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# Using Ecological Knowledge and Molecular Tools to Develop Effective and Safe Biocontrol Strategies

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## 1. Introduction

Today's farming systems undermine the well-being of communities in many ways: farming has destroyed huge regions of natural habitats, which also implies a loss of species and their ecosystem services (Sachs et al., 2010). Plant protection measures also causes problems for human health (Horrigan et al., 2002), and agriculture is responsible for about 30% of greenhouse-gas-emission (IPCC, 2007). Furthermore, emerging, re-emerging and endemic plant pathogens continue to challenge our ability to safeguard plant growth and health worldwide (Miller et al., 2009). Therefore, one of the major challenges for the 21st century will be an environmentally sound and sustainable crop production.

Microbial inoculants containing microorganisms with beneficial plant-microbe interactions have a great potential to contribute to this objective (Berg, 2009; Bhattacharjee et al., 2008). Over the past 150 years, research has demonstrated repeatedly that bacteria and fungi have an intimate interaction with their host plants and are able to promote plant growth as well as to suppress plant pathogens (Compant et al., 2005; Lugtenberg & Kamilova, 2009; Weller et al., 2002; Weller, 2007; Whipps, 2001). All plant-associated microenvironments, especially the rhizosphere, are colonized in high abundances by antagonistic microbes (Berg et al., 2005a). Between 1 and 35% of the microbial inhabitants showed antagonistic capacity to inhibit the growth of pathogens *in vitro* (Berg et al., 2002, 2006). The proportion of isolates, which express plant growth promoting traits is much higher in general, and was found up to 2/3 of the cultivable population (Cattelan et al., 1999; Fürnkranz et al., 2009; Lottmann et al., 1999). Diverse microbial inoculants, which were selected from this promising indigenous potential, are already on the market. In recent years, the popularity of microbial inoculants has increased substantially, as extensive and systematic research has enhanced their effectiveness and consistency (Berg, 2009).

New molecular and microscopic techniques are one reason for progress in biocontrol research. These techniques have enhanced our understanding about the plant and especially

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the rhizosphere as a microbial ecosystem and resulted into more effective screening strategies for bioactive microbes. In this chapter we will discuss these points first in general and in a second part with three representative examples.

## 2. Molecular and microscopic tools in biocontrol research

Molecular and microscopic tools can be used to study the ecology of single plant growth promoting rhizobacteria (PGPR) or biological control agent (BCA) strains or to analyse the structure and function of the target microbial community. In a first step we will analyse the use of methods for single strains (Table 1). Here, molecular fingerprints using repetitive elements in the genome (Rademaker & de Bruijn, 1997) can be used at several levels of biocontrol research. While the functions of many of these repetitive sequence elements are still unknown, they have proven to be useful as the basis of several powerful tools for use in microbial ecology. The repetitive, sequence-based PCR or rep-PCR DNA fingerprint technique uses primers targeting several of these repetitive elements and PCR to generate unique DNA profiles or 'fingerprints' of individual microbial strains (Ishii & Sadowsky, 2009). In screening strategies, these fingerprints can be applied to differentiate strains at population level and to select only unique isolates (Berg et al., 2006; Faltin et al., 2004). In a later stage, these highly reproducible fingerprints can be used for identity check and quality control. Genome sequencing also offers a tool to study PGPRs in great detail. Strains of *Pseudomonas fluorescens*, one of the dominant and cosmopolitan plant-associated species (Weller, 2007), were the first sequenced strains (Paulsen et al., 2005). Genomic information allowed the analysis of the mode of action, detailed investigations of interactions as well as optimisation of fermentation and formulation processes (rev. in Gross & Loper, 2009). De Bruijn et al. (2007) used genome mining to discover unknown gene clusters and traits that are highly relevant in the life style of *P. fluorescens* SBW25. Proteomic and transcriptomic studies are interesting to study the function of BCAs. For example, Garbeva et al. (2011) studied transcriptional and antagonistic responses of *Pseudomonas fluorescens* Pf0-1 to phylogenetically different bacterial competitors (*Bacillus, Brevundimonas* and *Pedobacter*), which demonstrated that Pf0-1 shows a species-specific response to bacterial competitors. In another transcriptomic study published by Hassan et al. (2010), a whole genome oligonucleotide microarray was developed for *P. fluorescens* Pf-5 and used to assess the consequences of a gacA mutation: GacA significantly influenced transcript levels of 10% of the 6147 annotated genes in the Pf-5 genome including genes involved in the production of hydrogen cyanide, pyoluteorin and the extracellular protease. Transcriptomic studies can also lead to new insights into plant responses on BCAs: *Pseudomonas*-primed barley genes indicated that, as is the case in dicots, jasmonic acid plays a role in host responses (Petti et al., 2010). A new tool is metabolomics, which allow the analysis of metabolites *in situ*. This is not only a technique to answer questions about the activity *ad planta*, it is also important for registration procedures, which are still a high hurdle on the way to the market. Frimmersdorf et al. (2010) used a metabolomic approach to show how *Pseudomonas aeruginosa* adapts to various environments. In addition, analysis of the mobilome of strains can result in interesting findings for biocontrol research as shown for *P. fluorescens* Pf-5 by Mavrodi et al. (2009), in which mobile genetic elements contain determinants that contribute to Pf-5's ability to adapt to changing environmental conditions and/or colonize new ecological niches. Studying the colonisation of plants has been greatly facilitated by the application of fluorescent proteins which are used as vital markers and reporter genes (rev. in Bloemberg, 2007). These new insights have changed our understanding about

colonisation; many of the strains analysed showed an endophytic life style (Chin-A-Woeng et al., 1997; Zachow et al., 2010), and the "root shield", which was hypothesized in former times, was rarely found in contrast to single cells and micro-colonies. Raman-FISH combines stable-isotope Raman spectroscopy and fluorescence *in situ* hybridization for the single cell analysis of identity and function (Huang et al., 2007a). This potential has been demonstrated through the discriminant functional analysis of Raman spectral profiles (RSP) obtained from the soil and plant-associated bacterium *P. fluorescens* SBW25; results suggests that SBW25 growth in the phytosphere is generally neither carbon-catabolite-repressed nor carbon-limited (Huang et al., 2007b).

Molecular tools were also used to analyse target habitats of biocontrol studies (Table 1). Cultivation-based methods to analyse plant-associated bacteria only address the culturable fraction, which are thought to represent only a small proportion (0.1 to 10%) of the total bacteria present in soil and in the rhizosphere (Amann et al., 1995). The analysis of nucleic acids directly extracted from plant microenvironments opened the chance to study a much broader spectrum of microbes (Table 1). Most frequently ribosomal RNA gene fragments are amplified from total community DNA and subsequently analysed by fingerprinting techniques: Terminal restriction fragment length polymorphism (T-RFLP), single-strand conformation polymorphism (SSCP), denaturing/temperature gradient gel electrophoresis (D/TGGE) using universal/specific primers (Schwieger & Tebbe, 1998; Smalla et al., 2007). Application of these fingerprinting techniques resulted in important findings such as plant-specific microbial communities (Smalla et al., 2001), the impact of cultivars on microbial communities (Milling et al., 2004) or the structure of endophytic communities (Rasche et al., 2006). Fingerprinting techniques are often used to analyse the structure of plant-associated communities and can also be used to study functional aspects. For example, Briones et al. (2002) found cultivar-specific differences for ammonia-oxidizing bacteria (AOB) in rice rhizospheres by a multiphasic approach including DGGE of the amoA gene, analysis of libraries of cloned amoA, fluorescently tagged oligonucleotide probes targeting 16S rRNA of

| Objective/Level | Isolates: BCAs and pathogens | Microbial communities |
|---|---|---|
| Molecular fingerprints | Rep-PCR (BOX) | T-RFLP, SSCP, D/TGGE using universal/specific primers |
| Genomic information | Genome sequencing | Metagenome |
| Functions<br>Functional diversity | Transcriptomics (RNA-based)<br>Proteomics (Protein-based) | Metatranscriptome<br>Metaproteome |
| Bioactive compounds | Metabolome | Metabolome |
| Adaptation/evolution | Mobilome | Metamobilome |
| Visualisation/activity | GFP/DsRed labelled strains, CLSM<br>Raman spectroscopy and fluorescence *in situ* hybridization (FISH) | FISH-CLSM |

Table 1. Molecular and microscopic tools in biocontrol research.

AOBs as well as metabolism rates obtained by the 15N dilution technique. Other techniques have a great impact on our functional understanding; this was shown for example for transcriptome profiling (Mark et al., 2005; Yuan et al., 2008), microarrays (Sanguin et al., 2006; Weinert et al., 2011) *in vivo* expression technology and differential fluorescence induction promoter traps as tools for exploring niche-specific gene expression (Rediers et al., 2005), new methods for the *in situ* analysis of antifungal gene expression using flow cytometry combined with green fluorescent protein (GFP)-based reporter fusions (de Werra et al., 2008), barcode pyrosequencing (Gomes et al., 2010), and ultra deep sequencing (Velicer et al., 2006). Stable isotope probing (SIP) used to determine bacterial communities assimilating each carbon source in the rhizosphere of four plant species resulted in plant species specific patterns (Haichar et al., 2008). Metagenomic approaches have been established to analyse the plant-soil interface (Erkel et al., 2006; rev. in Leveau, 2007).

## 3. Using ecological knowledge to screen and evaluate biocontrol agents

The advanced techniques discussed above should be integrated into strategies to screen and evaluate biocontrol agents (Fig. 1). Of primary importance is the life cycle of the pathogen. This can result in new targets for biocontrol; one example is the impact of zoospores on pathogenic oomycetes, which are primary targets for suppression (de Bruijn et al., 2007; Raaijmakers et al., 2010). Furthermore, it is also important to understand the target microenvironment of plants. Plant specificity is one critical point but also knowledge about the structure and function of the microbial communities. There are strategies to select BCAs from the indigenous antagonistic potential as well as to use ubiquitous, cosmopolitan BCAs (Zachow et al., 2010). If a BCA is selected, an evaluation strategy is needed to assess their potential for commercialization.

