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Indoor plant microbiome: microbial diversity, function and resistome analysis

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AFFIDAVIT

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Abstract

People in industrialized regions spend up to 90% of their time in indoor environments. Indoor environments generally have less microbial diversity due to cleaning procedures and an increasing degree of sterility. The indoor microbiome is mainly influenced by the living inhabitants such as humans and animals. Moreover, previous study demonstrated that indoor plants can substantially shape microbial communities in the environments. Despite the importance of plant microbiome, only limited studies have focused on indoor plant microbiome. Using a comprehensive analysis including metagenomics, amplicon sequencing and bacterial cultures, this study aimed to fill the gap in the microbial diversity in two models of indoor plant i.e. banana plant (*Musa acuminata)* and spider plant (*Chlorophytum comosum)*, and their potential beneficial effect on human health. This thesis provided three key findings (below) that revealed ecological function and potential beneficial effect of indoor plant associated microbiome on human health.

(i) Plant species and the built environment type, where the plants were grown, were the main factors influencing microbial community structure and richness in the indoor plant phyllosphere. The bacterial population showed substantially less variation between different built environments in comparison to the fungal and archaeal communities of the phyllosphere. Furthermore, two bacterial genera *Bacillus* (in *C. comosum*) and *Sphingomonas* (in *M. acuminata*) were identified as the microbial core microbiome.

(ii) Indoor plant phyllosphere is a source of antagonistic bacteria that can counter human opportunistic pathogens. Both the culture-dependent studies and the investigation of genes with functions related to the biosynthesis, transport and catabolism of secondary metabolites confirmed the potential of antimicrobial and antagonistic activities as well as the production of biosurfactants, siderophores and important export systems associated with multidrug efflux pumps. Using plant-associated beneficial microbes to increase microbial indoor biodiversity can contribute to reduce the settling of pathogens.

(iii) Indoor plant-associated bacteria can act as natural ARGs reservoirs. The occurrence of antibiotic resistances was examined more closely since there are still few studies on the plant resistome. Three quarters of the cultured isolates showed antibiotic resistances and the metagenomic data revealed multidrug

and macrolide-lincosamide-stretogramin (MLS) as the most predominant antibiotic resistance gene class. However, the total number of ARGs is relatively low and thus likely possess a reduced risk to be transferred to human pathogens, which mostly showed non-synergistic effect with the natural phyllosphere community.

Overall, this study has revealed stable bacterial community structure and suggested their beneficial role on human health. Indoor plant-associated bacteria can be used to counter opportunistic pathogens. Moreover, they are an important source of secondary metabolites.

Kurzfassung

Menschen in industrialisierten Regionen verbringen bis zu 90% ihrer Zeit in Innenräumen, welche aufgrund von Reinigungsverfahren und einer erhöhten Sterilität im Allgemeinen eine geringere mikrobielle Vielfalt aufweisen. Das Mikrobiom in Innenräumen wird hauptsächlich von den lebenden Bewohnern wie Menschen und Tieren beeinflusst. Darüber hinaus hat eine frühere Studie gezeigt, dass Zimmerpflanzen mikrobielle Gemeinschaften in der Umwelt wesentlich formen können. Trotz der Bedeutung des Pflanzenmikrobioms haben sich bisher nur wenige Studien auf das Mikrobiom von Zimmerpflanzen konzentriert. Unter Verwendung einer umfassenden Analyse, einschließlich Metagenomik, Amplikonsequenzierung und Bakterienkulturen, zielte diese Studie darauf ab, die Lücke in der mikrobiellen Vielfalt in zwei Modellen von Zimmerpflanzen, der Bananenpflanze (*Musa acuminata)* und der Grünlilie (*Chlorophytum comosum)*, und deren potenzielle vorteilhafte Wirkung auf die menschliche Gesundheit zu schließen. Diese Arbeit lieferte drei wichtige Ergebnisse (unten), die die ökologische Funktion und den potenziellen positiven Effekt des mit Zimmerpflanzen verbundenen Mikrobioms auf die menschliche Gesundheit aufzeigten.

