrying the risk of being human pathogen, (2) *in vitro* tests –plant growth-promotion (PGP) and biocontrol activities of selected EB against PSL and FOC (3) *in vivo* tests: testing the potentials of biocontrol and plant growth promotion of selected EB strains by pot tests under gnotobiotic conditions, (4) Monitoring the colonization and population dynamics of antagonistic EB (AEB) and pathogens in different part of plant tissues during the growing period, (5) molecular diagnosis of AEB, (6) Quantitative and molecular detection with Real Time PCR of gene expressions which are responsible for the production and activation of some enzyme, protein and metabolites in the cucumber tissues, triggered by AEB, and (7) to evaluate the performance for biocontrol and plant growth promoting and productivity in soilless culture of the AEB, which were induced the systemic resistance against PSL and FOC. In accordance with the obtained results from this research project, it would be able to develop an antilative and environment-friendly biopesticide against the two destructive pathogens of cucumber, and a biofertilizer for the plant growth and yield increase.

**Keywords:** Bacterial endophytes, plant growth promoters, inducing the systemic resistance (ISR), biocontrol, *Fusarium oxysporum* f. sp. *cucumerinum*, *Pseudomonas syringae* pv. *lachrymans*
A non-pathogenic *Fusarium solani* strain (Fs-K) was isolated from the roots of tomato plants grown on a suppressive compost. The fungus grows endophytically and is capable of protecting the plants against root pathogens and to elicit induced systemic resistance in tomato plants. The protective ability of strain Fs-K prerequisites plant responses is mediated by the well-known ethylene and ABA signalling pathways as determined by using a genetic approach of mutant tomato plant lines (1). On the basis of this disease protection capability, we hold a Greek patent and an international patent is pending, as regards the use of the endophyte. We are currently interested in developing effective formulations of the fungus to extend our field trials.

The pattern of root colonization was studied using a gfp-transformed isolate of FsK. Despite the dense hyphal network developed, which, at later stages, reached and completely filled the xylem, no disease symptoms were observed. On the contrary, root colonization by FsK resulted in a significant increase in plant growth compared to non-inoculated plants. Furthermore, the fungus was capable of promoting root growth under N deficiency and stem and leaf growth under Fe deficiency. The effect on plant growth was related to the total fungal biomass in the root system, as determined by Q-PCR (2). Our interest is to dissect the molecular mechanisms that govern the plant growth promotion effects.

The interaction between this beneficial fungus and an arbuscular mycorrhizal isolate, *Glomus* sp., was investigated in tomato roots, co-inoculated with both fungal strains. Root colonization ability of each fungal species was quantitatively assessed. Tomato plant mutant lines, compromised in their ability to establish effective mycorrhizal interactions, were also used. Results indicated a positive tripartite interaction. We are interested to determine factors that act commonly during plant colonization by endophytes (i.e. an ascomycete and arbuscular mycorrhizae) and distinguish key molecular components that lead to mutualistic and beneficial interactions. Furthermore, the mycorrhizal strain used in this study belongs in our collection of newly isolated strains from organically cultivated field sites that exhibit highly plant growth promoting effective capabilities. A new large-scale project in our group was funded to study the ecology and function of indigenous mycorrhizae isolates and other endophytic fungi from marginal ecosystems in Greece, aiming eventually to develop market-oriented single or mixed endophytic inocula. A main focus is on osmotic stress alleviation effects (salinity and water stress).

Root colonization by the endophytic FsK has an effect on the structure of the fungal community in the rhizosphere of tomato plants readily distinguished from the effect of pathogenic fungi, indicating that the endophytic nature of FsK may alter root physiology and exudation (4). Our interest focuses on the production of metabolites that produced both by the host plant and endophytes per se as well as on the role of such metabolites on the establishment of the interaction and the beneficial function of the endophyte.

References:


Our team studies fungi entering various interactions with plants and/or insects. These fungi are plant parasites and/or endophytes, but partially rely on insects for long-distance travel and transfer onto the most suitable plant host. The mechanism might be simple – the insect vector carries the fungal propagules on its surface or inside its guts (1, 2) – or the fungi may enter a symbiosis with the insect as xylariaceous fungi do with woodwasps (3).

Females of most species of siricid wood wasps (Hymenoptera: Siricidae) carry symbiotic fungi in their mycangia. Symbionts are inoculated in the wood during oviposition, grow in the larval galleries and serve as cellulose-digesters and nutrition for larvae. In Europe there are four species of *Xiphydria*: *X. longicollis* (oak) *Xiphydria camelus* (alder, birch), *Xiphydria prolongata* (willow), and rare *Xiphydria picta* (mainly alder).

The taxonomy and phylogeny of *Xiphydria* symbionts received little attention in the past. Therefore we screened symbionts from mycangia of emerging females of these four species. Each healthy female carried pure culture of a single fungus. In *X. longicollis*, *Daldinia childiae* was either the only fungus or a highly dominant one in the samples from moderately dry oak-hornbeam forests. Females from the alluvial sites harboured *D. childiae* and *D. decipiens* (approx. 1:1). *X. camelus* and *X. picta* (both from alder) shared the dominant symbiont *D. decipiens*, whereas *X. camelus* from birch carried *D. decipiens* and *D. petriniae* (approx. 1:1).

In *X. prolongata*, *D. childiae* was the dominant species followed by an yet undescribed *Daldinia* sp.; *D. decipiens* was rare and in three females *Hypoxylon macrocarpum* was found (3, 4).

No symbiont occurred in a significant number among endophytes from alder, oak, willow, and elm isolated from healthy wood or samples outside area affected by wood wasps. However, we found some hiding fungi. *Obolarina dryophila* was confirmed on willow as an asymptomatic endophyte, instead of forming fruiting bodies under oak bark, as usual (5). An entirely new coelomycetous endophytic genus *Liberomyces* was described, whose other members were found worldwide in associations with broad-leaved trees (2).

References:


A major fraction of primarily endophyllous fungi can be traced in litter samples and there is increasing evidence that leaf-endophytic fungi become saprobes during leaf senescence and participate in litter decomposition. Their role in decomposition is assumed to be mostly restricted to the early decomposition stages because the majority of endophytic fungi depend on the presence of readily available sugars or easily degradable structural compounds like non-lignified cellulose, which get soon depleted during decomposition. However, a functional role in the late stages of litter decomposition is conceivable for certain endophytic fungal taxa like the Xylariaceae, being capable of degrading lignin. In beech forests, the fungal communities in freshly fallen and highly decomposed leaves actually included similar numbers of previously endophytic fungi. However, evidence for active participation of these fungi in litter decomposition is still lacking.

The paucity in knowledge on the factors driving community composition of endophytic fungi and on their functional roles is mainly due to methodological issues. First of all, the diversity of endophytic and decomposer fungi mostly exceeds the available resources for obtaining statistically significant results in ecological cultivation-based studies. Up-to-date molecular methods based on ‘environmental PCR’ can easily cope with the taxonomic diversity at hand, but primer suitability and assignment of sequence data to taxa are still challenging issues. The function of decomposer communities may be assessed on the molecular level by analyzing gene expression profiles, but these meta-transcriptomes are too complex to be assessed de novo, even by high-throughput sequencing methods like 454 sequencing. The transcriptome targeting ‘EcoChip’ microarray, however, is suitable for simultaneously quantifying taxon-specific fungal activities and the expression of functional enzyme coding genes.