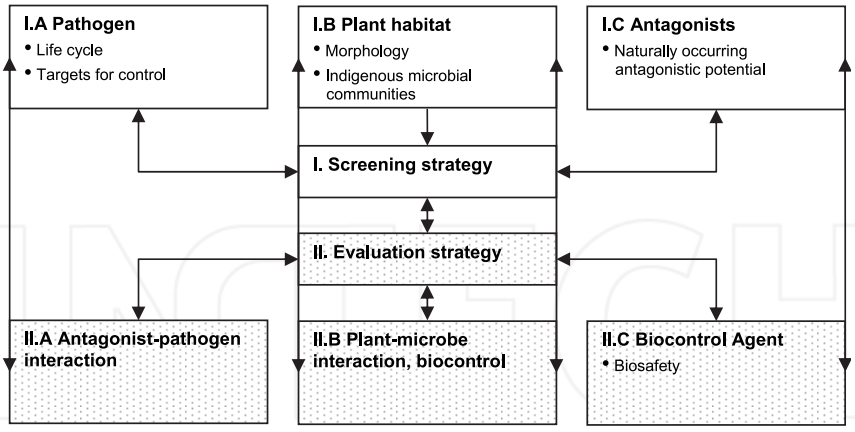


Fig. 1. Integration of ecological knowledge into screening and evaluation strategies.

Knowledge about the effect of BCAs under greenhouse and field conditions presents the basis for this evaluation. However, often inconsistent effects make the decision difficult. Detailed analyses of plant-microbe and pathogen-microbe interactions under different environmental conditions can help to optimize the biocontrol effect under practical conditions. Another aspect, which should be integrated in an early phase of evaluation, is

biosafety. Many BCAs fail here due to problems with human or environmental health. Due to the fact that the whole program to investigate toxicology is time-consuming and expensive, alternative test systems should be used, e.g. the *Caenorhabditis elegans* assay (Zachow et al., 2009) or Duckweed (*Lemna minor*) as a model plant system for the study of human microbial pathogenesis (Zhang et al., 2010).

## 4. Examples for screening and evaluation strategies

### 4.1 Strategy to control soil-borne pathogens on medical plants under organic conditions in Egypt

On the SEKEM farms in Egypt desert land was converted into arable land, and biodynamic agriculture is operated for over 30 years now (www.sekem.com). Today SEKEM is carrying out organic agriculture on more than 4100 hectares and has the largest market for organic products outside Europe and North America. They produce organic foods, spices, tea, cotton textiles and natural remedies. However, the cultivation especially of medical plants is more and more affected by soil-borne phytopathogens, which lead to significant yield losses. The objective of our study was to develop a specific biocontrol strategy for desert farming.

An important factor was to find out, whether and how the highly specialized natural microbial communities of the desert soil are affected by agriculture and watering. To examine the impact of organic agriculture on bacterial diversity and community compositions in desert soil, soil from a SEKEM farm in comparison to the surrounding desert soil were assessed by a pyrosequencing-based analysis of partial 16S rRNA gene sequences. When appropriate primers are chosen, in a pyrosequencing analysis with short reads the microbial diversity is represented almost as reliably as with near-full-length sequences (Will et al., 2010). Fragments encompassing the V4-V5 region of the 16S rRNA gene provide estimates comparable to those obtained with the nearly complete fragment (Youssef et al., 2009). In desert soil 19244 and in agricultural soil 33384 quality sequences with a read length of ≥ 150 bp were recovered. Using different data bases, 83.0% of all quality sequences could be classified below the domain level, in the range of the percentage of classified 16S rRNA gene sequences of other pyrosequencing-based studies (Lauber et al., 2009; Lazarevic et al., 2009; Will et al., 2010). The computed Shannon indices of diversity (H') (Shannon, 1997) were much higher for agricultural soil than for desert soil (H' at a dissimilarity level of 20%: SEKEM soil 4.29; desert soil 3.54); this indicates a higher bacterial diversity in soil due the agricultural use of the desert. A comparison of rarefaction analyses with the number of operational taxonomic units (OTUs) estimated by the Chao1 richness estimator (Chao & Bunge, 2002; Will et al., 2010) revealed that at this genetic distance the surveying effort in both soils covered almost the full extent (over 97% in both soils) of taxonomic diversity. This was also shown by a clear saturation of both curves in the rarefaction analysis (data not shown). The 43673 classifiable sequences obtained from both soil types together were affiliated with 18 different phyla. Dominant groups were especially Proteobacteria (30.2%), Firmicutes (27.3%) and Actinobacteria (10.5%). These dominant phyla were present in both soils. In detail, Firmicutes were highly enriched in agricultural soil (from 11.3% in desert soil to 36.6% in SEKEM soil), Proteobacteria (46.0% in desert soil and 21.0% in SEKEM soil) and Actinobacteria (20.7% in desert soil and 4.6% in SEKEM soil) occurred in SEKEM in lower abundances than in the surrounding desert. In addition, in both soils Bacteroidetes (4.6% and 5.3%) and Gemmatimonadetes (1.4% and 1.9%) were

present. Whereas Acidobacteria (7.9%) and Planctomycetes (1.1%) were only present in the agricultural soil, *Deinococcus-Thermus* (1.1%) was only detectable in the desert sand. These abundances of the phyla are coextensive with results from previously reported meta-analysis of bacterial community composition in soils and, despite the specific soil type of the desert, the composition covers rather well with studies of completely different soils (Hansel et al., 2008; Janssen, 2006; Lauber et al, 2009; Will et al., 2010). However, greatly different from all reported studies was the high abundance of Firmicutes. Janssen (2006) reported them to contribute only a mean of 2% (range 0 – 8%) in the total bacterial soil community. Most of the Firmicutes sequences were classified as belonging to the genus *Bacillus*; in the agricultural soil also the phylogenetically related genus *Paenibacillus* was found (5% of classified Firmicutes). In desert soil, *Ochrobactrum* was the most abundant genus within the (Alpha-)Proteobacteria (79% of classified Proteobacteria) and *Rhodococcus* among the Actinobacteria (90% of classified Actinobacteria). The Acidobacteria in the agricultural soil are affiliated only with subdivision 6.

Additionally to the pyrosequencing analysis, the composition of the bacterial as well as fungal community in the two different soil types was investigated by SSCP analysis of rRNA gene fragments (Bassam et al., 1991; Schwieger & Tebbe, 1998). Furthermore, the composition of the microbial community in rhizosphere and endorhiza of three different species of medical plants (*Matricaria chamomilla* L., *Calendula officinalis* L. and *Solanum distichum* Schumach. & Thonn.) grown under organic conditions on SEKEM farms were examined. According to the cluster analysis prepared on the basis of SSCP community fingerprints, the agricultural soil in bacterial as well as in fungal community composition strongly differed from the desert soil. As shown in the pyrosequencing analysis, in comparison to the desert in soil of the SEKEM farm an impressive diversity of bacteria, expressed as various bands in the gel, was found (data not shown). In the bacterial community of the desert soil, two dominant bacterial bands could be detected, which were also visible in all samples from the endorhiza of all three investigated medical plants. This shows that bacteria are taken up by the plants from the soil, and that soil is the main reservoir for biological control agents. The two dominant bands were identified by partial 16S rRNA gene sequence analysis as *Ochrobactrum* sp. (closest database match *O. grignonense*) and *Rhodococcus* sp. (closest database match *R. erythropolis*). Further, nearly in all samples *Bacillus* sp. was found (closest database match *B. subtilis*). By SSCP analysis and also by the pyrosequencing approach, *Ochrobactrum* and *Rhodococcus* could be detected as dominant bacteria. However, both genera include opportunistic human pathogens (*O. anthropi*, *R. equi*). Several studies provided evidence that similar or even identical functions are responsible for beneficial interactions with plants and virulence in humans (Berg et al., 2011). For *Ochrobactrum* was already detected the production of plant growth hormones and siderophores and also an antifungal activity towards several phytopathogens was described (Chakraborty et al., 2009). *Ochrobactrum* was found in diverse environmental niches, like rhizosphere, soil, sediments and activated sludge (Berg et al., 2005b). *Rhodococcus* could also be found in a broad range of environments, including soil, water and eukaryotic cells. This genus includes also a phytopathogenic species causing leafy gall formation on a wide range of host plants, *R. fascians* (Goethals et al., 2001). The fungal community fingerprints included a quite high diversity in all microenvironments. As an example, SSCP profiles of fungal communities in rhizosphere and endorhiza are shown in Figure 2. A dominant band, which was found nearly in all samples, was identified as

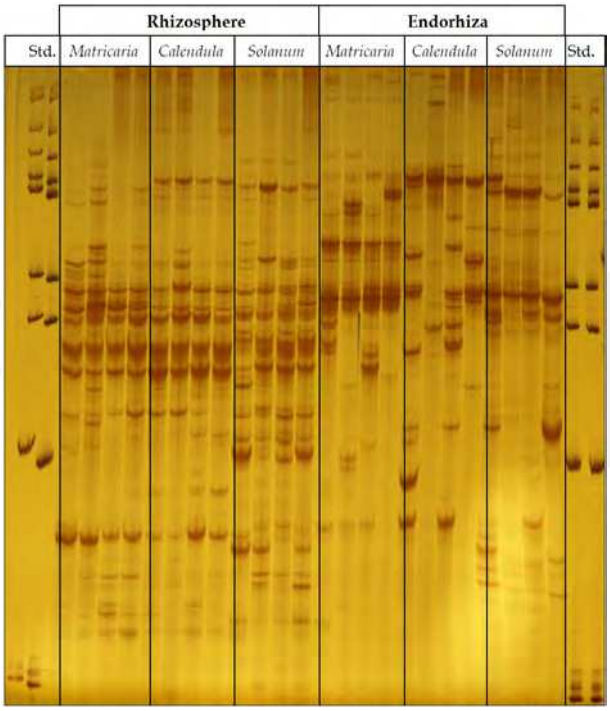


Fig. 2. SSCP profiles of the fungal communities in rhizosphere and endorhiza of the medical plants. Four independent replicates per plant and microenvironment were loaded onto the gel. Std.: 1 kb DNA ladder.