(i) Als Hauptfaktoren, die die Struktur und den Reichtum der mikrobiellen Gemeinschaft in der Phyllosphäre der Zimmerpflanzen beeinflussten, stellten sich die Pflanzenart und der Ort der Probennahme heraus. Die Bakterienpopulation zeigte im Vergleich zu den Pilz- und Archaea Gemeinschaften der Phyllosphäre eine wesentlich geringere Variation zwischen verschiedenen Innenräumen. Weiterhin wurden zwei Bakteriengattungen *Bacillus* (in *C. comosum*) und *Sphingomonas* (in *M. acuminata*) als mikrobielles Kernmikrobiom identifiziert.

(ii) Die Phyllosphäre von Zimmerpflanzen ist eine Quelle antagonistischer Bakterien, die opportunistischen Krankheitserregern beim Menschen entgegenwirken können. Sowohl die kulturabhängigen Studien als auch die Untersuchung von Genen mit Funktionen im Zusammenhang mit der Biosynthese, dem Transport und dem Katabolismus von Sekundärmetaboliten bestätigten das Potenzial antimikrobieller und antagonistischer Aktivitäten sowie die Produktion von Biotensiden, Siderophoren und wichtigen Exportsystemen für Multidrug-Effluxpumpen. Die Verwendung

pflanzenassoziierter nützlicher Mikroben zur Erhöhung der mikrobiellen Artenvielfalt in Innenräumen kann dazu beitragen, die Ansiedlung von Krankheitserregern zu verringern.

(iii) Pflanzen assoziierte Bakterien in Innenräumen können als natürliche Antibiotika Resistenzgen Reservoire (ARG) fungieren. Das Auftreten von Antibiotikaresistenzen wurde genauer untersucht, da noch wenige Studien zum Pflanzenresistom vorliegen. Drei Viertel der kultivierten Isolate zeigten Antibiotikaresistenzen und die metagenomischen Daten brachten Multidrug und Makrolid-Lincosamid-Stretogramin (MLS) als die vorherrschende Antibiotikaresistenz-Genklasse hervor. Die Gesamtzahl der ARGs ist jedoch relativ gering und birgt daher wahrscheinlich ein geringeres Risiko, auf humane Krankheitserreger übertragen zu werden, die meist einen nicht synergistischen Effekt mit der natürlichen Phyllosphärengemeinschaft aufweisen.

Insgesamt hat diese Studie eine stabile Struktur der Bakteriengemeinschaft gezeigt und ihre vorteilhafte Rolle für die menschliche Gesundheit nahegelegt. Zimmerpflanzenassoziierte Bakterien können verwendet werden, um opportunistischen Krankheitserregern entgegenzuwirken. Darüber hinaus sind sie eine wichtige Quelle für Sekundärmetaboliten.

Content

I. Introduction

1.1. Indoor microbiome

Many people spend most of their lifetime in indoor environments and live there with a large amount of microbial communities containing up to hundreds of bacterial, archaeal and fungal species as well as viruses (Täubel et al. 2009). The indoor microbiome is composed mainly of microorganisms from the living inhabitants such as humans, animals and plants and includes beneficial bacteria but also potential human pathogens (Kembel et al. 2012; Oberauner et al. 2013; Flores et al. 2011; Mora et al. 2016). Microbial diversity is the key to plant and human health, but it is less known how microbial diversity can be improved (Mahnert et al. 2018a). Indoor environments generally have less microbial diversity, especially in hospitals where accurate cleaning procedures are exerted. The loss of microbial diversity can lead to increase in antimicrobial resistance and this fact reinforces the importance of a diverse and balanced microbiome in built environments (Mora et al. 2016; Mahnert et al. 2019). Opportunistic pathogens, which are also part of the indoor microbiome, are defined as infectious bacteria that can cause diseases in immunocompromised patients and are therefore a major problem in indoor environment such as hospitals (Berg et al. 2005; Price et al. 2017). Under normal conditions, they can be commensal symbionts of humans such as *Streptococcus pneumoniae* and *Staphylococcus aureus* or originate from the environment like *Pseudomonas aeruginosa* and only become critical to humans after illnesses, medications or a previous infection (Brown et al. 2012). Common opportunistic pathogens such as *Acinetobacter baumanii*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus haemolyticus*, *Stenotrophomonas malthophilia* and *Pseudomonas aeruginosa* are able to produce biofilms (Raaijmakers et al. 2010; Zabielska et al. 2016; Danhorn und Fuqua 2007) as a common strategy to thrive in the harsh environment (Mora et al. 2016). Biofilm formation is becoming a health problem because it can be a reservoir for pathogens (Abdallah et al. 2014). In fact, Hu et al. 2015 showed that up to 93% of studied surfaces in the hospital carried bacterial biofilms.

