The "principal mode of action" of micro-organisms as agents between fertilization and plant protection

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Detailed Programme

Content (O= oral contribution, P= Poster)

O 1-01 Newly discovered micro-organisms that will change the face of agriculture

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Successfully addressing the challenge of providing future food security will require both improvements in crop yield as well as the cultivation of additional farmland. This may result in the steady increase of farming on marginal, arid, and semi-arid lands, especially in the developing world, leading in turn to greater biotic and abiotic stresses on crops. To enable crops to deal with these stresses, an ever-growing arsenal of chemicals will be needed to maintain acceptable yields, with consequent environmental damage and maybe even the loss of biodiversity. We have isolated fungal endophyte strains from wild populations of Irish plants and carried out a number of experiments which assessed the effect of inoculating these endophytes onto barley in a variety of stressful growing conditions. We have found that the endophytes induced improvements in important agronomic traits in nearly every situation, including 29% and 70% increases in grain yield and shoot biomass respectively in nutrient-stressed barley; 100% suppression of seed-borne barley diseases; 50% increase in both the number of shoots and grain yield in drought-stressed barley; and finally, a 600% increase in plant survival in multiply-stressed barley. We would propose that future work on developing crop inoculants from fungal endophytes may be most fruitful when the source and sink environments are more closely matched, thus maximising the environmental fit between host and symbiont. These impressive results suggest that the endophyte strains that we have isolated could provide the basis for the development of a commercially-viable biotechnological means of reducing chemical crop inputs, and we are currently working on such a project with industry partners. Benefits to barley provided by these organisms probably involve many different 'modes of action' and bringing this technology to market will rely on choosing an appropriate classification that will reduce time and cost.

O 1-02 Novel modes of plant-microbe interaction discovered by omics technologies

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Traditionally, plant-microbe interactions resulting in plant growth promotion (PGP) and biocontrol (BC) of plant pathogens studied by mutants and corresponding biochemical and molecular analyses are well-understood. Omics technologies supported by next generation sequencing and microscopy offer new possibilities to understand plants as meta-organisms but also the effect of PGP and BC agents in more detail. The majority of plant diseases is associated with a dysbiosis between the whole microbiome and its host as well as with reduced microbial diversity [1]. In contrast, PGP and BC treatments were shown to be able to enhance plant-associated diversity. Therefore, they are able to reduce the risk for pathogenic outbreaks [2-4]. According to these results, we suggest that plant microbiome shifts induced by microbial inoculants are an important mechanism for plant health. In addition, using transcriptomics approaches it is possible to detect new metabolites involved in beneficial interactions such as osmo and stress protectants [5].

[1] Erlacher *et al.* 2014 Front. Microbiol.

[2] Schmid *et al.* 2011 AEM

[3] Kröber *et al.* 2014 Front. Microbiol.

[4] Van Elsas *et al.* 2012 PNAS

[5] Alavi *et al*. 2013 Front. Plant Science

O 1-03 The right set of circumstances – selecting microbial bio-effectors for applications in alternative fertilisation systems – the BIOFECTOR Project.

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The practical application of plant growth-promoting microorganisms (PGPMs) as so-called biofertilisers to improve growth and nutrient acquisition of crops is frequently biased by low reproducibility of the desired effects. Various environmental factors including biotic and abiotic stresses, competition with the indigenous soil microflora and genotypic differences in host compatibility may limit the rhizosphere competence, the survival and thus the efficiency of the microbial inoculants.

BIOFECTOR is a collaborative project located within the 7^{th} EU framework programme with the aim to select suitable microbial partners for applications in various alternative fertilisation systems. It is expected that a strategic combination of microbial strains adapted to the culture conditions characteristic for the respective fertilisation systems will reduce the variability of host plant responses and thus the efficiency of the plant-microbial interaction.

Fist pomising results but also drawbacks and open questions are summarised with respect to PGPM use for improved nutrient acquisition from organic recycling fertilisers,in fertiliser placement strategies and mobilisation of sparingly soluble nutrients.

O 1-04 Market placement of microorganisms in CEUREG countries

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CEUREG stands for Central and Eastern Europe Registration. CEUREG FORUM is the name for technical conferences for pesticide regulation experts from Central and Eastern Europe with over 20 year tradition. The Forum provides an opportunity to exchange information and experiences between regulatory agencies in charge of placing plant protection products on the market in the member countries.

The subject of CEUREG meetings are not only plant protection products, but also other products used in plant protection. In the year 2014 the survey was performed as regards ways of market placement of certain groups of borderline products. The contribution will present the result of this survey as regards market placement of microorganisms.

O 1-05 Plant Strengtheners in Germany

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In Germany, plant strengtheners are defined in the national Plant Protection Act. Plant strengtheners have been known to the German legislation since 1986, however under various conditions. When Regulation (EC) No 1107/2009 came into force, the product group of plant strengtheners had to be re-defined. At present, according to § 2 No 10 of the Plant Protection Act, plant strengtheners are:

Substances and mixtures including microorganisms which are exclusively intended to maintain plant health in general as long as they are not plant protection products according to Article 2 (1) of Regulation (EC) No 1107/2009 or are intended to protect plants against non-parasitic impairments.

Products from the second group are, for example, products for reducing water evaporation or anti-freezing agents. Plant protection products according to Regulation (EC) No 1107/2009 cannot be plant strengtheners. When the major intention of uses is to provide plants with nutrients and trace elements and promoting their growth, products should rather classified as plant aids or soil improvers. These product groups are subject to the Act on Fertilisers.

The placing on the market of plant strengtheners is provided for in § 45 of the Plant Protection Act.

The BVL can prohibit products from being placed on the market if there are indications that they do not fulfil the definition of a plant strengthener or cause harmful effects on human and animal health, groundwater or the environment.

Detailed information can be found at: **http://www.bvl.bund.de** > Plant Protection Products > Plant Strengtheners & Adjuvants > Plant Strengtheners

O 2-01 Kill or cure? The role of plant growth conditions in studies of interaction between endophytic biocontrol agents and their host plant

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Verticillium wilt, which causes severe yield losses in a broad range of crops, is currently difficult to suppress. In order to develop a seed treatment against Verticillium wilt for oilseed rape and Brassica vegetables, five *Serratia* and five *Paenibacillus* isolates were compared for their plant growth-promoting (PGP) potential under different plant growth conditions. These strains were selected for their antagonistic properties against fungal pathogens shown by *in vitro* tests. The selected isolates were applied to the surface-sterilized seeds of oilseed rape and cauliflower using bio-priming. *Serratia* treatment resulted in different levels of PGP under all tested plant growth conditions, while *Paenibacillus* spp. damaged roots when plants were grown in sterile germination pouches. *Paenibacillus polymyxa* Sb3-1 did not have a significant effect on plant growth in non-sterile soil, however it did promote plant growth in the sterile soil. *Serratia plymuthica* 3RP8 and *P. polymyxa* Sb3-1 were selected for further testing of their biocontrol effect under field conditions. The study proposes that the choice of growth environments is crucial for the investigation of plant-bacterium interaction. Non-sterile soil was suggested as the ideal medium for use in studying the PGP effect as it best reflects the natural growth conditions.