*Verticillium dahliae*, which is one of the mainly occurring soil-borne phytopathogens on the SEKEM farms. In general, mainly potential plant pathogens were found within the fungal communities. The obligate root-infecting pathogen *Olpidium,* belonging to the fungal phylum Chytridiomycota, was found especially in the rhizosphere and endorhiza of *Matricaria chamomilla*. *Alternaria* and *Acremonium* were found primarily in the rhizosphere samples. According to the generated dendrograms, a clear plant specificity of the bacterial and fungal communities in the rhizosphere as well as in the endorhiza was found (Fig. 3). Furthermore, microenvironment-specific SSCP patterns of the bacterial and the fungal communities were detected (data not shown). There were significant differences between the rhizosphere and the endorhiza of the medical plants. In general, samples from the rhizosphere generated more bands than samples from the endorhiza of the medical plants, which indicate that a sub-set of rhizobacteria was able to invade the root.

The major problems in the cultivation of plants on SEKEM farms are caused by the soil-borne pathogenic fungi *Verticillium dahliae* Kleb., *Rhizoctonia solani* J.G. Kühn and *Fusarium culmorum* (Wm.G. Sm.) Sacc. as well as by the soil-borne pathogenic bacterium *Ralstonia solanacearum*. Although grown in organic agriculture, which aims to minimize the impact on the environment by practices such as crop rotation, using pathogen resistant cultivars, and the use of organic manure (compost) instead of synthetic fertilizers (Schmid et al., 2011), they have an increasing importance. One reason is an intensive growing of a limited number

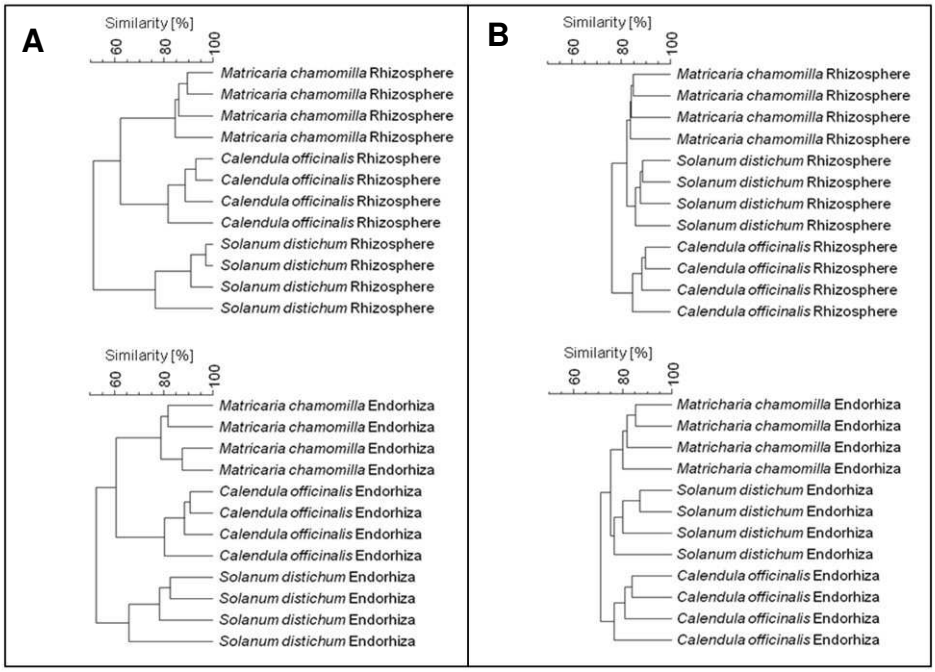


Fig. 3. UPGMA dendrograms of bacterial (A) and fungal (B) communities in rhizosphere and endorhiza of the medical plants. The dendrograms were generated from the SSCP community profiles with GelCompar II. The following settings were used: dendrogram type: unweighted pair group method with arithmetic mean (UPGMA); similarity coefficient: band based: dice; position tolerances: optimization: 4%, position tolerance: 1%.

of crops in short rotations. Here, biocontrol agents should solve these problems and help to suppress soil-borne pathogens on a natural way. Although BCAs are already on the market, our biocontrol product will be optimized for desert farming – regarding soil, weather, pathogen species, etc. For this reason, autochthonous bacteria were isolated from rhizosphere and endorhiza of medical plants as well as from bulk soil collected in SEKEM farms, and were evaluated for their potential for biocontrol. In a first step, the dual-culture assay was used to find out the antagonistic potential towards the pathogenic fungi (Berg et al., 2002, 2005a). A total of 1589 bacterial isolates were screened for their ability to inhibit *in vitro* the growth of *Verticillium dahliae*, *Rhizoctonia solani* and *Fusarium culmorum*. Bacterial isolates obtained from the soil of the SEKEM farm exhibited a higher *in vitro* antagonistic potential towards soil-borne phytopathogenic fungi in comparison to the bacteria isolated from the desert soil (SEKEM 21.6 ± 0.8%; desert 12.4 ± 0.7%). From the agricultural soil 17.4% (27 isolates) demonstrated antagonism towards all three pathogens, from the desert soil 10.6% (21 isolates) were able to suppress the growth of all fungi tested. Already the desert soil harbours a high proportion of antagonists, which were augmented by organic agriculture in SEKEM soil. The soil from the farm seems to be supplied with antagonists in such an optimal way, that there was no detectable enrichment of antagonists in the rhizosphere and endorhiza of the investigated medical plants. In general, *Matricaria*

*chamomilla* and *Solanum distichum* showed a better antagonistic potential than *Calendula officinalis*. Especially the endorhiza from *Matricaria chamomilla* harbours a high proportion of antagonists. Whereas in the soil and in the rhizosphere could be found most antagonistic bacteria towards *Fusarium culmorum*, in the endorhiza of the medical plants most antagonists were found towards *Verticillium dahliae*.

In a next step, the antagonistic mechanisms of all isolates, which showed an activity towards at least two of the investigated pathogenic fungi (162 isolates), were investigated *in vitro* with a special focus on fungal cell wall degrading enzymes (β-1,3-glucanase, chitinase and protease) (Chernin et al., 1995; Grube et al., 2009) and siderophore-production (Schwyn & Neilands, 1987). Production of chitinase could be detected for 8.0% of the antagonists; *Lysobacter enzymogenes* followed by all isolates of *Streptomyces* showed a high chitinolytic activity. Glucanase activity was shown for nearly all isolated antagonists (93.8%); only the isolates of the *Bacillus cereus* group were not able to degrade β-1,3-glucan. Casein degradation by protease could be shown at 80.9% (*Bacillus* sp. and *Lysobacter* sp.). The production of siderophores was shown for all antagonists except the isolates of *Paenibacillus* sp. (93.2%).

To avoid investigations with genetically similar strains, amplified rRNA gene restriction analysis (ARDRA) of the 16S rRNA gene with the restriction endonuclease *Hha*I (Zachow et al., 2008) and BOX polymerase chain reaction fingerprints (Berg et al., 2002; Rademaker & de Bruijn, 1997) of the antagonistic isolates were performed. A representative selection of promising biological control agents was identified by partial 16S rRNA gene sequencing. The use of ARDRA of the 16S rRNA gene with the restriction enzyme *Hha*I led to the separation of isolates clustered into five groups (data not shown); within groups the similarity of the band patterns was 100% identical: *Bacillus subtilis* group, *Bacillus cereus* group, *Paenibacillus*, *Streptomyces* and *Lysobacter*. Except *Lysobacter* (only one isolate from the rhizosphere of *Matricaria chamomilla*) only gram-positive antagonists were found. All microenvironments were dominated by antagonists from the Firmicutes branch. *Bacillus* and *Paenibacillus* could be isolated from all habitats. Antagonistic isolates of the genus *Streptomyces* were found exclusively in desert soil. Especially within the large ARDRA cluster of the *Bacillus subtilis* group containing 123 isolates, analysis of the BOX PCR fingerprints showed a high genotypic diversity. At a cutoff level of 80%, they could be divided into 39 genotypic groups. The genus *Paenibacillus* could be divided into 11 BOX clusters, *Streptomyces* was subdivided in three genotypes. According to the ARDRA and BOX dendrograms, 46 preferably genotypically different strains were selected to test them on their antibacterial activity towards *Ralstonia solanacearum* (Adesina et al., 2007) and *Escherichia coli*. The cluster of the *Bacillus cereus* group was completely excluded for further investigations, because of some human pathogenic strains belonging to this taxonomic group. Most isolates of the genus *Paenibacillus* (identified as *P. brasilensis* and *P. polymyxa*) were able to inhibit *in vitro* the growth of *E. coli* (7 of 11 isolates), but these strains showed no antagonistic activity towards *R. solanacearum*. The growth of *R. solanacearum* was inhibited by 32.6% of the selected antagonists: most isolates of *Streptomyces* (3 of 4 isolates) and some strains of the *Bacillus subtilis* group (12 of 30 isolates).