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Internal plant microbes, endophytes, have during the recent years become in the spotlight of research. Endophytes are essential for plant defense and numerous reports point to action of endophytes in plant growth and development. We have identified a unique plant-endophyte association in the meristematic tissue of Scots pine buds, where *Methylobacterium* spp. are the dominant species. These microbes are found in the cells of the apical meristem, needle and scale primordia, and around the resin ducts. Throughout the year they are associated with growing tissues. We have isolated endophytic products that potentially affect growth of pine tissue, and genome analysis of two isolates is revealing traits characterizing the endophytic life style. Infection of pine seedlings by the endophytic strain *Methylobacterium extorquens* sp. F increases lateral root formation, root length, and biomass to the same extent as mycorrhizal fungi and induces expression of several genes associated with development and defense. In potato, we have identified a *Methylobacterium* sp. that induces resistance towards *Pectobacterium atrosepticum* and *Pseudomonas syringae pv. tomato DC3000*. The induced resistance is dependent on the potato cultivar and on the dose of inoculated bacteria, and involves activation of the plant antioxidant system. Endophytes are diverse and can be involved in plant defense also through compounds produced. We have mapped the diversity of endophytic fungi in plants of the boreal forest, and by tools of genomics, identified antimicrobial peptides from endophytic bacteria and fungi. These peptides have activity towards e.g. *Micrococcus luteus*, *Escherichia coli* and *Staphylococcus carnosus*. Our results show that endophytes are potential tools for the biotechnology of plants, as well as a source of new compounds for e.g. pharmaceutical industry.

References:


A wide diversity of endophytic fungi has been associated with grapevines (Vitis vinifera L.). The composition of the endophytic microbial population changes greatly across plants and plant parts. The factors affecting the fluctuations of fungal communities composition in grapevine are as yet not understood. We compared fungal endophytic populations in Italian grapevines across farms using organic or integrated pest management (IPM). Both isolation of fungi on synthetic media and community analysis using an automated, DNA-based approach (ARISA) agreed in indicating that fungal populations from organic farms were significantly different from those from IPM farms. A multivariate statistical analysis showed that data obtained from DNA-based community fingerprinting ARISA was more information-rich than the analysis of cultured fungi.
Identification of Bacterial Endophytes in In-vitro Cultures of Prunus Avium L. Using 16S rDNA Sequence Analysis

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Up to now bacteria in plant in vitro cultures have mostly been treated as unwanted and harmful contaminants. The objectives of this project are to investigate the interaction between Prunus avium (wild cherry) in vitro plantlets and bacterial endophytes and to differentiate between bacteria with beneficial, neutral or negative properties for the plant. Prunus avium is cultivated as a fast growing hardwood for the production of high quality furniture. Fast-growing trees with a straight growth have the highest value on the market. To achieve these characteristics single trees with a good habitus are selected and propagated as in-vitro clones under the trademark silvaSELECT®.

During propagation, especially during rooting and acclimatisation, large-scale losses of plantlets have been observed which have been related to the presence of endophytes in in vitro cultured plantlets.

In a first step the bacterial community in six Prunus clones will be characterized and a PCR based quantification method will be developed. Secondly, different in vitro cultivation techniques and chemical and physical factors that affect plant growth and/or bacterial growth will be applied to analyse the changes within the bacterial community.

During the first months of the project we were able to isolate and purify endophytic bacteria from four of the six Prunus clones. The first three bacterial isolates were identified by sequencing of 16S rDNA fragments and comparison to database entries. One isolate from cherry clone ‘Neptun’ showed 99% sequence identity to α-Proteobacteria, two isolates from cherry clone ‘Demeter’ showed 100% sequence identity to the genus Bacillus (phylum Firmicutes) and Microbacterium (phylum Actinobacteria), respectively.

Additionally, a culture-independent approach was started by DNA extraction from plant material (clone ‘Neptun’) and PCR on 16S rDNA with bacteria-specific primers. Amplified fragments were cloned and 100 E. coli-colonies were analysed by ARDRA (Amplified Ribosomal DNA Restriction Analysis). First results show, that 14 groups of related bacteria species can be separated. One colony from each group was selected and will be identified by ongoing sequence analysis.

References:

Endophytes have been hardly used in commercial applications. Renewed interest was generated recently, particularly by recognizing that entomopathogenic fungi can be used for other purposes than the traditional one. They may be used as antagonists of plant pathogens and for control of nematodes, as endophytes against insects, and as plant growth promoting agents. Microbial disease control agents can also affect herbivorous insects through triggering the plant’s defense system. Broader use of existing products offers companies larger markets and a better return on investment. Secondly, endophytic use of these organisms eliminates important issues such as repeated and laborious applications, like spraying, and unfavourable environmental conditions needed for entomopathogenic fungi to be efficient, like a high relative humidity. Thirdly, they allow applications as seed treatment and/or early treated in the propagation phase. In terms of regulatory aspects, exposure to these endophytic microorganisms will be much less than when applied via spraying, and as a consequence risks for applicator and workers are greatly reduced. This should allow easier and less costly registrations.

Preliminary results will be presented on entomopathogenic fungi and their effect on insects when used as endophytes. The potential and advantages for a biopesticide industry will be highlighted as well as the needs in research and development.

References:
ENDOPHYTIC FUNGI – PLANT MUTUALISTS?

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Endophytic fungi living asymptotically within plant tissues have been found in virtually all plant species. Since livestock toxicoses in the USA and New Zealand in the mid-20th century were directly linked to be attributable to endophyte-origin mycotoxins, endophyte-plant symbiosis has become synonymous with ubiquitous “plant mutualism”. Endophytes receive nutrition and protection from the host plant while the host plant may benefit from enhanced competitive abilities and increased resistance to herbivores, pathogens, and various abiotic stresses. Statistical perusal of the endophyte literature, however, revealed that the reasons for the strong mutualistic stamp of endophytes are largely historical and system-based. Majority of the available literature has focused on systemic, vertically transmitted endophytes in grasses in which mutualism is more commonly detected compared to horizontally transmitted endophytes which are common in all plants. Furthermore, the grass endophyte literature appeared to be biased. Most of the conceptual framework for endophyte-plant interactions has been based upon studies of two, economically important and non-native grass species, tall fescue and perennial ryegrass, and generalist invertebrate pests. The grass endophyte literature is also biased toward short-term laboratory and greenhouse experiments and optimized growing conditions. In short, literature suggests that the agronomic grass model systems fail to capture the breadth of variability inherent in the wild grass–endophyte symbiosis. I propose that endophyte-grass interactions are much more complex than described in past literature. Like any other biological species-species interactions, endophyte-grass interactions may involve selfishness, cheating and power-struggle between the partners leading to a continuum of the interactions from antagonistic to mutualistic and occasional mutualism breakdown. Future studies should examine a “wider and wilder” range of plant-endophyte systems in long-term demographic field studies to better understand the key elements and the general importance of endophyte-plant interactions.

References
Drained peatlands in Finland represent harsh environmental conditions for seedlings and young trees due to uttermost variability in temperature, solar radiation and availability of water, and organisms surviving in such conditions need capacities against oxidative stress. In this study, we monitored antioxidative properties of endophytic fungi living in association with Scots pine roots growing on drained peatlands.