O 2-02 Acclimatisation of arbuscular mycorrhizal fungi to heavy metal stress

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Arbuscular mycorrhizal (AM) fungi are able to confer heavy metal (HM) tolerance to their host plants, but the extent of this ability depends on the site of isolation of AM fungal strains. Moreover, if strains are propagated under conditions without HMs, plants inoculated with such strains are less stress-tolerant compared to plants inoculated with the original isolates. Based on this observation, two hypotheses were formulated: (1) The ability to confer HM tolerance to plants depends on the HM tolerance of the AM fungus. (2) AM fungi cannot only loose, but also gain HM tolerance. The AM fungus *Rhizophagus irregularis* was cultivated in root organ cultures under increased Zn or Pb concentrations. Developing spores were transferred to new root organ cultures and fungal and root growth was monitored. The results showed that AM fungi can be acclimatised to HM stress and that this process also leads to increased tolerance of the inoculated roots. The process of acclimatisation was reflected by distinct AM fungal gene expression patterns. The acclimatisation to particular conditions will be transferred to practical AM fungal inoculum production in order to test the feasibility of the original idea for a 'Directed inoculum production process'. Moreover, particular marker genes could be used for quality control of produced inocula.

O 2-03 Transcriptomic profiling of B. phytofirmans PsJN colonizing potato plants in response to host plant drought stress

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It is widely accepted that bacterial endophytes actively colonize plants and interact with its host and positive effects on plant growth and health are well documented. However the mechanisms of plant-endophyte communication and bacteria´s adaption to the plant environment are still poorly understood. Here we investigated extracytoplasmatic function (ECF) group IV sigma factors as possible regulation elements allowing the endophytic bacteria B. phytofirmans PsJN to sense changing conditions inside a plant and to adjust its metabolism to the plant environment. A specific and efficient Taqman-quantitative PCR method was developed that allowed for identification of ECF sigma factors in PsJN that were activated in response to plant stress. Six ECF sigma factor genes were expressed in PsJN colonizing plants and the expression of one ECF sigma factor was significantly up-regulated when the plant was stressed. This indicates that B. phytofirmans PsJN actively senses the conditions in the host plant and therefore uses cell-surface signaling systems employing alternative sigma factors. Whole transcriptome sequencing of B. phytofirmans PsJN colonizing potato plants was used to analyze changes in gene expression in PsJN in response to plant stress. Transcripts significantly up-regulated in response to plant drought stress were mainly involved in transcriptional regulation, cellular homeostasis and the detoxification of reactive oxygen species whereas genes of the general metabolism were down-regulated. Collectively, our study indicates that the endophyte B. phytofirmans PsJN is affected by plant drought stress, that it senses plant stress signals and adjusts its gene expression accordingly. To the best of our knowledge this is the first study that investigates the transcriptome of B. phytofirmans PsJN during plant colonization. Data generated in this study improves our understanding on the interaction between endophytes and their host plant and the response of endophytes on plant stress.

O 2-04 Entomopathogenic fungi as endophytes: supporting their biocontrol activity by novel fermentation and formulation strategies

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Biocontrol of insect pests by entomopathogenic fungi like *Beauveria* spp. or *Metarhizium* spp. is challenging because of their lower efficacy, difficult handling and limited shelf life compared to synthetic pesticides. Recent studies have provided evidence that these fungi can grow endophytically in plant tissues, paving the way for novel plant protection measures. We hypothesize that specific formulations will enhance endophytic colonization of plant tissues thus protecting the plants from within. Fungal biomass can be applied as mycelium, microsclerotia, aero-, blasto- and/or submerged conidiospores which fundamentally differ in their cultivation requirements and pose different formulation challenges concerning their physicochemical and biological characteristics. For these "active ingredients" we evaluated three formulation strategies namely encapsulation, film coating and spray formulation.

To develop a spray formulation, different adjuvants were combined with selected fungal biomass and investigated with regard to UV stability, water retention, nutrition, viability, germination, penetration, colonization and insect mortality. By the use of sugar beet molasses as a UV-B protecting agent, the viability of *B. bassiana* spores could be increased up to 57±14 % and viability was even higher with 77±11 % when using titanium dioxide. Investigations into germination on tomato leaves revealed that only 2 % of aerial conidia germinated while also blastospores and submerged conidiospores germinated at a low percentage after 24 h. By the use of a spray formulation, this germination rate was increased up to over 35 % for blastospores and submerged conidiospores. In addition, germ tube growth was stimulated by a 45 fold increase in length. Furthermore, penetration of tomato leaves was enhanced up to 50 % using a spray formulation containing 0.1 % Triton X-114, 1 % molasses, 1 % titanium dioxide and 10^6/ml spores. Germination frequency and penetration were verified with GFP-labeled *B. bassiana*. Based on these promising findings, an integrated fermentation and formulation strategy with *Metarhizium* spp. will be developed to broaden the application spectrum of endophytic entomopathogenic fungi.

O 3-01 The mechanisms of beneficial plant-microbe interaction of the Stress Protecting Agent Stenotrophomonas rhizophila SPA P69 revealed by genome and transcriptome analysis

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Stenotrophomonas rhizophila SPA P69 (syn. DSM14405^T) promotes plant growth in a wide spectrum of crops under biotic and a-biotic stress conditions. Since little is known about the mode of interaction we have sequenced the genome of *S. rhizophila* SPA P69 as basis for studying the molecular and physiological mechanisms underlying the root system function by using both a transcriptomic approach and a root colonization assay with confocal laser scanning microscopy under different conditions. The competence to ecto- and endophytically colonize roots could be shown for all studied crops (oilseed rape, cotton, tomato and sweet pepper) and constitutes the key factor for beneficial effects on root and plant development. The genome of *S. rhizophila* SPA P69 comprises genes featuring ecological fitness and intimate interaction with plants. By analyzing the transcriptome in response to seedling extracts and salt shock, we identified functional genes that explain the colonization competence as well as the ability to promote plant growth and to enhance stress tolerance. This includes genes for host cell attachment, biofilm formation, synthesis of protective compounds and motility. Spermidine, described as a plant growth regulator, was also newly identified as a protector against stress. Furthermore, the production and excretion of the osmoprotective molecules trehalose and glucosylglycerol (GG) were found to mediated stress tolerance. Risk assessment studies reveal no health risks, mainly because SPA P69 is unable to growth at the human body temperature of 37°C due to the absence of heat shock genes and a temperature-regulated suicide mechanism. Taken together SPA P69 is a promising endophytic stress protecting agent (SPA) ready for commercial applications.

O 3-02 From lab to field: *Burkholderia phytofirmans* PsJN ameliorates drought tolerance in maize and wheat

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Burkholderia phytofirmans strain PsJN was originally isolated from surface-sterilized *Glomus vesiculiferum*-infected onion roots, and has been shown to colonize a wide variety of plants (e.g. potato, tomato, maize, wheat and grapevine). In many of its host plants strain PsJN stimulates plant growth and vitality likely due to the production of auxins, ACC deaminase, due to high antioxidative activities and additional, yet unknown mechanisms. In a genome comparison with other endophytic, proteobacterial genomes strain PsJN showed some outstanding features including a strong detoxification machinery, a high number of cell surface signaling and secretion systems as well as different quorum sensing systems.

In different experiments ranging from lab to field we tested the capacity of strain PsJN to improve drought tolerance of maize and wheat. Under greenhouse conditions strain PsJN efficiently colonized maize plants under drought conditions and improved root and shoot biomass production accompanied by improved leaf chlorophyll contents and relative water status, photochemical efficiency of PSII and photosynthetic activity. Similarly, but under field conditions, the effect of strain PsJN on drought stress amelioration of wheat was tested. Plants were exposed to drought stress at different growth stages by skipping the respective irrigation. The results showed that drought stress adversely affected physiological, biochemical and growth parameters of wheat seedlings. It decreased the CO_2 assimilation, stomatal conductance, relative water content, transpiration rate and chlorophyll contents in wheat. Inoculation of wheat with PsJN significantly reduced the adverse effects of drought on relative water contents and CO_2 assimilation rate thus improving the photosynthetic rate, water use efficiency and chlorophyll content over the uninoculated control. Grain yield was also decreased when plants were exposed to drought stress at the tillering and flowering stage, but inoculation resulted in better grain yield (up to 21 and 18 % higher, respectively) than the respective uninoculated control.