Organic amendments like manure, compost and cover crops positively affected the disease suppressiveness of SEKEM soil. During decomposition of organic matter in soil, the ecosystem is subjected to oligotrophication. The ratio of oligotrophic to copiotrophic organisms changes during microbial succession, and this has been associated with general disease suppression (van Bruggen & Semenov, 2000; Garbeva et al., 2004). Our cultivation-

independent approaches showed an extraordinary high Firmicutes level in SEKEM soils. By cultivation and characterization, the antagonistic role of *Bacillus* and *Paenibacillus* (both Firmicutes) was identified. Both are well-known and potent in biocontrol (Berg, 2009; Schisler et al., 2004; Tupinambá et al., 2008). These gram-positive bacteria have a natural formulation advantage due to their ability to form durable, heat-resistant endospores (Emmert & Handelsman, 1999). *Lysobacter* was the only gram-negative genus identified (Park et al., 2008). This is in contrast to the majority of other studies, where members of the *Pseudomonas* genus play a major role (Haas & Défago, 2005; Weller et al. 2007). Due to the fact that the proportion of antagonistic strains in soil and root is already high, biocontrol strategies could aim to enhance the diversity of the antagonistic community by application of *Lysobacter, Pseudomonas* or *Serratia* strains. However, in our study we selected promising candidates, which will be tested *ad planta* in comparison to these often used antagonists.

### 4.2 Strategy to control Fusarium wilt in bananas in Uganda

The banana family Musaceae includes monocotyledonous plants of the genera *Ensete*, *Musa* and *Musella*. Most important is the genus *Musa* comprising 50 to 100 species and cultivars including those with edible fruits like dessert or cooking banana, species with inedible fruits like ornamental bananas or those used for fibres production (Li et al., 2010). In many countries in Africa, Latin America, Asia or the Caribbean, banana production is an important source of income. Banana is the fourth important staple food after rice, wheat and milk in Uganda, the country with the highest per capita consumption per year of cooking banana and the second largest producer after India in the world. Farmers have to deal with several problems as plant pests and diseases, climate change or soil depletion. Diseases caused by fungi, bacteria and viruses are the most limiting factors of high quality production. Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*), is the most severe disease in banana plants, which leads to high yield losses (Ploetz, 2006). An infestation with the phytopathogen compromises the water and nutrient transport that can cause, in the worst case, the death of the plant. *Foc* belongs to the *F. oxysporum* species complex, which is distributed in a broad range of soils and causes serious symptoms on numerous host plants. Despite its ubiquitous occurrence, a morphological identification is difficult and is based primarily on the structure and abundance of asexual reproductive structures and on cultural characterizations (Fourie et al., 2011). The species is divided into more than 150 *formae specialis* and further subdivided in races, depending on the affected plant cultivars. *F. oxysporum* persists in soil as immobile chlamydospore until germinating by utilizing nutrients released from plant roots. The life cycle of the fungus commences with a penetration of the spore germ tube or the mycelium of the plants root tip. Further, wounds facilitate the endophyte an entrance of the potential host. When the mycelium entered the xylem vessel, it travels upwards through the plant. In later stages, microconidia are produced, which are distributed in the vessel system and germinate when their movement is stopped. This decreases water and nutrient transport, resulting in severe wilt and eventually death of the plant. Early symptoms of an infestation are reddish brown colouration of the xylem, a yellowing of old leaves and a beginning of wilt. In advanced stages, pseudostem coating leaves collapse and die. The pseudostem sometimes splits. Internally, xylem vessels of the roots and the rhizome turn reddish-brown as the fungus grows through the tissue (Aboul-Soud et al., 2004; Daly & Walduck, 2006). Different studies with bananas and banana plants *in vitro* and *in vivo* have shown that plants harbour fungal and bacterial organisms with antagonistic potential towards plant pathogens (Cao et al.,

2005; de Costa et al., 1997; Lian et al., 2008). However, an efficient strategy to control fungal pathogens especially *Foc* is still missing. In our study, we used molecular techniques to study banana-associated microbial communities in detail and focus on endophytes, which have a great potential for biocontrol of vascular diseases.

For screening of antagonists the rhizosphere, the endosphere and bulk soil of Ugandan banana plants were analysed. The term endosphere refers to the pseudostem of the plant, which is not lignified. Bananas grown in four different fields (variants) in Central Uganda characterized by different manure systems and/or agro-forest systems were sampled. In the first step, bacterial and fungal abundances in the microhabitats were examined. Surprisingly, the highest bacterial abundances with $\log_{10}$ 9.4 ± 0.1 $g^{-1}$ fw were calculated for the endosphere followed by the rhizosphere with $\log_{10}$ 8.4 ± 0.3 $g^{-1}$ fw and soil with $\log_{10}$ 7.7 ± 0.3 $g^{-1}$ fw from R2A medium. Similar values for all microhabitats ranging from $\log_{10}$ 6.2 ± 0.2 $g^{-1}$ fw for rhizosphere followed by soil and endosphere with almost same abundances of $\log_{10}$ 5.5 ± 0.3 $g^{-1}$ fw and $\log_{10}$ 5.4 ± 0.3 $g^{-1}$ fw were estimated for fungal isolates on synthetic nutrient-poor agar (SNA). A total of 1152 bacterial isolates from different media as R2A, MacConkey (for enrichment of Enterobacteriaceae) and King's B medium (for enrichment of *Pseudomonas*) and 586 fungi from SNA medium were randomly selected and screened *in vitro* for their antagonistic potential towards the pathogens. The target pathogen was also isolated from bananas in Uganda. Interestingly, different fungal species were identified: *F. oxysporum* f.sp. *cubense, Fusarium chlamydosporum,* and *Colletotrichum musae.* The latter are known as "low" pathogens; however, strains of all three species were integrated in the screening strategy. The antagonistic activity of bacteria or fungi towards the pathogen evaluated by the method of Berg et al. (2006) ranged from 3 – 6%. Altogether 37 highly active bacterial and 36 fungal strains were further characterized. ARDRA genotyping was able to distinguish bacteria on genus level into *Pseudomonas*, *Bacillus*, *Burkholderia* and *Serratia*. With repetitive BOX PCR a further characterization on population level was performed. Members of the genus *Burkholderia* were more diverse than those of *Serratia* (Fig. 4).

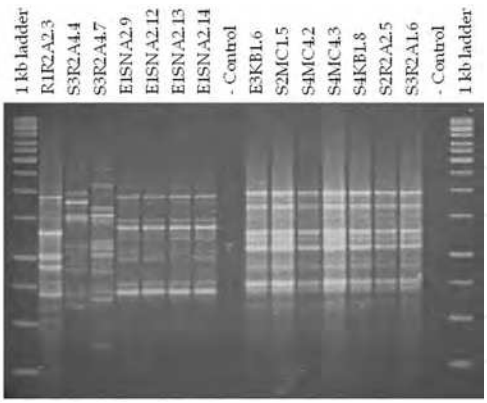


Fig. 4. BOX analysis on species level of bacterial antagonists. First seven isolates were identified as *Burkholderia* species and the other seven as *Serratia marcescens*. For identification of isolates the following abbreviations were used: a) habitat with R for rhizosphere, S for soil and E for endosphere, b) number of variant from 1 to 4, c) medium isolated from MC for MacConkey agar, KB for King's B agar, R2A for R2A agar and SNA for synthetic nutrient-poor agar d) number of replicate from 1 to 4 and e) number of isolate from 1 to 14.

Additionally, the best antagonists were screened for their ability to produce lytic enzymes like glucanase or protease, which are known for their positive influence in combating fungal pathogens by enzymatic degradation of the cell wall (Kamensky et al., 2003). Further, the production of siderophores, short-chained quorum sensing molecules and the auxin indole-3-acetic-acid (IAA) was investigated, which are involved in plant growth promoting processes. The results indicated that 100% of the tested isolates produced an active protease, while only a single isolate, which was identified as *Bacillus indicus*, was able to degrade glucan. Nearly all strains (94.6%) produced siderophores but only 21.6% isolates, belonging to the genera *Pseudomonas* and *Burkholderia*, released quorum sensing molecules. Seven isolates were positively tested for production of IAA, all of them identified as *Serratia marcescens*. To characterize fungal isolates, morphological groups were identified. Sequencing analysis of the ITS region indicated, that the majority of isolates belong to the genera *Penicillium*, *Paecilomyces*, *Fusarium* and *Mortierella*. All of them include known biocontrol strains, some actually tested in *Musa* spp. like non-pathogenic *F. oxysporum* strains (Kidane, 2008).