The aim of the study was to search for compounds with protective potential against oxidative stress related to neurodegenerative diseases like age-related macular degeneration. We investigated extracts of 26 fungal strains, mostly endophytic, isolated from the roots of Scots pine seedlings grown on Finnish drained peatland. The fungi were cultivated as pure cultures on agar plates for four weeks, collected and ground with liquid nitrogen followed by extraction with hot water. To screen antioxidant potential, the water extracts were assayed for total phenolic content (Folin-Ciocalteu’s phenol test), and for ferric reduction/antioxidant power (FRAP-test) using microplate applications. The extracts were also tested in an oxidative stress cell model, in which human retinal pigment epithelial cells (ARPE-19) were exposed to hydrogen peroxide, and the ARPE-19 cells were detected for cell division and viability using WST 1-test (Roche). Quercetin, which is a commercially available plant-derived antioxidant, was used as a positive control in the cell model testing.

In the oxidative stress cell model, the extracts from one of the fungal strains, *Phialophora lignicola*, protected efficiently ARPE-19 cells against overwhelming oxidative stress in the cytotoxicity assay. The level of the protection was dose dependent and comparable to the efficacy of quercetin.

According to our results, *Phialophora lignicola*, produces compounds, that might have therapeautical use, for example in treatment of retinal diseases associated with oxidative stress. The work for characterization of the bioactive compound(s) and monitoring of the therapeutic potential in other disease models are on-going.

References
An important part the project called EcoTechProduct, which is carried out at the Research Institute of Horticulture in Skierniewice (Poland), is to establish and maintain a Bank of Symbiotic Microorganisms, called the SYMBIO BANK. At present, the collected material of isolated spores of mycorrhizal fungi and PGPR bacteria comes from organic orchards and plantations located around Skierniewice, (Poland), and organic orchards and plantations in the Bieszczady and Białowieża areas (Poland). Results of studies to date have shown that there are large differences in the occurrence of mycorrhizal fungi depending on the species and plant cultivation method. At present, the material collected in the bank of isolated spores of mycorrhizal fungi and the bank of PGPR bacteria comes from less polluted regions in Poland. Trap cultures have been used to isolate and identify spores of the following species of arbuscular mycorrhizal fungi: Ambispora fennica, A. gerdemannii, Gigaspora margarita, Glomus aggregatum, G. caledonium, G. claroideum, G. constrictum, G. drummondii, G. fasciculatum G. macrocarpum, G. microaggregatum, G. mosseae, G. pallidum, G. rubiforme, Scutellospora dipurpurescens. The collection in the SYMBIO BANK contains (approximately): Spores isolated from the soil of the following plant species: strawberry 16.0 thousand, apple 6.5 thousand, sour cherry 1.1 thousand pear 8.1 thousand. Isolates of bacteria: Pseudomonas fluorescens -170, dissolving phosphorus compounds - 40, digesting cellulose - 40, producing spores - 110, fixing atmospheric nitrogen - 10, Actinomycetes - 40. Isolates of microscopic fungi - 50, including Trichoderma sp. - 30. The work of isolating and identifying species and strains of AM fungi and PGPR bacteria is continued. They are collected, catalogued and stored in a Bank of Symbiotic Microorganisms, called SYMBIO BANK, specially established for this purpose. The collected strains and species are identified, characterized and stored in a cryoprotectant (glycerol) at the temp. of -80°C. In the near future, a website of the SYMBIO BANK will be launched, which will contain a list of the isolates held in the collection and their descriptions, which will serve as a source of key information for the identification of the species of AM fungi and PGPR bacteria in Poland. This will contribute to the knowledge of the biodiversity of these symbionts and help in the formulation of microbiologically-enriched bioproducts for use in fruit-growing practice. The most effective strains and species of microorganisms will be patented and registered in Poland as bacterial and mycorrhizal inocula to be used in fruit production and in phytoremediation of heavy metal pollution. The establishment of the bank of PGPR bacteria and spores of mycorrhizal fungi will contribute to the understanding and maintenance of the biodiversity of these symbionts, the knowledge of their biology and ecology, as well as to the development of ecological technologies of fertilization of horticultural plants in Poland and the protection of the natural environment and human health.

**Keywords:** PGPR bacteria, microscopic fungi, AMF fungi, isolates

**References:**


TRICHODERMA SPP.: A MINE WHERE SEARCH FOR SOLUTIONS APPLICABLE IN SUSTAINABLE AGRICULTURE

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Trichoderma spp. are among the most important microbes beneficial for agriculture, and used worldwide as the active ingredient of bio-fertilizers and bio-pesticides. Selected fungi of the genus Trichoderma have the ability to interact simultaneously with plants and pathogens. Adequately selected strains of these antagonists have the ability to colonize the first few layers of the root tissues and/or act endophytically in the vascular system, therefore establishing a physiological interaction with the plant based on a molecular cross-talk. This results in triggering of systemic and localised resistance to diseases, as well as promotion of plant growth and development. Additional effects include the suppression of deleterious soil microflora/fauna, degradation of toxic compounds, direct stimulation of root development by producing hormone-like compounds or affecting plant synthetic pathways, and/or promotion of water and nutrient uptake. ‘Omics research on these symbionts are providing new opportunities for developing formulations that contain synergistic mixtures of bioactive Trichoderma metabolites and beneficial microorganisms. Analysis of the Trichoderma spp. “secretome” has permitted the identification of several key “MAMPs” involved in molecular cross-talk with the plant. Some of these MAMPs are well known secondary metabolites such as 6-pentyl-a-pyrone or harzianic acid, and proteins such us Cell Wall Degrading Enzyme (CWDE) or hydrophobins, having also a strong antibiotic effect.

The production of a specific secondary metabolite depends upon the species and strain considered, whereas the accumulation of the compounds is regulated by the sensing of other microbes and the balance between elicited biosynthesis and biotransformation rates. An improved knowledge on this class of plant symbionts and on the mechanisms and key factors that regulate their interactions with the plants and pathogens may have a significant impact on crop protection.

References

Bacterial intraspecies and interspecies communication is mediated by diffusible signal molecules. Many Gram-negative bacteria use N-acylhomoserine lactones (AHLs) as autoinducers in the quorum sensing response. While bacterial signalling is well described, the fate of AHLs in contact with plants is much less known. Thus, adsorption, uptake and translocation of N-hexanoyl- (C6-HSL), N-octanoyl- (C8-HSL) and N-decanoyl homoserine lactone (C10-HSL) were studied in axenic systems with barley (*Hordeum vulgare* L.).

AHL uptake and translocation into the plant was investigated by off-line UPLC, and with tritium-labelled compounds. To confirm the identity of the compounds, further measurements with FTICR-MS and alternatively with reporter strains were implemented. The experiments clearly showed an uptake of AHL by plant roots, and we were able to distinguish adsorption from true incorporation. The transport of AHL can be attributed to the vascular system, and is mediated by active processes. C8 was detected in the leaves of treated plants. AHL contact leads to changes in the antioxidative defense in plants, albeit in some enzymes only transiently.

Our work demonstrates that microbial signals are distributed in plants, and that plants are able to eavesdrop on the communication. Plant reactions might depend on the nature and intensity of the signal, and on other detected facts about the microbial partner.