O 3-03 Antagonistic activity of fungal root endophytes from solanaceous plants against potato late blight Phytophthora infestans

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Fungal endophytes have been shown to produce secondary metabolites that can protect plants from pathogens. The antifungal activity of 354 root-endophytic fungi isolated from four solanaceous species obtained from Kenya against *Phytophthora infestans* was screened *in vitro*. Accordingly, 60 isolates were selected and further evaluated in dual culture test. The results revealed that mycelial growth of *P. infestans* was differentially diminished by the tested endophytes. *Trichoderma harzianum* along with two endophytes (KB1S2-4 and KA1S1-1) suppressed mycelial growth of the pathogen by 84.5%, 78.2% and 76.5%, respectively. The other endophytes however, were either moderate (KB1S4-7, NA1S2-10, KT1S1-6) or slight (KT2S2-5, KB2S2-5) inhibitors. The potential of bioactive crude extracts of culture filtrates obtained from 10 endophytes was evaluated against sporangia germination of *P. infestans*. The results revealed that, sporangia germination was retarded by more than 88 % in the presence of extracts from the isolates KT1S1-2, KB1S1-10 and KB1S1-8 comparing to the control. The activity of crude extracts was further assessed using thin layer chromatography and bioautography techniques. Out of 10 crude extracts tested, two showed inhibition zones corresponded to the Rf values of 0.23, 0.43 for the isolate KB1S1-8 and 0.40 for the isolate KT1S1-2. Both isolates were identified as *Aspergillus aureofulgens* and *Aspergillus flavipes* using ribosomal gene sequence analysis of the ITS1, 5.8 and ITS2 regions.

O 3-04 Antagonists of fungal pathogens for control of root-knot nematodes

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Crop infestation by root-knot nematodes often facilitates infection by soil-borne fungal pathogens resulting in synergistic yield losses. Control by microbial antagonists with dual use against nematode and fungal pathogens might provide an environmentally friendly efficient solution.

To study the potential of bacterial antagonists of fungal pathogens to control the root-knot nematode *Meloidogyne incognita* on tomato and describe the mode-of-action.

Seven bacterial antagonists of *Rhizoctonia solani* and *Verticillium dahliae* applied as seed treatment were screened for their potential to control *M. incognita* on tomato. *Rhizobium etli* G12 served as positive control and *Escherichia coli* JM109 as negative control. The top isolates were further tested regarding their mode-of-action, i.e. nematicidal potential of bacterial supernatants, direct antagonism, repellence, and induced systemic resistance.

Following seed application three *Bacillus subtilis* isolates, i.e. Sb4-23, Mc5-Re2 and Mc2-Re2, plus the positive control *R. etli* G12 significantly reduced the number of galls and egg masses. Best control was achieved by Sb4-23 and *R. etli* G12 with over 90% reduction in number of egg masses. A soil drench with bacterial supernatants significantly reduced egg masses produced by *M. incognita* on tomato by up to 62% compared with the non-treated control. In choice tests bacteria treated plants showed a tendency to be less attractive for *M. incognita* than non-treated plants. All tested bacterial antagonists induced systemic resistance towards *M. incognita* as indicated in a split-root test system. When induced systemic resistance was combined with direct antagonism overall control potential was not enhanced. Therefore, the plant mediated effect is seen as the main mode-of-action of the tested bacterial antagonists in controlling *M. incognita* on tomato. In conclusion, bacteria known for their antagonistic activity against soil-born fungal pathogens also suppressed root-knot nematodes. Such multi-purpose bacteria might provide new options for controlling disease complexes of nematodes and fungi.

P-01 BORDERLINE PRODUCTS BETWEEN FERTILIZERS/BIOSTIMULANTS AND PLANT PROTECTANTS: THE STATE OF THE ART OF MICROBIAL CONSORTIA

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In the delicate normative balance of the borderline products (plant protectants and biofertilizers/bioeffectors) it has been stressed the intention of use in the first instance, and the mode of action in a second place. For the latter the basic difference between the two type of products is that a plant protectant has mostly a targeted activity on plant pathogens, while a biofertilizer acts indirectly by fortifying the host plant (healthier plant) thus inducing a generalized resistance to the onset of pathological status, irrespective of its origin and nature. This distinction is clearly reported by SANCO (Doc. 6621-99 rev.27 of May 22nd, 2005). Case-studies are presented on the effectiveness as biofertilizers/bioeffectors of Microbial Consortia on different crops. Biofertilizers exhibit a double effect, biotic and abiotic, leading to the fortification of the crop plant linked to its more effective water and nutrients uptake as well as to a generalized healthier status. This in turn means a higher resistance to diseases. In addition, biofertilizers play a relevant role for the reduction of the environmental impact of chemical fertilizers e.g. by facilitating the uptake of phosphorus, thus reducing the need of P fertilization. The phosphorus reservoirs on the earth are expected to estinguish by the year 2050, at the actual rate of extraction/consumption. Although finding a scientifically-based balance between regulatory needs and marketing constraints is not always an easy task, the availability of scientific advancements combined to common sense should help in describing the risk profile of Microbial Consortia satisfactorily for all interested parties.

P-02 Efficient detection and quantification of Verticillium dahliae in infected olive trees by Q-PCR

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Verticillium Wilt caused by *Verticillium dahliae* Kleb. is one of the most threatening diseases of olive worldwide. For pre-planting and post-planting control of *Verticillium* wilt in olive trees, availability of a rapid and reliable method for detection and quantification of *V. dahliae* is essential. In this study, presence of *V. dahliae in* different samples from top and lower parts of twigs or leaves from naturally infected trees was examined to investigate suitability of these samples for reliable and efficient detection and quantification of *V. dahliae* by Q-PCR with *V. dahliae* specific primers. Quantities of *V. dahliae* DNA in individual twigs and leaves appeared highly variable. However, it was concluded that analysis of DNA extracted from a combined sample containing small subsamples from 5 different shoots of infected trees gives reliable results. No significant differences were observed in results for top and lower parts of the twigs. In addition, it was shown that analysis of samples containing DNA extracted from 5-10 leaves of an infected tree also gives good results. Furthermore, using plastic bag and hammer for crushing leaves to enable extraction of DNA resulted in a higher relative quantity of *V. dahliae* DNA (compared to total DNA) than using liquid nitrogen and pestle and mortar did.

P-03 Evaluation of production of some secondary metabolites (in vitro) by four endophytics fungi and their effects on plants growth of barley (Hordeum vulgare L.)