Cultivation independent analyses include the fingerprint method SSCP, quantitative PCR (qPCR), metagenome analysis and confocal laser scanning microscopy (CLSM) in combination with fluorescence *in situ* hybridization (FISH). Using SSCP fingerprints, a high specificity was shown for each microenvironment of banana, particularly for the endosphere. The patterns obtained from the bacterial community using universal primers were highly diverse, especially for rhizosphere and soil. This is a typical picture for environmental samples, especially for soil. A detection of bacterial species ranges up to 100 most dominant ones. This problem can be solved by using of more specific primers, e.g. for *Pseudomonas* or Enterobacteriaceae. Using both in analyses, specific patterns for each habitat appeared. Surprisingly, comparing all fields with different treatments or environmental influence, bacterial, enteric and fungal community didn't show distinct patterns. This could be explained by a high specificity of banana-associated bacteria independent from the site. The *Pseudomonas* community was more sensitive, but each site showed an individual pattern. In our study, we found that Enterobacteriaceae were extraordinarily present in and around cultivated banana plants. Therefore, further investigations on the microhabitat-specific communities were performed using a metagenomic approach. The sequences (1944 – 23800) obtained after pyrosequencing were aligned with databases and identified on genus level. In Figure 5 taxa including more than 1% of the totally analysed community were presented. Each habitat harboured a specific arrangement of genera. In the two rhizosphere variants, more than 40% of the identified genera are members of the *Enterobacter* community, followed by *Serratia*, *Pantoea* and *Klebsiella* with almost 40% and some other genera making up less than 20%. The bacterial composition in the endosphere differed from the rhizosphere samples with a lower number of *Enterobacter* and higher presence of the genus *Raoultella*. The highest species richness was shown for the soil sample, with the dominant genus *Pantoea* with known plant growth promoting species (Bonaterra et al., 2005; Braun-Kiewnick et al., 2000). *Serratia*, *Klebsiella* and *Enterobacter* represented together more than 40% of the analysed species. The analysis illustrates that depending on the investigated microhabitat, different species dominated. For the majority of the listed genera, species with growth promoting abilities are described. In different parts of the plant, diverse species play a key role, like *Enterobacter* in rhizosphere or *Pantoea* in soil and endosphere. To complement pyrosequencing data, a further assessment of *Pseudomonas* and Enterobacteriaceae was performed with quantitative PCR. Similar results were measured for both communities; the highest copy numbers $g^{-1}$ fresh material of enterics and pseudomonads were detected in endosphere with $\log_{10}$ 8.4 ± 0.5 for *Pseudomonas* and $\log_{10}$ 7.9 ± 0.2 for Enterobacteriaceae

followed by rhizosphere with $\log_{10}$ 7.2 ± 0.6 and $\log_{10}$ 6.8 ± 0.4 and last with $\log_{10}$ 6.3 ± 0.8 for enterics in soil. In the *Pseudomonas*-specific analysis, no data for soil were received due to values under the detection limit. With confocal laser scanning analysis (CLSM) detection of different bacterial classes as Alpha-, Beta- and Gammaproteobacteria was performed to illustration our data. Due to the fact, that the number of enterics was extraordinary high in prior analyses, the focus in microscopy was also set on *Enterobacteriaceae*. The microscopic analysis confirmed the previous results, with the detection of a high number of *Enterobacteriaceae* in the endosphere and also lower detection in rhizosphere.

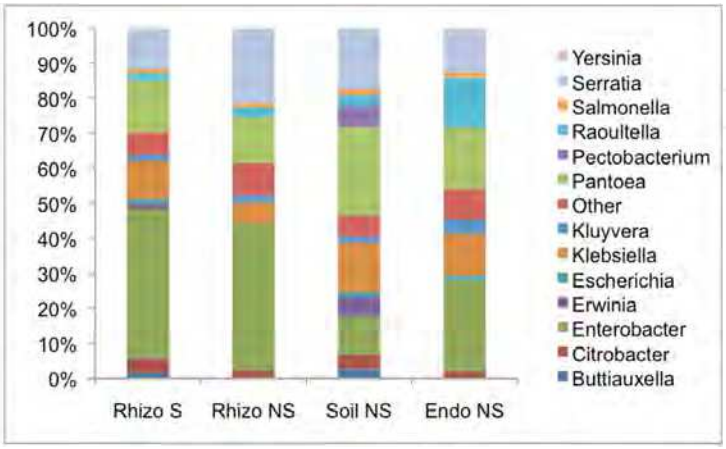


Fig. 5. Genera of the Enterobacteriaceae community associated with banana plants. Two rhizosphere samples under influence of agro-forest (shaded = S) and not (non-shaded = NS) and one sample from soil and endosphere in comparison. DNA was amplified with enterics-specific primers and analysed by pyrosequencing and identification with the web server SnoWMAn 1.7. The pipeline used was BLAT, NCBI database was selected and included taxa covering more than 1%. Phylogenetic groups accounting for ≤ 1 % of all quality sequences are summarized in the artificial group others.

This multiphasic approach showed that the pseudostem of banana – the endosphere – is a unique microenvironment in plants. It is characterized by extremely high microbial abundances, a high diversity and specificity, but a low proportion of antagonistic strains. Enterics play a key role in the bacterial community; they are dominant and represent a cluster of antagonists. However, they also contain human and plant pathogenic species. The endosphere should be the target habitat for biocontrol strategies: the number of strains with a beneficial plant impact should be enhanced here. We have isolated promising strains of *Pseudomonas*, *Bacillus*, *Burkholderia* and *Serratia,* which are interesting candidates for *ad planta* experiments. However, it is necessary to pay attention to the enteric community in bananas, especially to the pathogens.

### 4.3 Strategy to control a multi-species disease in the Styrian oilseed pumpkin

Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) is a pumpkin variety that bears its name according to its origin of cultivation that is the Austrian district Styria. The specialty of this cultivar is the absence of a wooden seed shell that facilitates the production of pumpkin seed oil. Beside the culinary aspect of this dark green oil it is

famous as a very healthy nutritional supplement containing high levels of polyunsaturated fatty acids, antioxidants, vitamins A, B1, B2, B6, C, D, E and counteracts diseases of bladder and prostate.

In recent years, dramatic yield losses of Styrian oil pumpkin were reported in Styria due to black rot of pumpkins caused by *Didymella bryoniae* (Auersw.) Rehm, anamorph *Phoma cucurbitacearum* (Fr.) (Huss et al., 2007). The ascomycete has a broad host range within the Cucurbitaceae and causes symptoms on vegetative plant parts known as gummy stem blight (Keinath et al., 1995). It spreads from temperate to tropical regions of the world (Sitterly & Keinath, 1996). Fruits, leaves and flower scars are invaded by the pathogen and it can also be seed-borne (Lee et al., 1984; Ling et al., 2010; de Neergaard, 1989; Sitterly & Keinath, 1996). By cultivation-independent SSCP fingerprinting of the fungal ribosomal internal transcribed spacer (ITS) region in combination with DNA sequencing and BLAST analysis (Altschul et al., 1997), it was detected as well in roots of oil pumpkin (data not shown). This underlines the potential establishment of the pathogen even in soils (Bruton, 1998). The analysis of the phenotypic and genotypic variability of the pathogen across different oil pumpkin fields in Styria resulted in a remarkable high morphological versatility in contrast to a low genetic diversity (Zitzenbacher, pers. communication). Styrian oil pumpkins are also affected by bacterial pathogens *Pectobacterium carotovorum* subsp. *carotovorum* and subsp. *atrosepticum*, *Pseudomonas* spp. and *Xanthomonas cucurbitae* causing soft rot of pumpkins and leaf diseases (Huss, 2011). The transport of these bacterial phytopathogens by the fungus was observed in vitro (Zitzenbacher, pers. communication) suggesting synergistic interactions between them in the course of co-infections.

In order to manage microbial diseases of Styrian oil pumpkin based on autochthonous bacterial and fungal antagonists, initial studies to discover the microbial diversity associated with this host plant were conducted. Roots, female flowers and fruit pulp from three different oil pumpkin cultivars ("Gleisdorfer Ölkürbis", "Gleisdorfer Diamant" and "GL Maximal") at a field site in Styria were collected. Root samples were taken at three time points (before flowering, time of flowering, fruits well developed). Bacterial genera *Pseudomonas* and *Bacillus* that are known for their plant beneficial interactions (Haas & Défago, 2005) were analysed by SSCP analysis. Data revealed a greater impact of the microhabitat on community structure for *Pseudomonas*, whereas the plant stage had a stronger impact for *Bacillus* populations. Female flowers as possible gates for bacterial and fungal infections were analysed in more detail. For *Bacillus* and *Pseudomonas* and ascomycete communities, no effect of the plant cultivar on population structure was observed. However, in the flower, the communities are well-structured. FISH-CLSM studies revealed a dense bacterial colonisation of pollen grains that act as propagation vehicles between pistils especially for Alphaproteobacteria (Fig. 6) and shaped in this way the bacterial community structure of the oil pumpkin anthosphere.

To obtain oil pumpkin-associated microorganisms for testing their antagonistic properties against *D. bryoniae* and bacterial pathogens, bacterial and fungal strains were isolated from oil pumpkin cultivars and microhabitats as described above. Endophytes were cultivated from roots and fruit pulp. In addition, seed borne microbial strains were obtained from aforementioned varieties by the isolation from roots, stems and leaves from plants that seeds were surface sterilized and grown under gnotobiotic conditions. Finally 2320 isolates (1748 bacteria and 572 fungi) were subjected to dual culture assays against *D. bryoniae* A-220-2b to test their antagonistic potential against this pathogen. Of tested bacteria, 7.3% inhibited growth, whereas 12.4% of observed fungi showed either growth inhibition or overgrowth of *D. bryoniae* (Fig. 7).