As the counterpart, plant associated microbes can increase indoor microbial diversity and stability and potentially improve human health because plants can

transmit their microbes into the environment without losing their own biodiversity (Mahnert et al. 2018a). This concept may be expanded in a way to use a beneficial microbiome derived from plants to avoid settlings of opportunistic pathogens in indoor environments (Mahnert et al. 2015).

1.2. Indoor plant microbiome

For a long time, the importance of house plants was only considered on a psychological level (Mahnert et al. 2015). Despite their positive effect on mood and the improved indoor air quality, only a few studies have described the importance of house plant-associated bacteria (Mahnert et al. 2015). In parallel, plants have been recognized as meta-organisms due to a close symbiotic relationship with their unique microbiome which forms a “second genome” that fulfils important functions such as growth stimulation, promotion of stress resistances and protection from pathogens (Berg et al. 2014; Vejan et al. 2016). Plants contain numerous microorganisms that act as antagonists or promote growth, but also potential human pathogens (Berg et al. 2005; Mendes et al. 2013).

The microbiome of the plant differs between the different parts. The rhizosphere is a nutrient-rich area, while the phyllosphere contains less nutrients, but is more dynamic and highly influenced by abiotic factors (Mahnert et al. 2018a). The phyllosphere comprises diverse microorganisms, which are dominated by bacteria, but also includes yeasts, fungi and algae (Vorholt 2012). Moreover, the phyllosphere microbial communities are highly influenced by the physiological properties, plant type and environmental factors, and the need for adaptation to abiotic and biotic factors suggests a functional diversity (Lindow und Brandl 2003). Due to their importance, the phyllosphere microbial community study was expanded. Given the fact that limited information about house plant associated microbes is available, this thesis attempted to fill the gap in understanding factors that are influencing the community structure.

1.2.1. Musa acuminata

Musa acuminata Colla represents the wild type of banana that comes from tropical and subtropical regions (Figure 1). The banana plant is a monocot and belongs to the family *Musaceae*. This plant was selected for the study because it is frequently used as a houseplant and has various health-promoting properties such as antioxidative, antidiabetic, immunomodulatory, anti-cancer and antimicrobial activities (Mathew und Negi 2017).

Figure 1: Musa acuminata from private houses.

1.2.2. Chlorophytum comosum

Chlorophytum comosum (Thunb.) Jacques, also known as spider lily, is one of the most common indoor plants and a monocotyledonous plant from the family *Asparagaceae* (Figure 2). The plant effectively reduces air pollution such as formaldehyde, toluene and ethylbenzene and thus significantly improves indoor air quality (Sriprapat et al. 2014).

Figure 2: Chlorophytum comosum from private houses.

1.3. Importance of indoor plant microbiome on indoor microbiome and human health

1.3.1. Potential role as counterparts against opportunistic pathogens

Growing body of research have demonstrated that the microbiome of plants is an enormous biological source of bioactive substances that could be important for medicine and agriculture i.e. biological control (Köberl et al. 2013). Numerous studies represented that plant-associated microorganisms can synthesize secondary metabolites for instance biosurfactants, antibiotics and volatile organic compounds and use them as defence mechanisms or signalling molecules for environmental interactions and stresses (Berg 2009; Miller et al. 2012; Singh et al. 2017).