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Four root endophytics fungi (*Penicillium crustosum, Aspergillus niger, Beauveria bassiana and Marasmiellus candidus*) were tested for their ability to promote plant growth of barely (*Hordeum vulgare L*. var. *Saïda*). This work consists of two parts: the first consists at the evaluation *in vitro* of the ability of endophytics fungi to produce secondary metabolites implied in the promotion of plant growth. Results reveal that endophytics fungi have a great metabolic capacity. However, all fungal strains solubilize phosphorus; *A. niger* and *M. candidus* produce AIA; *B. bassiana* and *M. candidus* are able to produce HCN. The second part consists to the inoculation of barley seeds (*Hordeum vulgare L.*var.) by this four endophytes and evaluation of their effects *in planta* on plant growth.The results obtained during the test carried out under greenhouse conditions show a variability of effects of these endophytes . Three of them act positively on plant growth of the seedlings of barley and a growth inhibition was recorded at the seedlings treated by *M. candidus*.The most important growth promotion was recorded at the seedlings inoculated by *Penicillium crustosum* with very high gain for the whole of the studied parameters whose gain in shoot and roots length is 42,06% and 56,34%. A gain in fresh and dry weight of the aerial part of 75,32% and 68,75%. While, the gain in fresh and dry weight of the root part was marked more at the seedlings treated by *A. niger* with 64,86% and 82,6%. *P. crustosum* registers a rate of germination of 94,82% and a vigor index of 7190,2006. Root colonization by the four endophytes was evaluated at the end of the experimentation. Interesting results were obtained where the highest colonization rate (100%) was recorded at the seedlings inoculated by *A. niger.*

P-04 Honeybee dispersal of the biocontrol agent Clonostachys rosea f. catenulate J1446: effectiveness in suppressing Botrytis cinerea under field conditions

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Honey bees can transfer fungal spores and bacteria among flowers of different plant species. The ability of bees to vector fungi, bacteria, and viruses can be turned to our advantage by using them to transport biological control agents from the hive to flower, a technique known as entomovector technology. The study was performed to determine the efficacy of biofungicide (*Clonostachys rosea* f. *catenulate* J1446) to be vectored by *Apis mellifera* to control gray mold (*Botrytis cinerea*) disease of strawberry.The experiments were conducted on experimental fields of Erzincan Horticultural Research Station in Erzincan, Turkey in 2013. The experimental design was completely randomized with 4 repetitions. The frigo plants of 'Aromas' strawberry cultivars were used. Each plot (no cage and cage) consisted of 70 strawberry plants planted in an area of 3.30 m × 4 m. The number of diseased fruit of each plot was recorded. The number of diseased fruit was averagely 283 in the treatment of *Clonostachys rosea* f. *catenulate* J1446 treatment disseminated with *A. mellifera*. On the other hand, the untreated plots were calculated 915 in the diseased fruit number. There were significant differences number of diseased fruit between treatments. BICOPOLL is the first application of the use of honey bees in entomovector technology in Turkey. Results have shown that *Clonostachys rosea* f. *catenulate* J1446 can be effectively delivered by honey bees for prevention of *B. cinerea* infection in strawberry. The project BICOPOLL was founded by transnational CORE Organic II Funding Body within the FP7 ERA-Network

P-05 The impact of plant breeding on the microbiome of sugar beet

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Abiotic stresses, breeding of stress-tolerant and pathogen resistant plant cultivars and the intrinsic and extrinsic microbiome affect the success in biocontrol performance. We used omics and microscopic technologies analysing a naturally composed microbiome as seed treatment for sugar beet plants against abiotic stresses and the late root rot caused by the soilborne pathogen *Rhizoctonia solani*. Two sugar beet cultivars (BERETTA/JENNA) were used, which are characterized by *R. solani* sensitiveness/tolerance and by different abundance, enrichment and interaction of a beneficial, sugar beet-specific *Pseudomonas* genotype. In the *Rhizoctonia*-tolerant cultivar JENNA, the *Pseudomonas* genotype was enhanced in seed, endorhiza as well as rhizosphere independent of the surrounding soil as example of naturally occurring biocontrol. In contrast the *Rhizoctonia*-sensitive cultivar BERETTA contains no detectable number of this genotype. One strain of the genotype was characterized in detail; the genome information of the endophyte *P. poae* RE*1-1-14 explains the endophytic lifestyle as well as the antagonistic effect. Additionally, we analysed the impact of genetic variation in ten cultivars on the composition of the microbiota. The overall results will contribute to integrated management strategies in modern sustainable agriculture.

P-06 Does the root endophytic bacterial community in Phragmites australis contribute to phytoremediation?

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Carbamazepine is a widely used antiepileptic and mood-stabilizing drug used widely. It is found as a persistent and recalcitrant contaminant, being one of the most prominent compounds in the list of hazardous PPCPs found in Waste Water Treatment Plants (WWTPs) where its concentrations range between 0,968 µg/L in influents and 0,674 µg/L in effluents. Unfortunately, the ultimate fate of this compound is the groundwater.

Until now, phytoremedation focused on the role of plants in carbamazepine removal and investigation about degradation of carbamazepine by endophytes is scarce. Since endophytes have been proposed to play an important role in phytoremediation, this study focuses on identification and characterization of endophytic candidates for biodegradation of carbamazepine in constructed wetlands.

Phragmites australis was exposed to carbamazepine (5 mg/L) for 9 days in semi-hydroponic conditions. After surface sterilization, bacteria were isolated from rhizomes and roots. Phylogenetic analysis based on 16S rDNA sequencing revealed that the majority of isolates belong to three groups: *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*. Carbamazepine uptake and PGP traits were analyzed among the isolates. Ninety percent of the isolates produce indole acetic acid (IAA) and all of them possess at least one PGP traits tested. One isolate *(Chryseobacterium taeanense*) combines good carba-mazepine uptake and all of the PGP traits. *Rhizobium daejeonense* can remove carbamazepine and produces 23 µg/mL of IAA. *Diaphorobacter nitroreducens* and *Achromobacter mucicolens* are suitable for carbamazepine removal while both, *Pseudomonas veronii* and *Pseudomonas lini* show high siderophore production and phosphate solubilization. These isolates might be applied as inoculates in constructed wetlands to enhance phytoremediation during wastewater treatment.

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P-07 Use of phytomolecules isolated from Rosmarinus officinalis in the protection and health of plants against microbial pathogenic agents

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Pesticides used in agriculture appear responsible of the pollution of most ecosystems that negatively affect biodiversity. To reduce the harmful effects of these chemicals, the use of biopesticides of microbial or plant origin, not dangerous for environment seems a way to minimize the damages and may be one of keys to the future. In this context, a study was undertaken to investigate the biological activities of Rosemary (*Rosmarinus officinalis* L.) plant belonging to the family Lamiaceae, endemic in the Mediterranean. To do this, a phytochemical study to highlight the major metabolites including those in the majority, flavonoids, tannins and saponins. The total polyphenols were extracted and tested against twelve isolated bacterial and fungal strains of agricultural soils and their metabolic potency was evaluated in vitro according to the diffusion technique on solid and liquid medium. Comparison tests have also been included in the tests using synthetic fungicides. The evaluation of antibacterial and antifungal activities of these polyphenolic natural molecules highlights a strong inhibitory power over some tested strains of microorganisms including the *Aspergillus*, *Fusarium* and *Botrytis*. The metabolic activity varies with the nature of the substance and the nature of the microbial agent tested. The inhibition diameters sometimes exceed those caused by synthetic chemicals.

P-08 Effects of ensilage to plant pathogens

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Processing of biomass leaves residual products consisting of plant material and soil particles, which might harbour phytopathogenic organisms. In biogas plants ensilaged material is mostly used. The following investigations studied separately the effect of microbial activity during ensilage to soil borne plant parasitic organisms.