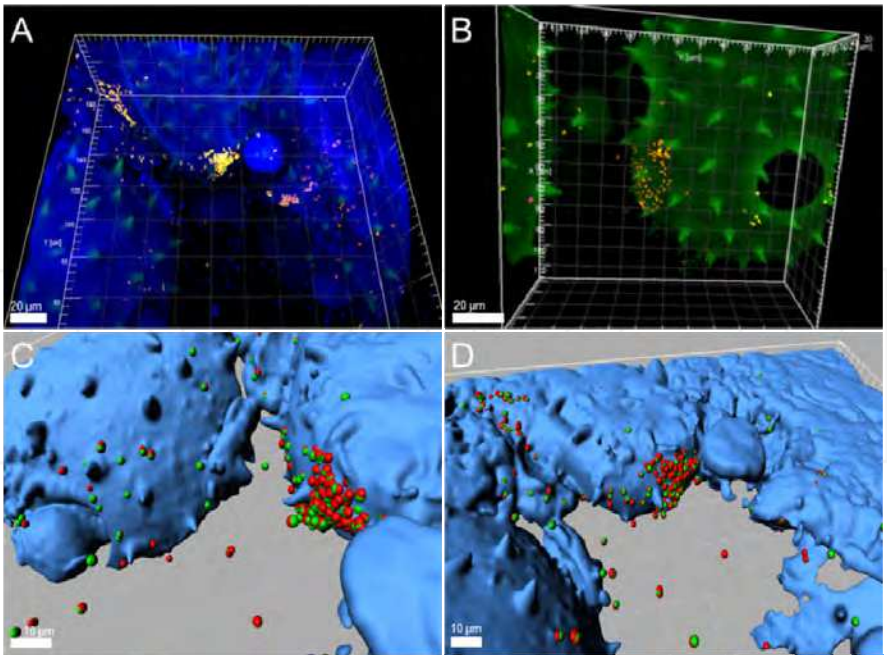


Fig. 6. FISH-stained bacteria colonising pollen grains located on pistils of oil pumpkin (GL Opal) visualized by CLSM. A) Alphaproteobacteria (in yellow) and not taxonomically classified bacteria (in red) labelled with ALF968-Cy5 and EUB338MIX-Cy3. B) Alphaproteobacteria labelled with ALF968-Cy5 (yellow), Firmicutes labelled with LGC354MIX-FITC (pink) and taxonomically undefined bacteria (in red) labelled with EUB338Mix-Cy3. C,D) 3D rendered image (Imaris software) of overall bacterial communities (in red) labelled with EUB338MIX-Cy3 and Alphaproteobacteria (red and green) labelled with ALF968-Cy5.

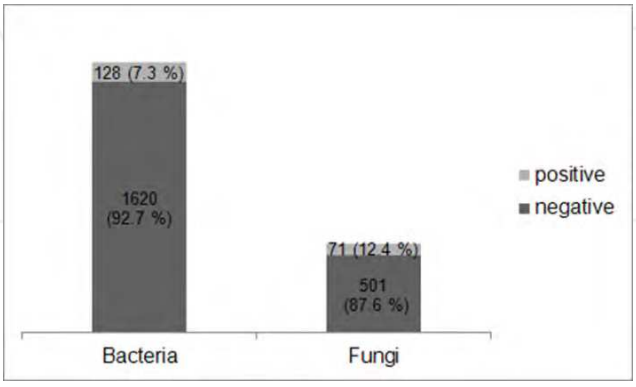


Fig. 7. Amount of oil pumpkin-associated bacterial and fungal isolates positively or negatively tested for *in vitro* antagonism against *D. bryoniae* A-220-2b.

Potential antagonists (128 bacteria and 71 fungi) were subsequently screened *in vitro* for effects on growth inhibition of *Pectobacterium carotovorum* subsp. *atrosepticum* 25-2, *Pseudomonas viridiflava* 2d1 and *Xanthomonas cucurbitae* 6h4 to find broad-spectrum antagonists. Altogether, 32% of fungal as well as 49% of bacterial *D. bryoniae* antagonists were positively tested against at least one, 34% of tested prokaryotes against at least two and 6% of investigated bacterial strains against all three bacterial phytopathogens, whereas no fungal *D. bryoniae* antagonist was effective against more than one bacterial pathogen (Fig. 8).

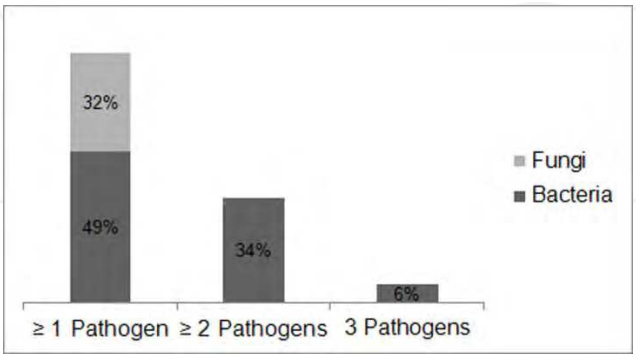


Fig. 8. Percentage of fungal and bacterial *D. bryoniae* antagonists positively tested against at least one, two or all three bacterial pathogens *Pectobacterium carotovorum* subsp. *atrosepticum* 25-2, *Pseudomonas viridiflava* 2d1 *and Xanthomonas cucurbitae* 6h4.

Broad-spectrum antagonists that have the potential to suppress *D. bryoniae* as well as at least two bacterial phytopathogens of oil pumpkin were characterized genotypically by ARDRA. This resulted in a grouping of 43 bacterial isolates into four different genera: *Pseudomonas*, *Paenibacillus*, *Serratia* and *Lysobacter*. As a relative high number of isolates belong to *Paenibacillus* and *Lysobacter* they were further analysed by BOX PCR (Rademaker & de Bruijn, 1997) to get insight into the intra-genera diversities. Within the group of *Paenibacillus* a negligible variability between BOX patterns was observed in contrast to strains of *Lysobacter* that were divided into five groups. Finally five potential broad-spectrum antagonists were chosen for further analysis: one representative for *Pseudomonas*, *Paenibacillus* and *Serratia* and two representatives from the *Lysobacter* cluster. Partial sequencing of 16S rRNA genes with subsequent BLAST analysis (Altschul et al., 1997) was performed for their identification and the following species could be affiliated to respective strains: *Pseudomonas chlororaphis* P34, *Paenibacillus polymyxa* PB71, *Serratia plymuthica* S13, *Lysobacter antibioticus* L175 and *L. gummosus* L101. To learn more about the mode of antagonism of chosen broad-spectrum antagonists against *D. bryoniae*, dual culture assays in which growth inhibition of *D. bryoniae* A-220-2b by either soluble or volatile antimicrobial compounds secreted by the five test strains was assessed were performed. Results suggest a high capability of broad-spectrum antagonists to synthesize bioactive compounds: sterile culture supernatants from *P. chlororaphis* P34, *L. gummosus* L101 and *P. polymyxa* PB71 as well as volatile organic compounds (VOCs) excreted from these bacteria and *S. plymuthica* S13 as well suppressed growth of the fungus significantly compared to control treatments (ANOVA; LSD, $p < 0.05$; data not shown).

Performances of broad-spectrum antagonists in terms of promoting plant growth and health will facilitate the selection of bacterial strains that will be analysed for the production of a biological strengthener for Styrian oil pumpkin. Studies with the model organism *C. elegans* (Zachow et al., 2009) will give insight into the potential pathogenicity of remaining test strains. The manufacture of the final product will further depend on the finding of an appropriate formulation procedure that guarantees a high stability of the ultimate BCAs/PGPRs.

## 5. Conclusion

Advanced ecological knowledge about plant-associated microorganisms and interactions of the biocontrol agent(s) with abiotic and biotic factors support the development of efficient biocontrol strategies. As shown in three examples, specific strategies have to be developed adapted to the life cycle of the pathogen and the autochthonous microbial communities in the target habitat. The latter varied strongly dependent on the plant species, microenvironment and climate.

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## 7. References

Aboul-Soud, M.A., Yun, B.W., Harrier, L.A. & Loake G.J. (2004). Transformation of *Fusarium oxysporum* by particle bombardment and characterisation of the resulting transformants expressing a GFP transgene. *Mycopathologia* 158, 475-482

Adesina, M.F., Lembke, A., Costa, R., Speksnijder, A. & Smalla K. (2007). Screening of bacterial isolates from various European soils for *in vitro* antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: site-dependent composition and diversity revealed. *Soil Biol Biochem* 39, 2818-2828

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J.H., Zhang, Z., Miller, W. & Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25, 3389-3402

Amann, R.I., Ludwig, W. & Schleifer, K.H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* 59, 143-169

Bassam, B.J., Caetano-Anolles, G. & Gresshoff, P.M. (1991). Fast and sensitive silver staining of DNA in polyacrylamid gels, *Anal Biochem* 196, 80-83

Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A. & Smalla, K. (2002). Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. *Appl Environ Microbiol* 68, 3328-3338

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A. & Hallmann, J. (2005a). Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol Ecol* 51, 215-229

Berg, G., Eberl, L. & Hartmann, A. (2005b). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ Microbiol* 7, 1673-1685

Berg, G., Opelt, K., Zachow, C., Lottmann, J., Götz, M., Costa, R. & Smalla, K. (2006). The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. *FEMS Microbiol Ecol* 56, 250-261

Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol* 84, 11-18

Berg, G., Zachow, C., Cardinale, M. & Müller, H. (2011). Ecology and human pathogenicity of plant-associated bacteria, In: *Regulation of biological control agents*, Ehlers, R.U., 175-189, Springer, Dordrecht

Bhattacharjee, R.B., Sing, A. & Mukhopadyay, S.N. (2008). Use of nitrogen-fixing bacteria as biofertilizer for non-legumes: prospects and challenges. *Appl Microbiol Biotechnol* 80, 199-209

Bloemberg, G.V. (2007). Microscopic analysis of plant–bacterium interactions using auto fluorescent proteins. *Eur J Plant Pathol* 119, 301-309

Bonaterra, A., Camps, J. & Montesinos, E. (2005). Osmotically induced trehalose and glycine betaine accumulation improves tolerance to dessication, survival and efficacy of the postharvest biocontrol agent *Pantoea agglomerans* EPS125. *FEMS Microbiol Lett* 250, 1-8

Braun-Kiewnick, A., Jacobsen, B.J. & Sands, D.C. (2000). Biological control of *Pseudomonas syringae* pv. *syringae,* the causal agent of basal kernel blight of barley, by antagonistic *Pantoea agglomerans*. *Phytopathology* 90, 368-375