Biosurfactants, which can also be used as biocontrol agents, are surface-active molecules made up of hydrophilic and hydrophobic units that can be produced and released by several microorganisms (Goswami und Deka 2019). For example, *Bacillus* and *Pseudomonas* are known to produce the lipopeptide surfactin and rhamnolipid, respectively (Raaijmakers et al. 2010). Biosurfactants can reduce the surface tension between two phases in a heterogeneous system and show antimicrobial, anti-biofilm and anti-adhesive properties. The main idea of this work represents the assumption that the indoor plant-associated microbes can compete with opportunistic pathogens for space and nutrients and thus prevent their spread. Moreover, the hypothesis of this study was, that bacteria associated with house plants can have a health-promoting effect on humans, since their secondary metabolites contain antimicrobial substances and biosurfactants that can counteract human pathogens.

1.3.2. Natural plant resistome

Antibiotic resistance is omnipresent (Martínez 2008, 2012). Resistance to antibiotics is ancient, since it was also found in 30,000-year-old DNA samples from permafrost sediments (Brown et al. 2012). Current knowledge suggests that environmental microbiota are the source of antibiotic resistances (Martinez 2008, 2012). Microorganisms show an enormous and rapid adaptability to new environmental influences, which is also underlined by today's global problem of antibiotic resistances (Bennett 2008). Due to the augmented use of antibiotics in medicine and agriculture, resistances to antibiotic substances have spread very quickly, which suggests that the selection pressure in humans is stronger than in nature (D'Costa et al. 2006; Bhullar et al. 2012). In natural systems, antibiotic resistance elements, for instance multidrug efflux pumps, are also involved in important metabolic functions such as the detoxification of intracellular metabolites, intercellular trafficking and biosynthesis of cell walls (Martínez 2008; Martinez et al. 2009). These coding genes are highly conserved and therefore represent an evolutionary old and general mechanism that is used as a natural defence mechanism in the environment. (Martinez et al. 2009). Despite the positive impact on humans due to the increased microbial diversity and the numerous beneficial bacteria, the risk factor such as the occurrence of antibiotic resistance embedded in the house plant

associated microbiome should not be overlooked. Therefore, in this study the phyllosphere of indoor plants and their resistome, which has so far been little investigated, was also examined in more detail.

1.4. Microbiome analysis

1.4.1. Cultivation dependent analysis

The cultivation of microorganisms is a useful method to perform direct inoculation experiments and thus leads to a better understanding of interactions between species or growth-promoting conditions (Armanhi et al. 2017). Culture collections are created by cultivating microbes directly from the environment to investigate various functions of the individual organisms on agar plates. Different nutrient media are used to obtain the highest possible diversity of microorganisms. Examples of these are Nutrient broth (NB), low-nutrient Reasoner's 2A agar (R2A) or selective and differential culture media such as Mac-Conkey or KingsB (Armanhi et al. 2017; Cernava et al. 2019; Moss et al. 2011). Nutrient agar (NA) is commonly used for cultivating a large number of microorganisms whereas R2A is advantageous for the enrichment of oligotrophic, slower growing microbes (Moss et al., 2011). However, cultivation dependent analysis cannot capture the whole diversity due to only a few microbes can be cultivated (Morgan und Huttenhower 2012; Hugenholtz und Tyson 2008).