Rhizoctonia solani, Sclerotinia sclerotiorum, Verticillium dahliae, Tilletia caries, Plasmodiophora brassica and the cystnematodes Globodera rostochiensis and *Heterodera schachtii* served as test organisms. The pathogens were separately encapsulated in bags made of membrans (Bioreba, mesh 250 µm). For mechanical protection they were placed in a stainless steel container with large pin holes, which was mounted together with a temperature recording data logger on a plastic carrier. This experimental device was press fitted in a tube fodder silo (6x2,5x1,5 m, 16 tons of weight) and submitted to ensilage of small waste components of sugar beet processing. Finally the vitality of the test material was analyst.

The investigations were carried out under worst case conditions in December 2012 at the end of the sugar beet harvesting season. 14 days after filling of the silo air temperature dropped below 10°C and the maximum temperature within the silo reached 25°C only for a few hours. The total exposure time of the test organisms to the ensilage process was 60 days. In spite of the limited weather conditions distinct results were found. Eggs and larvae of the cyst nematodes G. rostochiensis and H. schachtii seemed visually damaged. The invivo-test (hatch test) confirmed complete inactivation. There was no obvious morphological damage of the fungal pathogens *R. solani, S. sclerotiorum, V. dahliae* and *T. caries.* Subsequent invitro and invivo-tests showed complete inactivation. There was no impact on viability of *Plasmodiophora brassicae.* This test material kept infectivity almost completely.

P-09 Endophytic bacteria mediated resistance in cucumber plants to Fusarium oxysporum f. sp. cucumerinum and Pseudomonas syringae pv. lachrymans

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The aim of this study was to investigate biological control possibilites of some important pathogens of cucumber plants by endophytic bacterial (EB) isolates that can provide significant biological control of 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 under *in vitro* and *in planta* conditions. The endophytic bacteria were isolated from the internal tissues of roots, leaves and stems of healthy cucumber plants. In this study, EB strains were screened *in vitro* for their siderophore and hydrogen cyanide (HCN) production and antagonistic activity against FOC and PSL. The EB isolates exhibiting biocontrol activity against PSL and FOC and producing siderophore by *in vitro* tests were further tested against these plant pathogens in growth chamber experiments. EB inoculation took place two times; before sowing as seed coating and after transplanting as substrate drenching. Cucumber plants with treated EB were transplanted to peat inoculated with FOC spore suspension in order to observe the suppression of Fusarial wilt on cucumber plants. At the same time, cucumber plants with treated EB were spray inoculated with the suspension of PSL for their biocontrol activity. According to growth chamber test results, 38% of tested EB strains exhibited the disease reduction between 30 to 60%, comparing to only FOC inoculated plants, and 50% of tested EB decreased the symptom development of PSL between 30 to 50% while the disease severity was detected at the rate of 64 % on only PSL inoculated plants We suggest that some endophytes have the potential to activate inducible plant defense systems. Our work continues with molecular tests to determine whether the effects of EB strains involve the expressions of some defence-related genes lipoxygenase (LOX2), chitinaz (PR8), and pathogenesis related proeteins (PR1, PR3).

P-10 Aerial invasion: a devastating dialogue between the pathogen Verticillium longisporum and its antagonistic counterpart Paenibacillus polymyxa Sb3-1 via volatile compounds

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Paenibacillus polymyxa Sb3-1 is an endophytic bacterium showing strong *in vitro* antagonistic activity against *Verticillium longisporum,* which causes Verticillium wilt in a range of plants including oil seed rape and cauliflower resulting in substantial yield losses every year. Here, we demonstrated that volatile organic compounds (VOCs) emitted from bacterial antagonist *P. polymyxa* Sb3-1 significantly reduced the mycelial growth of *V. longisporum* ELV43. The VOC profiles of *P. polymyxa* Sb3-1 and *V. longisporum* ELV43 were obtained through headspace collection and analysis on gas chromatography – mass spectroscopy. Up to 39 organic compounds were produced by the *P. polymyxa* Sb3-1 culture and up to 15 by *V. longisporum* ELV43 culture. Among other VOCs, *P. polymyxa* Sb3-1 produced several pyrazines that were previously shown to have a strong antifungal effect. Many of the bacterial VOCs were not identifiable because they could not be matched with the mass-spectra of volatiles in the databases. In order to study the interaction of *P. polymyxa* Sb3-1 and *V. longisporum* ELV43 via the volatiles that they produced, both microorganisms were cultured so that their VOCs were able to intermingle, however at the same time the microorganisms did not have direct contact with one other. Both microorganisms produced VOCs in reaction to one another, and we speculate that the produced VOCs are involved in a dialogue between a potential biocontrol bacterium *P. polymyxa* Sb3-1 and its plant pathogenic target *V. longisporum*. The results will be integrated in a novel biocontrol strategy against *Verticillium*.

P-11 Microbial features to improve disease suppression in grain legumes

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Soil borne pathogens cause major problems to grain legumes what leads to high crop failures. Biological control agents received from organic fertilizer can help reducing the disease outbreak. Thus, in this study we performed experiments with a model system using *Pisum sativum* and 2 fungal pathogens to adress the following questions:

1. Which pathogen can be controlled due to application with composts in general and on the field?
2. Does the microbial community change due to infection with the pathogen?
3. Can we observe major BCA after application of different pathogens?

Different levels of suppression of the pathogens due to various compost applications were observed. With high-throughput molecular fingerprinting analyses the changes in bacterial and fungal communities structures regarding to phytopathogen infection were examined. Interestingly, the response of bacterial and fungal communities is different to the two different pathogens as well as with different composts and several indicator BCA could be detected.

P-12 Diversity of endophytic bacteria associated to cowpea root nodules

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Cowpea (*Vigna unguiculata*) tolerates high temperature, drought and low soil fertility and is, hence, one of the most important food legumes in semi-arid regions. Even though under favorable growth conditions high yields have been achieved, at low input management practices the productivity of cowpea remains rather low. Inoculation of seeds with plant growth promoting (PGP) bacteria, mainly rhizobia, was proposed as a sustainable alternative to enhance productivity. The aim of this study was to investigate the diversity of bacterial symbionts nodulating cowpea in Brazilian's semi-arid soils using 16S rRNA barcode sequencing of DNA directly extracted from nodule. Nodules, rhizosphere and soil samples were obtained from two soils (Petrolina, PE - argissol and Juazeiro, BA – vertissol) cultivated with the cowpea varieties BRS Acauã (low nodulation) and BRS Pujante (high nodulation). After surface sterilization, DNA was extracted and used for analysis of 16S rRNA genes via T-RFLP and barcode sequencing. The structure of bacterial communities found in the nodules was determined by the soil type rather than the variety. We detected a high bacterial diversity in the nodules. Most of the detected groups were isolated from cowpea nodules in previous studies. Surprisingly, although *Bradyrhizobium* sequences were highly abundant, OTUs affiliated to *Chryseobacterium* were predominant in all samples. Our study reveals that the bacterial diversity found in cowpea nodules is much higher previously observed in cultivation-dependent methods. We speculate that these non-rhizobial endophytic bacteria improve plant fitness by promoting the increasing of infection sites for rhizobial colonization.