Briones, A.M., Okabe, S., Umemiya, Y., Ramsing, N.B., Reichardt, W. & Okuyama, H. (2002). Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. *Appl Environ Microbiol* 68, 3067-3075

van Bruggen, A.H.C. & Semenov, A.M. (2000). In search of biological indicators for soil health and disease suppression. *Appl Soil Ecol* 15, 13-24

de Bruijn, I., de Kock, M.J., Yang, M., de Waard, P., van Beek, T.A. & Raaijmakers, J.M. (2007). Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in *Pseudomonas* species. *Mol Microbiol* 63, 417-428

Bruton, B.D. (1998). Soilborne diseases in *Cucurbitaceae*: pathogen virulence and host resistance, In: *Cucurbitaceae '98*, McCreight, J.D., 143-166, ASHS Press, Alexandria

Cao, L., Qiu, Z., You, J., Tan, H. & Zhou, S. (2005). Isolation and characterization of endophytic streptomycete antagonists of Fusarium wilt pathogen from surface-sterilized banana roots. *FEMS Microbiol Lett* 247, 147-152

Cattelan, A.J., Hartel, P.G. & Fuhrmann, J.J. (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sci Soc Am J* 63, 1670-1680

Chakraborty, U., Chakraborty, B.N., Basnet, M. & Chakraborty, A.P. (2009). Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease. *J Appl Microbiol* 107, 625-634

Chao, A. & Bunge, J. (2002). Estimating the number of species in a stochastic abundance model. *Biometrics* 58, 531-539

Chernin, L., Ismailov, Z., Haran, S. & Chet, I. (1995). Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. *Appl Environ Microbiol* 61, 1720-1726

Chin-A-Woeng, T.F.C., de Priester, W., van der Bij, A. & Lugtenberg, B.J.J. (1997). Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. *Mol Plant Microbe Interact* 10, 79-86

Compant, S., Duffy, B., Nowak, J., Clement, C. & Barka, E.A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl Environ Microbiol* 71, 4951-4959

de Costa, D.M., Amaradasa, B.S. & Wegiriya, R.N.B.P.M.R.C.L. (1997). Anatgonists *of Colletotrichum musae* associated with banana fruit skin. *J Natn Sci Coun Sri Lanka* 25, 95-104

Daly, A. & Walduck, G. (2006). Fusarium wilt of bananas (Panama disease) (*Fusarium oxysporum* f.sp. *cubense*). *Agnote* 151, Available from: https://transact.nt.gov.au/ebiz/dbird/TechPublications.nsf/1717DB3D7CD580C06925723C004EA6DD/$file/786.pdf?OpenElement

Emmert, E.A. & Handelsman, J. (1999). Biocontrol of plant disease: a (gram-) positive perspective. *FEMS Microbiol Lett* 171, 1-9

Erkel, C., Kube, M., Reinhardt, R. & Liesack, W. (2006). Genome of rice cluster I archaea – the key methane producers in the rice rhizosphere. *Science* 313, 370-372

Faltin, F., Lottmann, J., Grosch, R. & Berg, G. (2004). Strategy to select and assess antagonistic bacteria for biological control of *Rhizoctonia solani* Kühn. *Can J Microbiol* 50, 811-820

Fourie, G., Steenkamp, E.T., Ploetz, R.C., Gordon, T.R. & Viljoen, A. (2011). Current status of the taxonomic position of *Fusarium oxysporum formae specialis cubense* within the *Fusarium oxysporum* complex. *Infect Genet Evol*, In press

Frimmersdorf, E., Horatzek, S., Pelnikevich, A., Wiehlmann, L. & Schomburg, D. (2010). How *Pseudomonas aeruginosa* adapts to various environments: a metabolomic approach. *Environ Microbiol* 12, 1734-1747

Fürnkranz, M., Müller, H. & Berg, G. (2009). Characterization of plant growth promoting bacteria from crops in Bolivia. *J Plant Dis Protect* 116, 149-155

Garbeva, P., van Veen, J.A. & van Elsas, J.D. (2004). Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu Rev Phytopathol* 42, 243-270

Garbeva, P., Silby, M.W., Raaijmakers, J.M., Levy, S.B. & Boer, W.D. (2011). Transcriptional and antagonistic responses of *Pseudomonas fluorescens* Pf0-1 to phylogenetically different bacterial competitors. *ISME J*, In Press

Goethals, K., Vereecke, D., Jaziri, M., van Montagu, M. & Holsters, M. (2001). Leafy gall formation by *Rhodococcus fascians*. *Annu Rev Phytopathol* 39, 27-52

Gomes, N.C., Cleary, D.F., Pinto, F.N., Egas, C., Almeida, A., Cunha, A., Mendonça-Hagler, L.C. & Smalla, K. (2010). Taking root: enduring effect of rhizosphere bacterial colonization in mangroves. *PLoS ONE* 5, e14065

Gross, H. & Loper, J.E. (2009). Genomics of secondary metabolite production by *Pseudomonas* spp. *Nat Prod Rep* 26, 1408-1446

Grube, M., Cardinale, M., de Castro, J.V. Jr., Müller, H. & Berg, G. (2009). Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *ISME J* 3, 1105-1115

Haas, D. & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 3, 307-319

Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T. & Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME J* 2, 1221-1230

Hansel, C.M., Fendorf, S., Jardine, P.M. & Francis, C.A. (2008). Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl Environ Microbiol* 74, 1620-1633

Hassan, K.A., Johnson, A., Shaffer, B.T., Ren, Q., Kidarsa, T.A., Elbourne, L.D., Hartney, S., Duboy, R., Goebel, N.C., Zabriskie, T.M., Paulsen, I.T. & Loper, J.E. (2010). Inactivation of the GacA response regulator in *Pseudomonas fluorescens* Pf-5 has far-reaching transcriptomic consequences. *Environ Microbiol* 12, 899-915

Horrigan, L., Lawrence, R.S. & Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ Health Perspect* 110, 445-456

Huang, W.E., Stoecker, K., Griffiths, R., Newbold, L., Daims, H., Whiteley, A.S. & Wagner, M. (2007a). Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence *in situ* hybridization for the single cell analysis of identity and function. *Environ Microbiol* 9, 1878-1889

Huang, W.E., Bailey, M.J., Thompson, I.P., Whiteley, A.S. & Spiers, A.J. (2007b). Single-cell Raman spectral profiles of *Pseudomonas fluorescens* SBW25 reflects *in vitro* and *in planta* metabolic history. *Microb Ecol* 53, 414-425

Huss, H., Winkler, J. & Greimel, C. (2007). Der Pilz *Didymella bryoniae* schädigt steirischen Ölkürbisanbau: Fruchtfäule statt Kernöl. *Der Pflanzenarzt* 60, 14-16

Huss, H. (2011). Krankheiten und Schädlinge im Ölkürbisbau. *Der fortschrittliche Landwirt* 3, 30-33

IPCC (2007). Fourth assessment report of the intergouvernmental panel on climate change, In: *Climate Change 2007*, Cambridge University Press, Available from: www.ipcc.ch

Ishii, S. & Sadowsky, M.J. (2009). Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution. *Environ Microbiol* 11, 733-740

Janssen, P.H. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* 72, 1719-1728

Kamensky, M., Ovadis, M., Chet, I. & Chernin, L. (2003). Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biol Biochem* 35, 323-331

Keinath, A.P., Farnham, W. & Zitter, T.A. (1995). Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma* spp. isolated from cucurbits. *Phytopathology* 85, 364-369

Kidane, E.G. (2008). *Management of Fusarium wilt diseases using non-pathogenic Fusarium oxysporum and silicon*, Doctoral Thesis, University of KwaZulu-Natal, Pietermaritzburg

Lauber, C.L., Hamady, M., Knight, R. & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 75, 5111-5120

Lazarevic, V., Whiteson, K., Huse, S., Hernandez, D., Farinelli, L., Østerås, M., Schrenzel, J. & François, P. (2009). Metagenomic study of the oral microbiota by Illumina high-throughput sequencing. *J Microbiol Methods* 79, 266-271

Lee, D., Mathur, S.B. & Neergaard, P. (1984). Detection and location of seed-borne inoculum of *Didymella bryoniae* and its transmission in seedling of cucumber and pumpkin. *J Phytopathol* 109, 301-308

Leveau, J.H.J. (2007). The magic and menace of metagenomics: prospects for the study of plant growth-promoting rhizobacteria. *Eur J Plant Pathol* 119, 279-300

Li, L.F., Häkkinen, M., Yuan, Y.M., Hao, G. & Ge, X.J. (2010). Molecular phylogeny and systematics of the banana family (Musaceae) inferred from multiple nuclear and chloroplast DNA fragments, with a special reference to the genus *Musa*. *Mol Phylogenet Evol* 57, 1-10

Lian, J., Wang, Z. & Zhou, S. (2008). Response of endophytic bacterial communities in banana tissue culture plantlets to Fusarium wilt pathogen infection. *J Gen Appl Microbiol* 54, 83-92

Ling, K.S., Wechter, W.P., Somai, B.M., Walcott, R.R. & Keinath, A.P. (2010). An improved real-time PCR system for broad-spectrum detection of *Didymella bryoniae*, the causal agent of gummy stem blight of cucurbits. *Seed Sci Technol* 38, 692-703

Lottmann, J., Heuer, H., Smalla, K. & Berg, G. (1999). Influence of transgenic T4-lysozyme-producing plants on beneficial plant-associated bacteria. *FEMS Microbiol Ecol* 29, 365-377

Lugtenberg, B. & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 63, 541-556

Mark, G.L., Dow, J.M., Kiely, P.D., Higgins, H., Haynes, J., Baysse, C., Abbas, A., Foley, T., Franks, A., Morrissey, J. & O'Gara, F. (2005). Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proc Natl Acad Sci USA* 102, 17454-17459