References:


Species of the genus *Fusarium* (Ascomycota, Hypocreales, Nectriaceae) are well known causal agents of diverse plant diseases and producers of secondary metabolites that can cause mycotoxicoses in humans and animals. Because of its agricultural importance, *Fusarium* belongs to one of the best studied fungal genera. It accommodates more than 1000 species. The taxonomic structure in *Fusarium* is highly complex. It had been subdivided into various sections and has been linked with several teleomorphic genera. Recent phylogenetic analyses have shown that current concept of *Fusarium* is not monophyletic and that the genus divides mainly into two large groups. One of these groups comprises taxa mainly associated with insects, lichens, and other fungi, while the species of the other group predominantly inhabit plants. The biology of the species of the latter clade is, however, remarkably diverse and the clade is phylogenetically subdivided into various lineages. Although the *Fusarium* clade accommodates potent plant pathogens, highly aggressive necrotrophism can be observed only in some lineages. The representatives of other lineages are only weakly or not plant pathogenic at all. The issue is discussed on the bases of few poorly studied, however, ecologically interesting and purportedly endophytic or biotrophic *Fusarium* species that have not yet emerged as devastating plant pathogens.

The *Fusarium dimerum* species group presents a strongly supported phylogenetic lineage among other lineages of the *Fusarium* clade. Most species of this group originate from dead plant material or from soil, while *Fusarium dimerum sensu stricto* is known mainly from clinical specimens or as an opportunistic human pathogen. However, the lineage also accommodates at least three purportedly endophytic and probably plant host specific species. They have been isolated from cladodes of living Cactaceae (*Opuntia sp. / Gymnocalcium damsii*) or from living potato leaves, respectively. Surprisingly, these species produce normally looking colonies only on vitamin and growth factor supplying agar media. They grow weakly on synthetic vitamin deficient agar media. Because of their vitamin dependency these species may badly compete with other soil organisms and live only in association with their living plant hosts.

A similar situation is encountered in another poorly known lineage of the *Fusarium* clade accommodating *Pycnofusarium rusci* described from and known to occur on the Mediterranean shrub *Ruscus aculeatus*. *Pycnofusarium rusci* poorly grows on synthetic nutrient medium while it develops normally looking colonies on media to which vitamins and growth factors are added. The species was isolated from healthy plants but produces fruiting bodies only on decaying or dead stems that are still attached to living bushes. *Pycnofusarium rusci* produces submerged anamorphic fruiting bodies that are entirely embedded by plant material, which is not known to occur in other *Fusarium*-like species.

The observed vitamin deficiency indicates endophytism or biotrophism of the mentioned fungi, while a mutualistic interaction between the plant and the *Fusarium*-like fungi cannot be inferred on the basis of these data.

References:

Plants are colonized by complex bacterial endophyte communities, which to a large extent are not easily to cultivate. We therefore have studied how endophyte communities are structured and affected by various parameters including soil, vegetation stage, plant genotype and plant stress (1, 2). To obtain better understanding on the functional potential of endophyte communities we applied metagenomic approaches (3, 4) as well as the analysis of individual strains (5). In particular *Burkholderia phytofirmans* strain PsJN (6) has been investigated. This strain proved to efficiently colonize many plants, strongly promote plant growth and to induce systemic resistance responses in plants. Our studies revealed that the beneficial effects of endophytes include improvement of plant growth and health as well as degradation of pollutants.

References:


ENDOPHYTES OF SPHAGNUM MOSSES: RESOURCES FOR DEVELOPMENT HIGH-EFFECTIVE BIOFERTILIZERS

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Previously the investigations of Austrian colleagues were shown that Sphagnum mosses associated with unique endophytic microorganisms which have important functions for Sphagnum mosses and for the bog ecosystem as a whole [1, 2]. The aim of our study was to isolate promising bacterial strains associated with Sphagnum mosses possessing beneficial for agricultural crops properties as perspective objects for agricultural microbiology. About 250 strains of endophytic bacteria were isolated from green tissues of Sphagnum plants by using original methods of surfaces sterilization. The investigations of beneficial for agriculture properties allowed to select the number of strains with high antifungal and bactericidal activity, capable to produce auxins and stimulate plant growth, solubilize the inaccessible for plants phosphates, show the increased enzymatic activity. The analysis of 16S rRNA gene nucleotide sequences of strains with beneficial properties revealed their close homologues among the genera Pseudomonas, Serratia, Flavobacterium, Burkholderia and Collimonas.

Development of new biofertilizers for agriculture had four main ways. Firstly we study the colonization activity of isolated strains and their PGPR-properties. The colonization activity was studied using gnotobiotic systems with tomato and wheat plants whereas localization of introduced bacteria on the roots of cultivated plants was demonstrated by methods FISH and CSLM. Three strains of bacteria (Pseudomonas brenneri, P. fluorescens and Collimonas sp.) demonstrated the powerful simulative effect and increase the green masses of tomato by 60-90% in greenhouse experiments.

The second part of work included the creation of biofertilizers with protective effect. There are there strains with high fungicide activity were selected for future work. It was shown that strain P. fluorescens RF13H demonstrated fungicide effect in planta and decrease the rot root of wheat caused Fusarium culmorum from 90 to 40%. The strain Flavobacterium saccharophilum demonstrated the bactericide activity in vitro against the wide range Phytopathogenical bacteria from genera Erwinia, Pseudomonas, Clavibacter and can be use as biocontrol agent.

About 20 strains of bacteria, mostly from genes Pseudomonas had the ability to solubilize the calcium orthophosphate and phosporite powder on Muromcev medium. These strains will be tested in planta for development biofertilize with P-solubilizing effect.

The fourth part of our work included the searching the strains of bacteria with high cellulase activity for biodegradation of crop residues (straw). The aerobic acidophilic bacterial community with cellulosolitic properties was isolated from Sphagnum plants extracts. Community consist of about five different morphotypes of Gram-negative bacteria and able to grow only on acid liquid medium with cellulose as single carbon source.

Thus, selected strains can be used for further study in greenhouse and fields experiments with agricultural crops.

Work was supported by grant RFBR 09-04-91007- ANF_a.
References:
**THE BIOSYNTHESIS OF ALLANTOFURANONE – A NEW FUNGISTATIC NATURAL PRODUCT ISOLATED FROM ALLANTOPHOMOPSIS LYCOPODINA**

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Allantofuranone was found in culture extracts from the ascomycete *Allantophomopsis lycopodina* and showed promising antibiotic activity against filamentous imperfect fungi like *Paecilomyces variotii* and *Magnaporthe grisea*.¹ This fungus was isolated from a piece of wood from a deciduous tree collected in France. Originally described as a pathogenic fungus to cranberries² this species was additionally described as an endophyte by Shiono.³ In the course of biosynthetic studies, feeding experiments gave evidence for phenylalanine as a direct precursor of allantofuranone. Isotopic enrichment confirmed these findings but also suggested an unexpected biosynthetic pathway with polyporic acid being the key intermediate.⁴ Feeding *A. lycopodina* with synthetic difluoropolyporic acid resulted in fluorinated allantofuranone. In addition to the formation of allantofuranone, its abiotic and metabolic degradation were investigated.