P-13 Effect of Rhizobacteria Selected for Plant Growth Promotion and Biological Control of Fusarium oxysporum f. sp. radicis-lycopersici on Yield of Tomato Plants

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Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) results in severe losses in the greenhouses and open field tomato crops.. In this study, plant growth-promoting rhizobacteria (PGPR) were tested on tomato plants for the biological control of FORL and for their ability to promote plant growth and yield. Firstly, rhizobacteria strains isolated from tomato plants in commercial greenhouses were screened *in vitro* for their plant growth promoting traits like production of indole-3-acetic acid and siderophore, phosphate solubilization and the effects on seed germination and vigor index. Also their effects on FORL were investigated. According to the results of *in vitro tests*, 10 PGPR isolates were selected for *in vivo* tests realized in growth chamber. A sensitive and a resistant tomato varieties to FORL were used as plant material. PGPR strains gave rise to significant increase in growth of tomato seedlings, TR2/1 and TR18/1 gave higher values in this respect. PGPR strains suppressed disease symptoms significantly in sensitive variety, TR21/1 and 14/1y were most effective strains for control of FORL. TR 21/1 suppressed disease symptoms by 75.7%. Four PGPR were selected for greenhouse experiment, and were tested to determine their effect on growth and productivity of tomato plants in greenhouse under healthy conditions without FORL inoculation. Among the tested PGPR; TR 2/1, TR 18/1 and TR 21/1 were found to be effective for increasing seedling growth. Tomato plants inoculated with PGPR gave higher yield compared with the control treatment during the first 4 weeks of harvesting period. It was determined that PGPR selected for the ability of plant growth promotion and biological control of FORL according to the results of *in vitro* and *in vivo* tests could also increase the yield of tomato plants without FORL inoculation. It was concluded that yield increase would be more evident in FORL sensitive varieties under FORL pressure.

P-14 Quantitative and qualitative synthesis of siderophores by Pseudomonas fulva in the presence of Cd2+

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Siderophores are low-molecular-weight chelating agents (200–2000 Da) synthesized by microorganisms to facilitate uptake of iron. Despite their preference for iron, they can also chelate numerous other metals e.g. Cd, Cu, Pb, Al or Zn, implicate in the homeostasis and heavy metal tolerance.

We hypothesized that: (1) production of siderophores by *P. fulva* can be activated or inhibited by Cd^{2+} ions and level and (2) diversity of siderophores depend on the concentrations of Cd^{2+} ions in the medium (0, 0.5, 1, 2 and 3 mM Cd^{2+}). The analysis were carried out using a spectrophotometric chemical assays, high performance liquid chromatography (HPLC) as well combined techniques - LC-Q-TOF/MS.

The results revealed the ability of *P. fulva* to synthesize both hydroxamic, catechol as well as phenol siderophores under exposure to high concentrations of Cd^{2+}. The presence of metal ions in the medium significantly stimulated the synthesis of catechol and phenolic siderophores compared to the control medium (not supplemented with Cd^{2+}). Qualitative analysis performed by liquid chromatography combined with UV/VIS detection has allowed the separation of seven compounds in extracts of culture. Comparing the retention times obtained for the standards mixture of ferrioxamines B, G_1, D_1 and E (FOX-MIX) have been identified in the analyzed extracts only ferrioxamine E. In order to confirm applied UHPLC coupled with the mass spectrometer. The obtained mass spectrum confirmed the presence of ferrioxamine E and allowed the identification of ferrioxamine G_2.

P-15 Evaluation of V. dahliae distribution and colonization in olive trees by using Q-PCR and GFP-labelled isolates

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Verticillium Wilt, caused by *Verticillium dahliae* Kleb., is a major disease of olive (*Olea europaea* L.) in most of the olive producing countries. Symptom severity depends on the virulence of the infecting *V. dahliae* pathotype, being classified as defoliating (D) or non-defoliating (ND). In this study, differences in distribution of V117 (D) and V4 (ND) isolates of *V. dahliae* in susceptible ('Picual') and resistant ('Frantoio') cultivars of olive were studied by using qPCR technique and GFP-labelled isolates. We observed lower quantities of pathogen DNA (V4 or V117) in 'Frantoio' corresponding to reduced disease severity compared to 'Picual' that showed higher disease severity and quantities of pathogen DNA. Difference in disease severity caused by D and ND isolates was not significant in each cultivar. We also observed that from 5dpi to 30dpi quantity of V4 isolate increased in 'Frantoio' and 'Picual' cultivars, whereas quantity of V117 isolate decreased in both cultivars. By the end of the experiment (106dpi) quantity of V4 in both cultivars was higher than V117. We also illustrated that *V. dahliae* is spread through the xylem system of infected trees by sporulation and upward movement of conidia, and subsequently colonizes the tree by local development of mycelium. These results indicate that both of the olive genotype and *V. dahliae* pathotype are determinative factors in disease progress and quantity of pathogen DNA in infected olive trees.

P-16 Mitigation of salt stress in Brasica napus by halotolerant endophyte - Pseudomonas stutzeri

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Salinization of arable soils is increasing and by the middle of the 21st Century, it is predicted to lead to the loss of 50% of agricultural land. Salt-affected soils lead to a reduction in crop productivity by e.g., lowering the osmotic potential, increasing Na^+ and Cl^- ions to toxic concentrations, disturbances in the uptake and transport of nutrients. Plants have evolved a wide range of mechanisms to allow them to grow in environments of high soil salinity. The co-existence of plants with highly specialized microorganisms can also increase the resistance of the host to unfavorable soil conditions.

The aim of our research was to assess the effect of endophytic bacterial strain *P. stutzeri* isolated from roots of obligatory halophytic plant (*Salicornia europaea* L.) on growth *Brasica napus* L. under salt stress conditions (0, 50, 150, 300 mM NaCl). *P. stutzeri* used for inoculation of *B. napus* was selected based on metabolic properties i.e. ability for N2 fixation. Mitigation of salt stress in *B. napus* by halotolerant endophyte was assessed using ecological (germination, biomass, chlorophyll, number of leaves, length of shoots and roots) and biochemical analysis (lipid peroxidation, glutathione and proline levels in leaves, shoots and roots).

Received results revealed strong effect of inoculation on ecological parameters of plants e.g., 4% higher germination, 132% higher biomass, 38% increased level of chlorophyll. All parameters were strongly related with the level of NaCl concentration. The highest levels of lipid peroxidation were observed in the leaves of plants (both inoculated and control). Values of lipid peroxidation increased with an increasing concentration of NaCl in the growth substrates. We have revealed also the influence of inoculation and salt concentration on the level of glutathione and proline in plant tissues.

P-17 Endophytes in biological control of citrus diseases

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Endophytic actinomycetes and fungi encompass microorganism groups that are well known for the production of a diverse range of secondary metabolites. Vochysia divergens is a medicinal plant in Brazil and Schinus terebinthifolius and Maytenus ilicifolia are also well known for the production of secondary metabolites. An endophytic actinomycete strain isolated from V. divergens, and four endophytic fungi strains isolated from S. terebinthifolius and M. ilicifolia were investigated for their potential biological activity. The actinomycete strain was characterized as Microbispora sp. LGMB259 by morphology and molecular. The fungi strains were identified as Diaporthe endophytica (LGMF928 and LGMF935) and D. terebinthifolii (LGMF907 and LGMF914) using multigene analysis. Microbispora sp. was cultivated in R5A medium, and produced four β-carboline and three indole derivatives. The major compound was identified as 1-vinyl-β-carboline-3-carboxylic acid. It displayed moderate activity against the fungi Phyllosticta citricarpa and Colletotrichum gloeosporioides. The indole compounds (indole-3-carbaldehyde, indole-3-acetic acid, and indole-3-carboxylic acid) were correlated with plant growth promotion and induced resistance upon infection by fungi in plants. These data suggest the possibility of strain LGMB259 to be used as biological control system, since this microorganism produces compounds with antimicrobial activity and improves the defense systems in plant hosts. The fungi strains reduced the pathogen inoculum against P. citricarpa in in vitro tests, in fruit detached and were transformed by A. tumefaciens with plasmid PcamDsRed for in vivo studies. Furthermore, D. endophytica and D. terebinthifolii were able to colonize citrus plants. This capability was confirmed by re-isolation of transformants after 14 days and 28 days of inoculum on inoculated and on uninoculated leaves. It was also ensured by light microscopy, showing fungus mycelium colonizing intercellular regions and glands of citrus oil. This shows that these two new species are a valuable addition to control the plant pathogens and are capable of colonizing citrus plants.