1.4.2. Cultivation independent analysis

Numerous culture-independent techniques are used today in which the entire DNA of an environmental sample is isolated. Culture-independent techniques using next generation high throughput sequencing provide information about the taxonomic diversity as well as the putative biological functions of the community members (Morgan und Huttenhower 2012). Amplicon sequencing is commonly used to investigate microbial community structure in defined environments (Schlaeppi und Bulgarelli 2015). This technique is based on amplification of specific marker genes such as the 16S rRNA gene in bacteria and the ITS region in fungi (Callahan et al. 2016; Turner et al. 2013). The variable regions of these genes are sequenced and

a taxonomic identification can be obtained through comparisons with various databases (Turner et al. 2013). However, no functional characteristics of the community can be extracted from this phylogenetic information (Schlaeppi und Bulgarelli 2015). On the other hand, shotgun metagenomics use the entire genomic DNA from various organisms from a defined environment and sequence them massively in parallel (Schlaeppi und Bulgarelli 2015). Public databases such as eggNOG and Uniprot are commonly used to annotate and predict gene functions in the metagenome (Huerta-Cepas et al. 2019; UniProt: the universal protein knowledgebase 2017). In this way, new information can be obtained from microbes that cannot be cultivated, such as novel insights into metabolic pathways.

Despite the large amount of information obtained from the culture-independent methods, the relationships and interactions between individual strains are difficult to specify (Sommer 2015). For a comprehensive research approach, the combination of cultivation technologies and culture-independent methods is necessary to improve the understanding of microbial interactions (Overmann et al. 2017).

1.5. Objectives of this research

Studies on the beneficial effects of indoor plants on human health are still limited. Recent work suggests that the plant phyllosphere can act as a reservoir of antibiotic resistance and likely possess risk for human through direct contact (Chen et al. 2019). Therefore, in addition to the potential beneficial effect on human's health, the risk factor such as the occurrence of antibiotic resistance embedded in the indoor plant associated microbiome should not be overlooked.

To fill the gap, this study was aiming to investigate the population structure and diversity of indoor plant associated microbiome as well as factors that shape them. This study focused on the phyllosphere microbiome, as this compartment is likely to affect surrounding abiotic surfaces and gets exposed with humans. We obtained two representative indoor plants species, *Musa acuminata* and *Chlorophytum comosum* from three different built environments i.e. public houses, and the botanical garden to represent less and more controlled environments respectively in terms of different size, visitor number as well as local climate and commercial stores which is the environment where people normally obtain the indoor plants.

This thesis specifically addresses the following questions: (i) What factor influences the indoor plant associated microbiome? (ii) Is there a specific core microbiome of indoor plant associated microbes? (iii) If so, what is their putative function and their importance toward plant and human health? Overall, this study provides key insights of ecological functions and potential beneficial effects of indoor plant associated microbiome on human health.