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**References:**

Endophytic bacteria may play a positive role for plant growth. On the other hand latent contamination is a problem for preservation and exchange of plant genetic resources as well as for micropropagation. DSMZ maintains more than 750 undifferentiated cell cultures, IPK has more than 1300 frozen accessions of old potato varieties and the Institut für Pflanzenbiologie preserves valuable embryogenic cell lines of barley and wheat. All institutes face the problem of latent infections. Therefore the investigation of latent infections and their physiological impact is planned.

So far research at the different institutes was focused on initiation of cell lines and cryopreservation. Most recently at IPK the epigenetic stability of cryopreserved potato has been investigated. At DSMZ transgenic tobacco and potato cell lines have been established to investigate salt and osmotic stress in relation to cryopreservation. A potato cell line overexpressing a pr-10a gene showed increased salt and osmotic tolerance, modified proline and glutathione metabolism and increased cryotolerance.

Apart from plant work in the field of stress metabolism DSMZ offers expertise in bacterial taxonomy and classical as well as molecular techniques for the identification of bacteria. As service collection DSMZ may serve as resource center to obtain microbial strains as well as for deposit. Plant and microbial competences at DSMZ should be combined in the project. Techniques should be developed to detect, localize, quantify and identify latent microbial contaminations in cell and tissue cultures. The influence of contaminants on growth, preservation and stress physiology of the plant material should be investigated as well as physiological mechanisms leading to a latent state of contaminations or their switch to active growth.

In addition the planned project may offer opportunities to search for novel endophytes, to develop screening systems based on cell, tissue and organ cultures and to create strategies for co-cultivation and cryopreservation of endophytes and cell culture.

References:
The “model endophyte” family Xylariaceae - polythetic taxonomy, functional biodiversity and bioprospecting

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We have been studying the (chemo-)taxonomy, phylogeny and secondary metabolism of the ascomycete family Xylariaceae for many years [1]. These fungi, traditionally well-known for their ability to form conspicuous ascostromata on dead wood, are among the most frequently encountered endophytes of seed plants. Moreover, recent evidence points toward their being closely and specifically associated with insect vectors, which mediate the dispersal of their propagules. Perhaps owing to their interesting ecology, the Xylariaceae are prolific sources for unique secondary metabolites [2]. The antiparasitic agents, PF-1022A and nodulisporic acid are among the very few endophyte-derived compounds that have entered into drug development and – and in contrast to certain plant metabolites that were detected tentatively in the endophytes, the aforementioned metabolites were accessible by large scale fermentations in multi-gram scales.

In an attempt to find additional useful compounds, which can be sustainably, our future research will focus on the natural roles of secondary metabolites during the ecological interactions of the Xylariaceae; these model endophytes will be studied intensively by HPLC-DAD/MS profiling (relying on a comprehensive database including datasets for thousands of known and yet unknown metabolites, derived from analyses of 8000 herbarium specimens and hundreds of well-characterised strains from around the world). Moreover, GC-MS profiling is presently being also employed to study the volatilome of these fungi. These analytical data have been very helpful in the past to link taxonomic and phylogenetic data to secondary metabolite production and to discover numerous new genera and species. They will now also be employed to fully elucidate the life cycles of selected xylariaceous endophytes and to establish a phylogeny based on a polythetic approach, taking also into account the evolution of secondary metabolite biosynthesis. Aside from powerful analytics, we also rely at HZI on various methods of biological characterization, ranging from antagonism assays to cellular or target-based screens to evaluate antimicrobial, antiviral and anticancer activities. Interesting compounds will be produced by fermentation (up to 300 litre scale) using the facilities of the in-house pilot plant. Sophisticated methods for downstream processing and preparative isolation are also readily available at HZI.

Nevertheless, extensive field work, in-depth morphological studies, and international, interdisciplinary collaborations with leading mycological taxonomists, molecular ecologists, geneticists, and molecular biologists, are indispensable for success of these projects. Needless to say, the above facilities can also be employed for collaboration on virtually any other group of fungal or bacterial endophytes. Therefore, I would greatly encourage respective proposals from other members of our Action.

References:
The canker of kiwifruit, caused by the Gram negative bacterium *Pseudomonas syringae* pv. *actinidiae* (*Psa*), is the most destructive disease of *Actinidia* spp.: *Actinidia chinensis*, the yellow fleshed kiwifruit, is highly susceptible to the disease, whereas *Actinidia deliciosa*, the green fleshed kiwifruit, is somewhat less susceptible. In the last few years the disease destroyed several hundreds of hectares in Italy and New Zealand, the two most important kiwi fruits producers worldwide, but several orchards have been also found affected and destroyed in Chile, France, Spain, Portugal and Turkey. During two growing seasons (2009 and 2010) inspections made to severely affected orchards located in central Italy and prior to their complete eradication, allowed the finding of a few actinidia trees with almost no symptoms. Ten of those trees were uprooted and the trunk was cut to a length of about 160 cm, from the grafting point to the permanent cordon. From each trunk, woody disks were cut at different heights, approx. every 20 cm, and isolation of bacterial endophytes was attempted from each single disk on nutrient sucrose agar (NSA). A total of 45 bacterial isolates were obtained and purified from the ten plant trunks collected in affected orchards. Those isolates were subject to a number of nutritional, biochemical and phytopathological assays and they proved to be different strains. Their difference was confirmed by genetic fingerprints obtained with rep-PCR. The different 45 isolates were then assayed for their ability to inhibit the growth *in vitro* of the bacterial pathogen *Psa*: a total of 15 isolates proved to significantly inhibit the growth of *Psa*. Additional inhibition assays were performed on six other important phytopathogenic bacteria, like *Erwinia amylovora*, the causal agent of the fireblight of pome fruits and *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of the bacterial canker of tomato. Four out of the fifteen *Psa*-inhibiting isolates were also able to significantly inhibit *in vitro* all the phytopathogenic bacteria tested. The identity of those four highly inhibiting strains was confirmed by fatty acid methyl ester (FAME) profiles: two of them belong to the family of *Enterobacteriaceae* and two to the family of *Pseudomonadaceae*. Their activity *in vivo* is being currently assayed on infected *Actinidia deliciosa* under controlled conditions.

**References:**


IMPROVEMENT OF PLANT HEALTH BY BENEFICIAL ENDOPHYTES

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Endophytes are microorganisms that live within host plants for at least part of their life and do not cause apparent symptoms of diseases. They may promote host plant growth, increase plant nutrient uptake, inhibit plant pathogen growth, reduce disease severity, and enhance tolerance to environmental stresses. 

\textit{Burkholderiaphytofirmans}\textsuperscript{PsJN} is able to efficiently colonize the rhizosphere, root, and above-ground plant tissues of a wide variety of genetically unrelated plants, such as potato, tomato, canola, maize, and grapevine. Strain \textsuperscript{PsJN} shows strong plant growth-promoting effects and was reported to enhance plant vigor and resistance to biotic and abiotic stresses (Weilharter et al. 2011).