P-18 Pathogenic activity of fungal strains from the genus Phoma sp. on the synthesis of phytoalexins by the host plant

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Fungi are common causative agents of plant diseases and use a variety of strategies to infect host plants. Moreover, pathogenic fungi can have different sensitivities to the natural defence mechanisms of the host plant, which include the synthesis of phytoalexins. The genus *Phoma* includes fungal species that are important pathogens and infect crops intemperate climate region. Due to the wide variety of fungi belonging to this genus, untangling their phylogeny is a challenge.

Main aim of presentend work was: molecular identification of five strains of pathogenic fungi belonging to the genus *Phoma* sp. (supported with morphological observations) (1), differentiation of these strains based on their enzymatic activity (2), and the effect of *Phoma* sp. species on biosynthesis of phytoalexin in *Hippeastrum* scales (3).

Sequence analysis showed a close relationship between our strains and at least five species of *Phoma* and/or two *Peyronellaea* species. Their phenotypic traits suggest that all of them can either belong to *Peyronellaea curtisii* or to three other *Phoma* sp. species. The strains differed significantly in their tested enzymatic activities. A high level of amylase activity was positively correlated with cellulase activity but negatively correlated with pectolytic activity. Moreover, some strains suppressed phytoalexin production in *Hippeastrum* scales and were resistant to various concentrations of phytoalexins added to the culture medium. Based on these results, we suggest that pathogenicity in strains of *Phoma* may be associated with their interactive physiological potential for enzymatic attack and defence capabilities against phytoalexin.

P-19 Establishment of a test system to evaluate the effect of endophytes on phytoplasma infections of fruit trees

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Plant-pathogenic phloem-limited phytoplasmas cause important diseases of fruit trees such as apple proliferation (*Candidatus* Phytoplasma mali), European stone fruit yellows (*Candidatus* Phytoplasma prunorum) or pear decline (*Candidatus* Phytoplasma pyri). These diseases are difficult to control and so far only insecticide treatments against the transmitting psyllid vectors are applied. Direct curative treatments do not exist. However, symptomatic diseased trees may recover and recent data indicate that this recovery phenomenon might be linked to specific endophyte populations. This initiated search for endophytes as biocontrol agents against phytoplasma diseases in fruit trees. To evaluate the effect of selected endophytes on phytoplasmas a test system is needed which comprises the entire tree. Here we report the use of micropropagated fruit trees as such a test system which is independent from the vegetative season, standardisable, time-saving and enables larger numbers of repetitions. Phytoplasma-infected fruit tree cultures are available and virulence of different strains can be evaluated by measuring symptoms and growth parameters as well as the phytoplasma titer using quantitative real-time PCR. Different methods have been tested to inoculate selected endophytes into these micropropagated plants. Specific primers have been developed to monitor the multiplication efficiency of these endophytes in the plantlets via quantitative real-time PCR. First results were obtained with a *Pseudomonas* strain intrinsic to the tissue cultured plants. For this, we compared phytoplasma-infected, symptomatic apple plants with normal growing plants of the same culture line which spontaneously recovered from the phytoplasma infection. Interestingly, the *Pseudomonas* titer per gram fresh weight is significantly lower in phytoplasma-infected plants than in non-infected plants. Thus, the *in vitro* system can be used to study the interaction between endophyte and phytoplasma under defined conditions. This test system is now used to test field-isolated endophytic bacteria obtained from recovered fruit trees.

P-20 Effects of biogas fermentation to plant pathogens

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Processing of biomass leaves residual products consisting of plant material and soil particles, which might harbour phytopathogenic organisms. In biogas plants ensilaged material is mostly used. The following investigations studied separately the effect of microbial activity during biogas fermentation to soil borne plant parasitic organisms.

Rhizoctonia solani, Sclerotinia sclerotiorum, Verticillium dahliae, Tilletia caries, Plasmodiophora brassicea and the cyst nematodes Globodera rostochiensis and *Heterodera schachtii* served as test organisms. The pathogens were separately encapsulated in bags made of membrans (Bioreba, mesh 250 µm). For mechanical protection they were given altogether in one plastic container with large pin holes. Several of these test kits were placed in an experimental biogas fermenter of the Prüf- und Forschungsinstitut in Pirmasens. The flow medium consisted of ensilaged small waste components of sugar beet processing (mesophilic anaerobic decomposition with a temperature between 38-40°C). The test kits were removed in weekly intervals in order to analyse the vitality of the pathogens.

The cyst nematodes and most of the fungal pathogens were inactivated after a storage period of merely one week (microscopy, INVITRO- and INVIVO-test). After two weeks *Plasmodiophora brassicae* inactivated as well (biotest). Only the spores of *P. brassicae* seemed to survive the fermentation processing (microscopy).

An additional experiment based on batch fermentation (changeless medium) INVIVO-testing of the thick walled spores of P. *brassicae* showed after two weeks no more activity. Additionally a rapid inactivation of *Claviceps purpurea*, *Phytophthora infestans* and several plant seeds was found (*Avena fatua, Brassica napus, B. juncea, Rumex crispus, Solanum lycopersicum*).

The results demonstrate a high disinfection potential of biogas fermentation. A preceding lactic acid fermentation of the organic material should have a cumulative effect.

P-21 A root endophyte induces tolerance towards root herbivory in rice

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The root endophytic fungus *Piriformospora indica* recruits gibberellin (GA) signaling to colonize roots and systemically promotes plant growth under pathogen attack and salt stress. The rice water weevil (RWW) is a major insect pest of wetland rice. The adults feed on leaves without major impact on the plant, but the root-feeding larvae cause severe yield loss. We conducted two glasshouse experiments to investigate whether *P. indica* can protect rice plants against RWW. Root colonization by *P. indica* attenuated the negative impact of RWW on root and shoot biomass without affecting RWW performance, and suppressed larval induction of jasmonic acid (JA) in roots. Using the JA insensitive *COI1-18* and the GA-deficient *EUI1-OX* mutant, we observed that JA led to the depression of root growth by adult and larval feeding; an effect that was counteracted by GA. On the other hand, GA was required for the growth promoting effect of *P. indica*, while JA was uncovered to be a negative regulator of this pattern. We propose that crosstalk between GA and JA mediates the endophyte-induced tolerance towards root herbivory in rice.

P -22 Phosphorous mobilisation and biocontrol of plant pathogens combined in one strain - results of a fungal and a bacterial inoculant

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Mycorrhizal fungi are mainly known for supporting phosphorous uptake by plants, whereas *Pseudomonas* species are often selected as biocontrol strains. However, both microbial groups have also other mode of actions.

Our research showed that by inoculating peat substrate with the mycorrhizal fungus *Rhizophagus irregularis* strawberry roots were colonized by mycorrhiza, which was not the case in non-inoculated peat substrate. These mycorrhiza-colonized strawberry plants showed significantly reduced *Phythophthora cactorum* infections.

An antagonistic *Pseudomonas chlororaphis* strain was selected for its phosphorous (P) mobilisation capacity. In greenhouse assays, this strain elevated P levels in young tomato plants, and infection due to *Pythium aphanidermatum* was repeatedly reduced.