Mavrodi, D.V., Loper, J.E., Paulsen, I.T. & Thomashow, L.S. (2009). Mobile genetic elements in the genome of the beneficial rhizobacterium *Pseudomonas fluorescens* Pf-5. *BMC Microbiol* 9, 8

Miller, S.A., Beed, F.D. & Harmon, C.L. (2009). Plant disease diagnostic capabilities and networks. *Annu Rev Phytopathol* 47, 15-38

Milling, A., Smalla, K., Xaver, F., Maidl, K., Schloter, M. & Munch, J.C. (2004). Effects of transgenic potatoes with an altered starch composition on the diversity of soil and rhizosphere bacteria and fungi. *Plant Soil* 266, 23-39

de Neergaard, E. (1989). Histological investigations of flower parts of cucumber infected by *Didymella bryoniae*. *Can J Plant Pathol* 11, 28-38

Park, J.H., Kim, R., Aslam, Z., Jeon, C.O. & Chung, Y.R. (2008). *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Microbiol* 58, 387-392

Paulsen, I.T., Press, C.M., Ravel, J., Kobayashi, D.Y., Myers, G.S., Mavrodi, D.V., DeBoy, R.T., Seshadri, R., Ren, Q., Madupu, R., Dodson, R.J., Durkin, A.S., Brinkac, L.M.,

Daugherty, S.C., Sullivan, S.A., Rosovitz, M.J., Gwinn, M.L., Zhou, L., Schneider, D.J., Cartinhour, S.W., Nelson, W.C., Weidman, J., Watkins, K., Tran, K., Khouri, H., Pierson, E.A., Pierson, L.S., Thomashow, L.S. & Loper, J.E. (2005). Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat Biotechnol* 23, 873-878

Petti, C., Khan, M. & Doohan, F. (2010). Lipid transfer proteins and protease inhibitors as key factors in the priming of barley responses to Fusarium head blight disease by a biocontrol strain of *Pseudomonas fluorescens*. *Funct Integr Genomics*, 10, 619-627

Ploetz, R.C. (2006). Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f.sp. *cubense*. *Phytopathology* 96, 653-656

Raaijmakers, J.M., de Bruijn, I., Nybroe, O. & Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol Rev* 34, 1037-1062

Rademaker, J.L.W. & de Bruijn, F.J. (1997). Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer-assisted pattern analysis, In: *DNA markers: protocols, applications and overviews*, Caetano-Anollés, G. & Gresshoff, P.M., 151-171, John Wiley & Sons, New York

Rasche, F., Velvis, H., Zachow, C., Berg, G., van Elsas, J.D. & Sessitsch, A. (2006). Impact of transgenic potatoes expressing antibacterial agents on bacterial endophytes is comparable to effects of soil, wildtype potatoes, vegetation stage and pathogen exposure. *Can J Microbiol* 43, 555-566

Rediers, H., Rainey, P.B., Vanderleyden, J. & de Mot, R. (2005). Unraveling the secret lives of bacteria: use of *in vivo* expression technology and differential fluorescence induction promoter traps as tools for exploring niche-specific gene expression. *Microbiol Mol Biol Rev* 69, 217-261

Sachs, J., Remans, R., Smukler, S., Winowiecki, L., Andelman, S.J., Cassman, K.G., Castle, D., De Fries, R., Denning, G., Fanzo, J., Jockson, L.E., Leemans, R., Lehmann, J., Milder, J.C., Naeem, S., Nziguheba, G., Palm, C.A., Pingali, P.L., Reganold, J.P., Richter, D.D., Scherr, S.J., Sircely, J., Sullivan, C., Tomich, T.P. & Sanchez, P.A. (2010). Monitoring the world's agriculture. *Nature* 466, 558-560

Sanguin, H., Remenant, B., Dechesne, A., Thioulouse, J., Vogel, T.M., Nesme, X., Moënne-Loccoz, Y. & Grundmann, G.L. (2006). Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on *Agrobacterium* spp. and bacterial communities. *Appl Environ Microbiol* 72, 4302-4312

Schisler, D.A., Slininger, P.J., Behle, R.W. & Jackson, M.A. (2004). Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology* 94, 1267-1271

Schmid, F., Moser, G., Müller, H. & Berg, G. (2011). Functional and structural microbial diversity in organic and conventional viticulture: organic farming benefits natural biocontrol agents. *Appl Environ Micobiol* 77, In press

Schwieger, F. & Tebbe, C.C. (1998). A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. *Appl Environ Microbiol* 64, 4870-4876

Schwyn, B. & Neilands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160, 47-56

Shannon, C.E. (1997). The mathematical theory of communication. 1963. *MD Comput* 14, 306-317

Sitterly, R.W. & Keinath, A.P. (1996). Gummy stem blight, In: *Compendium of cucurbit diseases*, Zitter, T.A., Hopkins, D.L. & Thomas, C., 27-28, APS Press, St. Paul

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H. & Berg, G. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Appl Environ Microbiol* 67, 4742-4751

Smalla, K., Oros-Sichler, M., Milling, A., Heuer, H., Baumgarte, S., Becker, R., Neuber, G., Kropf, S., Ulrich, A. & Tebbe, C.C. (2007). Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: do the different methods provide similar results? *J Microbiol Methods* 69, 470-479

Tupinambá, G., da Silva, A.J., Alviano, C.S., Souto-Padron, T., Seldin, L. & Alviano, D.S. (2008). Antimicrobial activity of *Paenibacillus polymyxa* SCE2 against some mycotoxin-producing fungi. *J Appl Microbiol* 105, 1044-1053

Velicer, G.J., Raddatz, G., Keller, H., Deiss, S., Lanz, C., Dinkelacker, I. & Schuster, S.C. (2006). Comprehensive mutation identification in an evolved bacterial cooperator and its cheating ancestor. *Proc Natl Acad Sci USA* 103, 8107-8112

Weinert, N., Piceno, Y., Ding, G.C., Meincke, R., Heuer, H., Berg, G., Schloter, M., Andersen, G. & Smalla, K. (2011). PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. *FEMS Microbiol Ecol* 75, 497-506

Weller, D.M., Raaijmakers, J.M., Gardener, B.B. & Thomashow, L.S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu Rev Phytopathol* 40, 309-348

Weller, D.M. (2007). *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology* 97, 250-256

de Werra, P., Baehler, E., Huser, A., Keel, C. & Maurhofer, M. (2008). Detection of plant-modulated alterations in antifungal gene expression in *Pseudomonas fluorescens* CHA0 on roots by flow cytometry. *Appl Environ Microbiol* 74, 1339-1349

Whipps, J. (2001). Microbial interactions and biocontrol in the rhizosphere. *J Exper Bot* 52, 487-511

Will, C., Thürmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M., Gutknecht, J., Wubet, T., Buscot, F. & Daniel, R. (2010). Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Appl Environ Micobiol* 76, 6751-6759

Youssef, N., Sheik, C.S., Krumholz, L.R., Najar, F.Z., Roe, B.A. & Elshahed, M.S. (2009). Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. *Appl Environ Microbiol* 75, 5227-5236

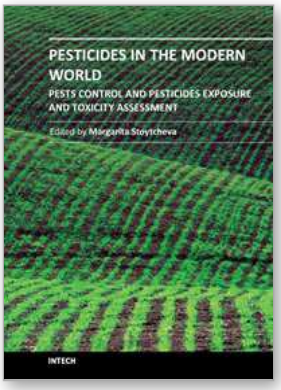
Yuan, Z.C., Liu, P., Saenkham, P., Kerr, K. & Nester E.W. (2008). Transcriptome profiling and functional analysis of *Agrobacterium tumefaciens* reveals a general conserved response to acidic conditions (pH 5.5) and a complex acid-mediated signaling involved in *Agrobacterium*-plant interactions. *J Bacteriol* 190, 494-507

Zachow, C., Tilcher, R. & Berg, G. (2008). Sugar beet-associated bacterial and fungal communities show a high indigenous antagonistic potential against plant pathogens. *Microb Ecol* 55, 119-129

Zachow, C., Pirker, H., Westendorf, C., Tilcher, R. & Berg, G. (2009). The *Caenorhabditis elegans* assay: a tool to evaluate the pathogenic potential of bacterial biocontrol agents. *Eur J Plant Pathol* 125, 367-376

Zachow, C., Fatehi, J., Cardinale, M., Tilcher, R. & Berg, G. (2010). Strain-specific colonization pattern of *Rhizoctonia* antagonists in the root system of sugar beet. *FEMS Microbiol Ecol* 74, 124-135

Zhang, Y., Hu, Y., Yang, B., Ma, F., Lu, P., Li, L., Wan, C., Rayner, S. & Chen, S. (2010). Duckweed (*Lemna minor*) as a model plant system for the study of human microbial pathogenesis. *PLoS ONE* 5, e13527




**Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment**

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The present book is a collection of selected original research articles and reviews providing adequate and up-to-date information related to pesticides control, assessment, and toxicity. The first section covers a large spectrum of issues associated with the ecological, molecular, and biotechnological approaches to the understanding of the biological control, the mechanism of the biocontrol agents action, and the related effects. Second section provides recent information on biomarkers currently used to evaluate pesticide exposure, effects, and genetic susceptibility of a number of organisms. Some antioxidant enzymes and vitamins as biochemical markers for pesticide toxicity are examined. The inhibition of the cholinesterases as a specific biomarker for organophosphate and carbamate pesticides is commented, too. The third book section addresses to a variety of pesticides toxic effects and related issues including: the molecular mechanisms involved in pesticides-induced toxicity, fish histopathological, physiological, and DNA changes provoked by pesticides exposure, anticoagulant rodenticides mode of action, the potential of the cholinesterase inhibiting organophosphorus and carbamate pesticides, the effects of pesticides on bumblebee, spiders and scorpions, the metabolic fate of the pesticide-derived aromatic amines, etc.

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