The AHL-producing \textit{B. phytofirmans} strain was inoculated on two potato varieties, which respond differently to strain \textsuperscript{PsJN}. Furthermore, a derivative of strain \textsuperscript{PsJN} was included, which contains a lactonase gene and which showed reduced AHL production. In addition to determining the effect of bacterial inoculation on plant growth, gene expression was analyzed by using an EST-based microarray targeting more than 4000 potato sequences. Results clearly showed a different response of the two varieties to the microbial inoculation treatments, particularly to a \textsuperscript{PsJN}-derivative containing a lactonase gene indicating that the composition and abundance of AHLs modulate cultivar specific plant responses. The effects on the modern, disease-resistant cultivar Bionta were smaller, it responded by mainly up-regulated gene expression, whereas the transcription rate in the susceptible Russet Burbank was reduced (Trognitz et al. 2008).

Furthermore plant response was monitored after infiltration with \textsuperscript{PsJN}, the lipopolysaccharide of \textsuperscript{PsJN}(LPS) and PBS medium. Salicylic acid (SA), nitric oxid (NO), reactive oxygen species (ROS) and the change of the plant transcriptome was measured after infiltration with \textsuperscript{PsJN}, LPS and PBS. We have shown that both LPS and \textsuperscript{PsJN} treatment reduced the SA level compared to the mock infiltration, whereas the \textsuperscript{PsJN} treatment exhibits a longer lasting effect. NO and ROS showed a significant higher level after inoculation with the pathogen \textit{Phytophthora infestans} in \textsuperscript{PsJN} and LPS treatments compared to PBS. Defense related genes were down regulated after two days infiltration with \textsuperscript{PsJN} or LPS compared to the PBS treatment. The infiltration with the endophyte \textsuperscript{PsJN} showed a stronger effect in the plant than the LPS alone.

**References:**

The fungal species composition of non-systemic and systemic endophytes was examined in seeds from some healthy grasses. Surface disinfestation of the seeds by soaking them for 10 min. in 2.5% sodium hypochloride solution followed by a 1 min. soak in 70% ethanol and final 2X rinse in sterile water was efficient for eliminating epiphytes to obtain fungal endophytes. A total of 324 fungal isolates from 42 taxa and 25 genera were isolated and identified from seven genera of native grasses from 95 collection sites in Turkey. The genera of fungal endophytes isolated were *Alternaria*, *Fusarium*, *Cladosporium*, *Bipolaris*, *Epicoccum*, *Stemphilum*, *Neotyphodium*, *Penicillium* and *Acremonium*. The plant pathogenic fungal endophytes that occurred most frequently in the seed samples were *Alternaria alternata* 45.9% (in 51 seed samples), other *Alternaria* spp 75.7% (in 84 seed samples), *Fusarium* spp. 28.8% (in 32 seed samples), *Cladosporium* spp. 19.8 % (in 22 seed samples), and *Bipolaris* plus *Drechslera* 13.5% (in 15 seed samples). The non-pathogenic systemic fungal endophytes were *Neotyphodium typhinum*, *N.starrii*, *N. lolii* isolated from *Triticum* spp., *Lolium* spp., an *Elymus* sp. and a *Festuca* sp. A total of 42 fungal species were identified, of which *Bipolaris sorokiniana*, *Drechslera graminea*, *Fusarium culmorum*, *F. graminearum*, *F. avanaceum*, *F.poae*, are known plant pathogens, and *Aspergillus*, *Chaetomium*, *Epicoccum*, *Penicillium*, *Preussia*, *Septonema* spp. are known saprophytic fungi. Some fungal isolates were identified only to genus level. Some fungi were found only in one grass specimen such as *Botrytis* sp., *Gliocladium* sp., *Preussia aemulans*, *Rhizoctonia* sp. *Torula* sp.. Thirty one isolates did not sporulate and could not be identified. The possible ecological roles of these endophytes are not yet known.
The purpose of my research is to acquire information about the identity, ecology and functioning of endophytic communities in an agricultural context. Many knowledge gaps still exist, e.g. on plant colonization, specificity of particular microbial groups involved in plant associations, and on microbial functioning inside plants. These are key questions that need to be addressed before we can utilize and manipulate plant-endophyte interactions. Plant-endophyte interactions are important because they contribute to higher crop yields and improved plant performances, which are particularly useful in agricultural regimes under low/no input of agricultural chemicals (fertilizers, pesticides).

Endophytic bacteria has been isolated in my laboratory from different crop plants (Arabidopsis, potato, tomato, lettuce, chrysanthemum, leek, rice) and tested for in vitro antagonism towards important phytopathogens. Many of these isolates showed in vitro antagonistic activities towards phytopathogens and some of these also showed in situ activities against phytopathogens upon association with plants. The ecology of endophytic strains has been studied at several occasions within plants (roots and shoots), but also in the rhizosphere. In general, endophytes appeared to be excellent rhizosphere colonizers. Shifts in endophytic communities (by making use of molecular fingerprinting techniques) upon changes in important plant parameters (plant genotype, growth stage, soil type) has been studied by making use of multivariate analyses. We could identify the importance of these parameters on bacterial community structures inside plants. The endosphere metagenome of rice recently has been unravelled in collaboration with other groups involved in this action. Further, new bacterial groups belonging to the genus of Enterobacter has been isolated from rice. We coined the term ‘competent endophytes’ for those soil bacteria for which the internal plant environment favours growth of these bacteria. To be competent, these bacteria must be equipped with mechanisms that enables them to enter plants, to colonize internal tissues and to proliferate in there. This in contrast to soil bacteria that become resident in plants because of stochastic events (e.g. via wounding). Currently novel plant-associated groups belonging to Verrucomicrobia subdivision 1 and Acidobacteria subdivision 8 are explored for their roles in plant associations. Eventual endosphere competence for these groups would reveal new insights in the interactions between plants and hitherto unexplored bacterial groups dominating in soils.

References:
Bacteria that live as ‘residents’ in plants are believed to undergo extensive communication hence how bacteria and plants talk with each other is an important subject of present-day research. A question that we are addressing is whether incoming plant pathogens interact with harmless or beneficial endophytes. As a model for these studies we are using \textit{Pseudomonas savastanoi} pv. \textit{savastanoi} (PSV) which is a pathogen of olive trees that can cause tumors once it gets to the inside of the plant. We have recently reported that the PSV pathogen and a harmless resident endophyte engage in interspecies cell-cell signaling and mutually benefit each other (Hosni et al., 2011); this is the first major example of such an interaction in plants. PSV in the presence of the endophytic bacteria \textit{Erwinia toletana} could induce a significantly bigger tumor; importantly both bacteria proliferated more as a result of this interaction signifying mutual benefit. The mechanism(s) of this interaction are currently unknown; however, both bacteria make the same quorum sensing (QS) N-acyl homoserine lactone (AHL) signal molecule. QS cell-cell signaling is pivotal in PSV pathogenesis as QS mutants unable to produce AHLs induce smaller tumors; this phenotype can be rescued by the presence of \textit{E. toletana} indicating AHL signal sharing and interspecies signaling. We are currently studying this interaction between pathogenic and resident bacteria \textit{in vivo} and possible mutualisms that are taking place; working models and unpublished results will be presented and discussed.

We have also recently initiated a project on the isolation of rice endophytic bacteria; a large set of potential endophytes have been isolated from Italian rice varieties and these are currently being characterized. The final aim of this 3-year funded project is to identify potential endophytes to be used as biofertilizers and/or biocontrol agents. Results and directions of this work will also be presented and discussed.