These two examples demonstrate the multiple mode of actions of one fungal and one bacterial inoculant, both combining P mobilisation and biocontrol of plant pathogens.

P-23 Bio-effectors enhance growth of tomato in low-P soil and transiently modify its rhizosphere microbiome

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Bio-effectors are a viable microorganism or active natural compounds which directly or indirectly affect plant performance and can contribute to a reduced fertilizer and pesticide use in crop production based on biological mechanisms interfering with soil-plant-microbe interactions. Low phosphorus availability limits plant growth in many soils across the world and is a common constraint to agricultural productivity. A greenhouse experiment was conducted in the framework of the EU-project "BIOFECTOR aiming to study the effect of four bio-effectors (B1: *Trichoderma harzianum* T-22; B2: *Pseudomonas* sp.; B3: *Bacillus amyloliquefaciens* FB01, B4: *Pseudomonas jessenii* RU47) on the growth of tomato plants in a phosphorus limited soil and their effects on the indigenous rhizosphere bacterial community compared to non-inoculated plants (B0). At each sampling time (t)2, 3, 4 and 6 (weeks after sowing), the rhizosphere competence and colonization patterns of the bio-effectors were monitored in rhizosphere samples using CFU counts and by confocal laser scanning microscopy (B3, B4). Effects on the bacterial community composition were determined using denaturing gradient gel electrophoresis (DGGE) and amplicon pyrosequencing of 16S rRNA gene fragments amplified from total rhizosphere community DNA of B0, B4 (t=3) and B0, B1, B2, B3, B4 (t4). All bacterial bio-effectors showed a good rhizocompetence and promoted the growth of tomato with B3 and B4 showing the best plant growth promoting activity. The DGGE and amplicon sequencing revealed that only RU47 belonged to the dominant population in the rhizosphere. The UPGMA analysis for DGGE and amplicon sequences showed significant differences between the bacterial community composition B0 and the inoculated samples. Amplicon sequencing allowed us not only to reveal bacterial genera with significantly increased or decreased relative abundance in the tomato rhizosphere compared to the bulk soil dominant genera enriched in the rhizosphere but also the taxa with increased or decreased relative abundance in response to the inoculation. At t3 B4 inoculation caused strong but transient bacterial community changes. The effect of all bio-effectors at t4 revealed that inoculation cause significant increase in the relative abundance of *Bacteroidetes* and *Betaproteobacteria*. Interestingly, the relative abundance of *Lysobacter* was significantly increased for all bacterial bio-effector treatments at t4. Our data showed that inoculants cause major but often transient shifts in the bacterial community which might also contribute to the PGP effects observed.

P-24 Study of the inhibitory power of a strain of Trichoderma sp. on some pathogenic fungi vegetable crops

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Plant diseases are caused by various organisms and in particular, fungi and bacteria. These organisms are normally present in large quantities and often in their environment, sometimes are beneficial and symbiotic to the plant.

Among the microorganisms known in the biocontrol of plant or biological control methods, which compete with pathogenic microorganisms for the invasion of the host or trigger its resistance mechanism creating a premunition the genus Trichoderma. Its wide use in protection of plants against microbial diseases and thereby it should be put to the test of serious investigations.
In this context, a study was undertaken for the evaluation of the inhibitory power of a strain of Trichoderma sp. towards some pathogenic fungal strains. To do this, the isolation was made from soil samples from a cultivated plot by vegetable crops (tomato and potato). After several purification steps on different culture media, identification was made by key determinations (Botton et al, 1983), evaluation of the Trichoderma sp antifungal activity was then tested in vitro by the method broadcast on solid culture medium in five pathogenic fungal strains. Control experiments were performed by contacting the five strains tested with solutions of semisynthetic chemical pesticides.

Antifungal activity tests highlighted the inhibitory potency of Trichoderma sp. Indeed, the inhibition of the growth depends on the nature of the strain. The inhibition diameters sometimes exceed those caused by chemical pesticide solutions.

P-25 Effect of saprophytic bacteria of wastewater on natural substances of onion "*Allium cepa* L.".

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In this work we have realized a test on onion (*Allium cepa*) in order to check the effects of treated water on some natural substances. Two treatments were chosen, firstly irrigation by treated wastewater, compared to a second one which is with treated water as a "check". The tests concerned the chlorophyll content, soluble sugar and proline. We have found that, the total chlorophyll content has been superior in the treated plants. This shows the ability of plants to react favourably under worn water irrigation. The soluble sugars, were often taken as reference's tolerance, to abiotic stress, were accumulated more than at leaves and roots level of the treated plants. The content of proline at the leaves and roots of the treated plants were superior to check, leading to the probable explanation that there is an ability of the cultivars to sustain abiotic conditions. Eventhough the results that have been obtained are somewhat positive in the expression of the varieties, a wareness has to be considered. Numerous studies and experiments have permitted these last decades, to establish standards more and more precise when it comes to deal with treated wastewater in agriculture purpose.

P-26 Study of the effect of a phytohormone on biochemical quality of *Hordeum vulgare* contaminated plants by soil *Aspergillus*

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This study aimed to test the effect of a natural plant hormone isolated from leaves of , on some biochemical parameters in leaves of Hordeum vulgare cultivated massively in the North-eastern Algeria. To do this, isolation of phytohormon was made from a green algaby on absolute methanol, followed by hot extraction in ether at alkaline pH. Young seedlings of Hordeum vulgare infected by Aspergillus were then treated with different doses of the extract plant hormone. Witnesses infected and untreated seedlings were included in the trials.

The levels of chlorophylls (a) and (b), were determined by the method of Holden (1975), the dosage of carbohydrates was performed according to the technique of Shields and Burnett (1960), total protein and proline were evaluated respectively according to Bradford (1976) and Monneveux and Nemmar (1986). The results are validated by a statistical analysis of Dunnett's test.

The results show that the levels of chlorophylls (a) and (b) of the treated plants are higher than those of infected plant and not treated. The rates of carbohydrates and proline are significantly higher compared against those of witnesses. The levels of total protein were significantly higher in plants treated with low doses, statistical analysis showed highly significant differences between treated plants and witnesses.

P-27 Chaetomium spp., potential biocontrol agents with multiple modes of action

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A number of *Chaetomium* species, especially *C. globosum,* have been reported as endophytes and producers of metabolites with antimicrobial activities. *Cheatomium* comprises a very large number of species, and it appears worthwhile to also evaluate other members of the genus regarding antagonistic capabilities and potential as biocontrol agents.

Seven isolates of *Chaetomium* from the culture collection of JKI were employed in the present study. Depending on the *Chaetomium* / pathogen combination, interactions observed in dual culture assays varied from indifferent to overgrowth of the pathogen by the antagonist. In some combinations, intracellular growth and coiling indicated a parasitic relationship. When added to agar media, the culture filtrates of all *Chaetomium* species caused at least some inhibition of the mycelial growth of *B. cinerea*, *P. infestans* or *F. oxysporum*. The most effective species in this respect were *C. aureum*, *C. globosum* and *C. indicum.* More drastic effects were observed when the phytopathogens were directly grown in culture filtrate. In order to study the ability to grow endophytically, barley, wheat and tomato seeds were sown into potting substrate amended with the antagonists. Most species could be successfully re-isolated from parts of young plants placed on agar media. However, the ability to grow endophytically depended largely on the *Chaetomium* / host plant combination. Overall, endophytic growth appeared to be rather erratic.