II. Materials and methods

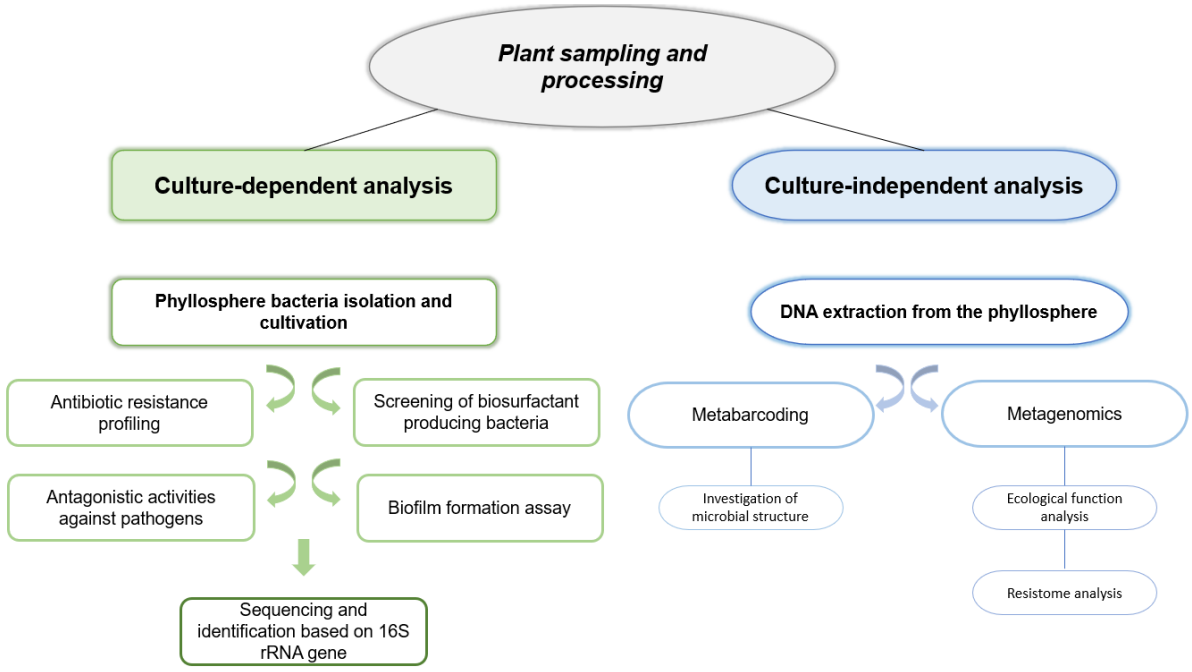

Figure 3: Workflow of the project for microbiome investigation of indoor plants.

2.1. Sampling and processing of plant material

Chlorophytum comosum and *Musa acuminata* were collected between July and August 2019 from multiple locations within Styria, Austria including Graz Botanical Garden (n=1), private homes (n=3) and commercial shops (n=2) (Table S 1). Several healthy leaves from an individual plant of *C. comosum* were chosen randomly whereas only one leaf from *M. acuminata* was sampled by using sterile gloves and instruments resulting in a total of 37 samples. The leaf samples were kept in 25 x 32 cm bags (ARO freezer bags, Düsseldorf, Germany) and kept cool at 4°C until laboratory processing.

The leaf samples were processed within 24 h after collection as described previously (Ortega et al. 2016). Approximately 10 g of leaf samples were transferred to a freezer bag containing 50 ml 0.85% NaCl solution with 0.01% Tween 80. The bag with leaf samples were subjected twice to washing treatment in a stomacher

(BagMixer; St. Nom, France) for 3 min and followed by sonication, using a Transsonic Digital T910 DH sonicator (Elma, Singen, Germany), at 60 Hz for 5 min. The leaf wash-offs were then transferred to a 50 ml Sarstedt tube and used for total community DNA extraction and cultivation of phyllosphere bacteria as described below.

2.2. Preparation of amplicon and shotgun metagenomic sequencing

2.2.1. DNA extraction

The leaf wash-offs were centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge; DuPont Instruments, USA) at 6000 g for 20 min to pellet cells. The pellets were then transferred to 2 ml sterile Eppendorf tubes and were further centrifuged at 16 000 g for 20 min. DNA extractions were performed using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instructions. The DNA quality and quantity were measured by using the NanoDrop™ 2000/2000c Spectrophotometers and Qubit dsDNA BR (ThermoFischer Scientific), respectively.

2.2.2. Amplicon sequencing of total microbial communities

Extracted DNA were subjected for specific PCR to target the bacterial, fungal and archaeal community. The set of bacteria-specific primers 515f/926r (Caporaso et al. 2011; Parada et al. 2016) was used to amplify the V4-V5 region of bacterial 16S rRNA gene. A set of fungi-specific primers ITS1f/ITS2 (White et al. 1990) was used to amplify the ITS1 region of fungal intergenic transcript spacer (ITS) gene and the specific primer pair 349f/519r was used to amplify 16S rRNA gene fragments of the archaeal community. Amplicon library construction including attachment barcode sequences for multiplexing was performed as described previously in protocols of the Earth Microbiome Project (Walters et al. 2016). PCR of each sample were performed in independent duplicates. PCR products were visualized on 1% agarose gel to verify amplification and purified using Wizard® SV Gel and PCR Clean-Up kit (Promega). DNA concentrations were measured by using the NanoDrop™

2000/2000c Spectrophotometers and then all of samples were pooled in an equimolar ratio. The pool library was sequenced using the Illumina MiSeq platform (2 × 300 bp paired-end) at the commercial provider Genewiz (Leipzig, Germany).