References:

EFFECTS OF SYSTEMIC GRASS ENDOPHYTES ON RESISTANCE AND TOLERANCE OF HOST GRASSES TO FUNGAL PATHOGENS

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Systemic grass endophytes (Epichloë/Neotyphodium -fungi) have been shown to enhance the resistance of their host plants against various stressors. We explored whether these endophytes affect the resistance and tolerance of host grasses to some fungal pathogens. We used 1. agricultural meadow fescue (Festuca pratensis)-Neotyphodium uncinatum and 2. wild red fescue (Festuca rubra)-Epichloë festucae symbioses as study systems. Resistance and tolerance of grasses to snow molds were tested with meadow fescue system and the effects of endophyte on susceptibility of powdery mildew, rusts and ergot were studied with red fescue system. In agricultural meadow fescues snow mold infested heavily the grasses in highly fertilized field plot. Surprisingly the endophyte infected grasses were more intensively colonized by one of the snow mold (Typhula ishikariensis) and expressed more pink snow mold (Microdochium nivale) induced damages than endophyte free grasses indicating negative effects of endophyte for pathogen resistance of host grasses. The re-growth of the endophyte infected grasses exceeded the growth of endophyte free ones so that after the following growing season the endophyte infected grasses had higher biomass compared to endophyte free grasses showing the positive effect of endophyte on performance of the meadow fescues. With the grasses from natural populations we found no clear effects of endophyte on susceptibility of red fescues to rusts (Puccinia sp.), powdery mildew (Blumeria graminis) or ergot (Claviceps purpurea), but the results were dependent on the geographic origin and the habitat of the grass-endophyte combinations. As a conclusion, we didn’t found positive effects of endophyte on host grasses in terms of resistance to fungal pathogens.

References:
THE POPLAR ENDOPHYTE *PSEUDOMONAS PUTIDA* W619 AS A KEY TO A SUCCESSFUL PHYTOREMEDIATION OF VOLATILE ORGANIC CONTAMINANTS: FROM THE LAB TO THE FIELD

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Background
Phytoremediation of volatile organic contaminants is often unsatisfactory because plants and their rhizosphere do not completely degrade these compounds resulting in evapotranspiration through the leaves causing secondary contamination of the ambient air and by consequence undermining the merits of phytoremediation. Under laboratory conditions, endophytic bacteria equipped with the appropriate degradation pathway can be used to improve the *in planta* degradation of organic contaminants resulting in decreased phytotoxicity and evapotranspiration.

Materials and methods
In this work, we describe the different experiments that were performed in order to move endophyte-enhanced phytoremediation from lab-scale experiments towards application on a TCE-contaminated field site. Poplar cuttings were exposed to trichloroethylene (TCE) in 3 different experimental set-ups, more specifically a short-term (days) hydroponics experiment, a midlong-term (weeks) pot experiment, and finally in a long-term (months) field trial. In order to improve the phytoremediation efficiency, poplar cuttings were inoculated with the TCE degrading root endophyte *Pseudomonas putida* W619.

Results
In the short-term hydroponics experiment, *P. putida* W619 colonized all plant parts except the leaves which resulted in a very significant plant growth promoting effect and a slightly diminished (23%) TCE evapotranspiration. In case the cuttings were grown in pots filled with potting soil, a growth promoting trend and a 41% decreased TCE evapotranspiration were observed after the roots and the stem were colonized by *P. putida* W619. In the field experiment, 3 months after inoculation, the inoculated root endophyte *P. putida* W619 could only be re-isolated from the roots. However, also some natural abundant stem endophytes got equipped with the TCE metabolic activity by horizontal gene transfer. Although the inoculation did not significantly affect plant growth, a 90% reduced TCE evapotranspiration was achieved after inoculation.

Conclusions
These results demonstrated that the endophyte *P. putida* W619 could be the key to both, increasing biomass production on marginal, nutrient poor land and also to an optimized phytoremediation of volatile organic contaminants. Furthermore, we can conclude that although we can learn a lot from lab-experiments, it is often very difficult to extrapolate lab-scale observations to real life field applications. However, the combination of the results obtained from these experiments at different levels of complexity provided us with new, interesting knowledge that can be exploited in future field applications.
The Iberian Peninsula is a center of diversity of Festuca rubra, a perennial grass commercially used for sports and ornamental lawns. In natural grasslands of western Spain about 70% of the plants of F. rubra are infected by Epichloë festucae, an endophyte which systemically colonizes leaves and stems, and is vertically transmitted by seed. Alkaloids and other unknown factors have been linked to an improved performance observed in some grasses infected by epichloë endophytes. Our work has focused in understanding why high endophyte infection rates occur in natural populations, and how wild endophyte germplasm can be used for turfgrass cultivar improvement. In wild populations the genetic variation of endophytes depend on the distance among populations, and clonal lineages occur at a local scale, reflecting seed transmission and vegetative plant expansion (1). The variation in alkaloid contents of infected plants depends on the type of alkaloid. The content of peramine, an insect toxic alkaloid is less dependent on environmental factors than the content of ergovaline, toxic to mammals (2). Experiments have shown no differences in biomass between Epichloë infected and non infected spaced plants. However, E. festucae increased the phosphorus concentration of plants and their allelopathic potential, reducing the growth of several competing legumes (3). These factors could benefit infected plants under interspecific competition in grassland communities, and help to understand the high infection rates observed in wild populations.

Surveys of non-systemic endophytes in wild grasses, revealed high species diversity. About 100 endophytic taxa were identified in some grasses, and only a small fraction of these endophytes were pathogens (4). Dominant endophytes on each grass tend to be multihost generalists, and some of these are also important airborne fungi. Hypothetically, this suggests that some ubiquitous airborne fungi are endophytes in part of their life cycle.

Resembling plant-endophyte associations, numerous endophytic species are infected by apparently asymptomatic viruses. The identification of these viruses and their effects on endophyte biology is another area of study in our laboratory (5).

References:
THE BIOSYNTHESIS OF STRAWBERRY FLAVOUR AND THE ROLE OF THE ENDOPHYTE *METHYLOBACTERIUM EXTORQUENS*

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The symbiotic relationship was studied between strawberry cells and *Methylobacterium extorquens* (CABI. former International Mycological Institute, UK, registration number: IMI 369321, isolated and identified from strawberry tissue cultures) \[1\]. The emphasis was given on how bacterial and plant cells collaborate in production of metabolites that are used in the biosynthesis of furanones in strawberry. It was found that the strawberry-derived 1,2-propanediol is oxidised by the bacteria to 2-hydroxy-propanal (lactaldehyde) which is then used by the plant cells in the biosynthesis of one of the most important strawberry flavour components: 2,5-dimethyl-4-hydroxy-2\(H\)-furan-3-one (DMHF) \[1\]. It was found that the bacterial and plant alcohol dehydrogenases (ADH) follow a symbiotic pattern in cell-free extracts of *Methylobacterium extorquens* and strawberry \[2,3,4\].

The biosynthesis of DMHF in strawberry extract alone, and in the strawberry together with bacterium was followed by HPLC quantitative analysis \[3\]. Our results on the enzymatic activities indicated a co-operation between the plant and the bacterial cells \[4\].