2.2.3. Shotgun metagenomics sequencing

For this approach, samples from the botanical garden were deliberately chosen because the plants were grown under the most controlled conditions. Extracted DNA from these samples was used for the shotgun metagenomics sequencing. Six shotgun libraries (1 µg DNA per library) consisted of two different plant species with three biological replicates for each plant species were used. DNA library preparation and shotgun metagenomics sequencing using the Illumina NovaSeq (2 × 150 bp paired-end) were performed by the commercial provider Genewiz (Leipzig, Germany).

2.2.4. Bioinformatic analyses

For amplicon sequencing, initial quality filtering and removal of primer sequences were performed using Trimmomatic and cutadapt, respectively (Bolger et al. 2014; Martin 2011). Due to high occurrence of low-quality reads on the reverse read, only the forward read was used for bacterial community analysis. Quality filtering, trimming, denoising, merging and chimera removal of paired end sequences were performed using DADA2 algorithm (Callahan et al. 2016) through the integrated plugin in the open-source QIIME2 version 2019.10 (Bolyen et al. 2019). The resulting amplicon sequence variants (ASVs) were taxonomically assigned using VSEARCH classifier (Rognes et al. 2016) against the Silva ribosomal RNA gene database v128 (Quast et al. 2013) for the bacterial and archaeal datasets and UNITE + INSD (v6_sh_97) (Abarenkov et al. 2010) for the fungal dataset. Plant-derived sequences such as chloroplasts and mitochondria were removed prior further statistical analyses.

For shotgun metagenomic sequencing, adaptor removal and quality filtering of raw reads were performed using Trimmomatic and VSEARCH (Bolger et al. 2014; Rognes et al. 2016). Filtered reads were used as input for taxonomic profiling using Kaiju (Menzel et al. 2016) and co-assembled using Megahit for each plant species

(Li et al. 2015). The assembled contigs were then annotated using the blastX algorithm in DIAMOND (Buchfink et al. 2015) against the eggNOG database (Powell et al. 2014) for ecological function profiling. The assembled contigs were further aligned to the manually curated antibiotic resistance gene database (deepARG, Arango-Argoty et al. 2018) for antibiotic resistance gene (ARG) profiling. To minimize the risk of false positives, reads were defined as ARG-like reads at the cutoff of E-value of 10-10 and similarity of 50% as previously described by Cernava et al. 2019. Annotated contigs were then filtered to remove redundancy and converted to the GTF format using MGKit package (Rubino und C.J. Creevey 2014). Bowtie2 (Langmead und Salzberg 2012) and featureCounts (Liao et al. 2014) were used to aligned individual metagenomic dataset to the annotated contigs and counted the number of reads, respectively. Only reads mapped to contigs with eggNOG and deepARG features were used for further analysis.

2.2.5. Statistical analysis

Microbial community analyses were performed in R v3.3.1 (R Core Team 2017) through the Rstudio IDE (using http://www.rstudio.com/) with the ggplot and phyloseq packages (Wickham 2016; McMurdie und Holmes 2013). For alpha and beta diversity analyses, the number of sequences was normalized by randomly selecting an equal number of sequences according to the lowest common number of sequences in the respective datasets. Kruskal-Wallis test and pairwise Wilcoxon rank sum test were used to evaluate effects of plant species and sampling site on the microbial richness according to the observed Shannon index. Bray-Curtis dissimilarity matrix distances were generated and subjected to permutational analyses of variance (PERMANOVA, 999 permutations) along with the adonis test to evaluate effect of plant species and sampling site on the microbial community structure. A non-metric multidimensional scaling (NMDS) plot was generated to ordinate the normalized Bray-Curtis dissimilarity matrix.