Our current efforts focus on the study of the localization of endophytic *Methylobacterium* in strawberry tissue by in situ hybridization \[5\]. Our aim is to localise the ADH genes of *Methylobacterium extorquens* in strawberry cells and compare the levels of DMHF and the presence (or not) of the bacteria in different berry species. Our ultimate scope is to confirm if these two genes are activated in the same cells (or even in the same location inside a cell) and conclude if the lactaldehyde is translocated during the synthesis or not.

References:


THE “HIDDEN” ENDOPHYTES IN PROTOPLAST CULTURES – A CRITICAL THINKING ABOUT

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Somatic hybridisation is a promising tool for crossing of far related plant species and for creation of new genetic variability. The process from protoplast isolation, protoplast fusion and regeneration of somatic hybrids is influenced by a lot of factors. The induction of the totipotency of the (fused) plant cell is a highly complicated process and a lot of observations remain unclear until now. Here, we will discuss about the appearance of endophytes during the regeneration process after somatic hybridisation of Pelargonium and Hydrangea ssp., respectively.

For the protoplast fusion we keep the parent plants in vitro. Pelargonium plants growth up well without the addition of antibiotics. After fusion, the development is slowly but in the most cases successfully until the proliferation of the first shoot primordia from the callus. In this stage endophytes appear on the explants. Only a fast separation of the developing shoots from callus and transferring them to fresh medium secures their survival and the further development to plants. There are then no endophytes more. An addition of antibiotics is not necessary.

In Hydrangea protoplast fusions we find a similar phenomenon. After a successful development of proliferating callus endophytes are present. However, the calluses turn brown rapidly and die. A supplement of the antibiotic Timentin has no effect. Up to now, only roots could be induced from hortensia hybrid callus.

Some efforts were done to indentify the endophytes e.g. by means of electron microscopy and the sequencing of regions of bacterial 16S rRNA gene. The results have so far been modest. One of the reasons could be that the bacteria did not grow at least on the common bacterial media. From our observations we conclude careful that the endophytes coexist in the in vitro plants without them to damage. The stress situation like the switch from callus proliferation to organogenesis seems to disturb this balance situation. Not only for the plant tissue culture will it be useful to increase the understanding about the “hidden” endophytes and the associations with the plants.

References:

Cankers diseases of grapevine (Eutypiose, Esca and BDA) cause serious losses in vineyards. The banning of two active substances, i.e. sodium arsenite and carbendazim, used to control Cankers diseases was made in 2001. Today, an increase of these diseases is observed, it causes severe damages and winegrowers try to control them to maintain the quality of products and sustain their wine. To meet their need, Agrauxine has developed an alternative method, Esquive®WP, based on the specific strains *Trichoderma atroviride* I-1237. This strain, selected in partnership with French research laboratories, have also a great efficacy on other diseases. Today, *T. atroviride* I-1237 product, is registered against eutypiose dieback and new development are pending. Efficacy trials conducted in post-AMM vine point out, a significant decrease in foliar symptoms of Eutypiose dieback, and also significant reduction in Esca and BDA developments. Indeed, after several years of Esquive®WP application in the vineyard, the first significant results point out a reduction in foliar symptoms expression of Esca / BDA (up to 90% from the control). Good results had also obtained on open field against soil disease of vegetables. This I-1237 strains, selected also for this great capacity of production, can today provided different viable commercial products for the development of IPM strategy.
Authors Index

Aapola U. 55
Abel S. 77
Afzal M. 61
Ahmadinejad N. 4
Ait Barka E. 9, 13
Akkopru A. 45
Alonso A. 17
Amann R. 4
Andrade D. 15
Anke T. 64
Aragón I.M. 36
Assenza F. 4
Baffoni L. 16
Bailleul F. 13
Bartsch M. 52
Baum C. 24
Berg C. 3, 62
Berg G. 3, 27, 62
Berner D.K. 69
Bertani I. 71
Bianco P. A. 5
Biavati B. 16
Bills G. 1
Blal B. 78
Bogumil A. 56,
Bonaldi M. 2
Bordiec S.
Brader G. 61
Bragina A. 3, 62
Brazil D. 14
Bulgarelli D. 4
Bulgari D. 5
Buonaurio R. 71
Burlak O. 6
Çakır B. 45
Campisano A. 51
Cardinale M. 3
Casati P.
Castañeda-Ojeda P. 36, 71
Castillo-Lizardo MG. 36
Chebotar V. K. 3, 7, 62
Ciudad AG. 75
Clément C. 9, 13
Compant S. 8, 37, 61
Cooper J. 34
Corneo P E. 51
Cortesi P 2
Couillerot O. 9
Criado BG 75
Czarnołęski M. 35
da Silva DP. 71
Dąbrowska G. 24
de Vera J-P. 6
Derkowska E. 56
Dhont-Cordelier S. 13
Di Gioia D. 16
Dinelli G., 16
Döring M. 10
Doupis G. 28
Dow M. 38
Dowling D.N. 14, 19
Dunne G.G.C. 14
Efremova N. 11
Ehaliotis C. 46
Eickhorst T. 4
Eid A.M. E. 58
Ezra D. 12
Fel R. 13
Fernandez O. 13
Fontaine F. 13
Frank C.A. 50
Franken P. 15
Gaggìa F. 16
Garcia E. 17
Gat T. 12
Gavrilova O.P. 7
Germaine KJ. 14, 19
Giovanardi D. 67
Glassner H. 20
Gluszek S. 56
Götz C. 59
Graillon C. 37
Grosh R. 15
Gruden Kristina 30
Gül A. 45
Hackl E. 40
Häggman H. 50
Hans-Henrik K. 50
Harbuzov A. 56
Hardoim P.R. 21
Heine-Dobberneck E. 65
Helander, M. 23, 73
Hokkanen J. 50
Hryniewicz K. 24
Hubert J. 9
Huettel B. 4
Huhtala A. 55
Jacquens L. 13
Jiménez-Diaz, R. M 25, 26
Jiménez-Fernández, D, 25, 26
Kajula M. 50
Kansu B. 69
Smalla K. 27
Spiteller M. 32, 33
Spröer C. 65
Stadler M. 66
Stasik O. 6
Stefani E 67
Sumorok B. 56
Taghavi S. 74
Tejesvi M. V. 50
Theocharis A. 13
Tikhonovich I. A. 62
Trapero-Casas, J. L. 25
Trognitz B. 68
Trognitz F. 61, 68,
Trzciński P. 56
Tunali B. 69
Tunç C. 44
Tyburski J. 24
Uusitalo H. 55
van der Lelie D. 74,
van Elsas JD. 21, 43
van Overbeek L 70
van Themaat E.V. L. 4
Vangronsveld J. 74
Varlese R.58
Vázquez de Aldana B.R. 75
Venturi V. 71
Verbarg S. 65
Viitala K. 55
Vinale F.58
Wäli PR. 73
Wäli, PP. 73
Weber F. 53
Weiharter A. 61
Weiharter A. 31
Welters P. 11
Weyens N. 74
Weinzierl K. 77
Winkelmann T. 52
Woo S. L.58
Yaron S. 20
Yildirim A. 69
Yolageldi L. 44, 45
Yousaf S. 42
Yousaf S., 61
Yu.Gagkaeva T. 7
Zabalgogeazcoa I. 75
Zabetakis I. 76,
Zaets I. 6
Zchori-Fein E. 20
Zloch M. 24
<table>
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