2.3. Functional characterization of cultivable phyllosphere bacteria

2.3.1. Isolation of bacteria from the plant phyllosphere

For isolation of bacteria, samples from botanical garden were chose to limit the number of isolates and allow us to facilitate comparisons with the putative bacterial functions derived from the metagenomic dataset. A total of 100 µl of the leaf wash-offs was serially diluted 10-fold and plated on both Reasoner's 2A (R2A) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and Nutrient Broth II agar (NA) media (SIFIN, Berlin, Germany) in triplicates. Isolated bacteria were streaked on NA and incubated on 25 °C for four days. Single colonies were picked and subcultured on NA to ensure purity of the isolates. Grown colonies were then transferred to 96-well plates with NB II medium and 30% glycerol for long-term storage and the plates were kept at −70 °C at the Institute of Environmental Biotechnology, TU Graz, Graz, Austria. In total, 389 isolates (n= 264 from *C. comosum* and n= 125 from *M. acuminata)* were collected and used for functionality assays. Prior to use in functionality assay and antibiotic resistance profiling, each isolate was subcultured in 200 µl LB in 96-well plates and incubated at 25 °C for 2 days. All phyllosphere isolates were tested in three replicates to allow the reliability of the screening assay.

2.3.2. Antagonistic activity against opportunistic human pathogens

The human opportunistic pathogens *Acinetobacter baumanii, Enterococcus faecalis, Escherichia coli, and Pseudomonas aeruginosa* were obtained from culture collection of Department of Internal Medicine, Medical University of Graz. *Staphylococcus haemolyticus* strain 48-6 was previously isolated from a hospital setting (Oberauner et al. 2013) while *Stenotrophomonas malthophilia* was previously isolated from eyecare solution and is closely related to a clinical strain, *S. maltophilia K279a* (Lira et al. 2017). Both isolates are part of the microbial culture collection of the Institute of Environmental Biotechnology (Graz University of Technology). Isolated bacteria were spotted on NA pre-inoculated with human opportunistic pathogens and inhibition zone was assessed after 4 days of incubation at 25°C (Figure 4). All isolates that produced visible inhibition zones were determined as antagonists of the model pathogens.

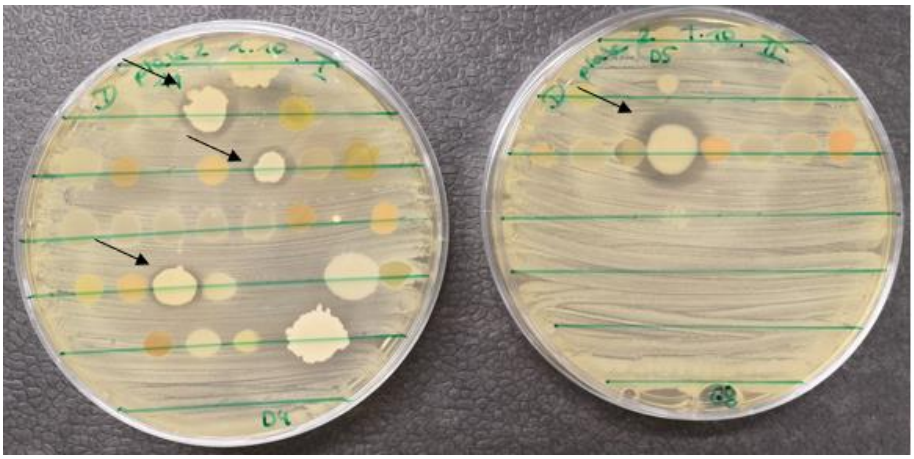

Figure 4: Antagonistic assay. Isolated bacteria were spotted on pre-inoculated plates with Staphylococcus haemolyticus. Inhibition zones were evaluated after 4 days of incubation at 25 °C.

2.3.3. Screening for biosurfactant producing bacteria

A qualitative screening of biosurfactant producing bacteria was performed using the drop-collapsing assay as previously described (Bodour und Miller-Maier 1998) Briefly, the 96 wells of the lid of a 96-well microtiter plate were covered with 2 µL mineral oil and incubated for 2 hours to equilibrate. Afterwards 5 µL of a liquid culture of the phyllosphere bacteria were added to the mineral oil and the result was evaluated after 1 min. A drop that remained beaded was determined as negative while collapsed drops were determined as positive (Figure 5).

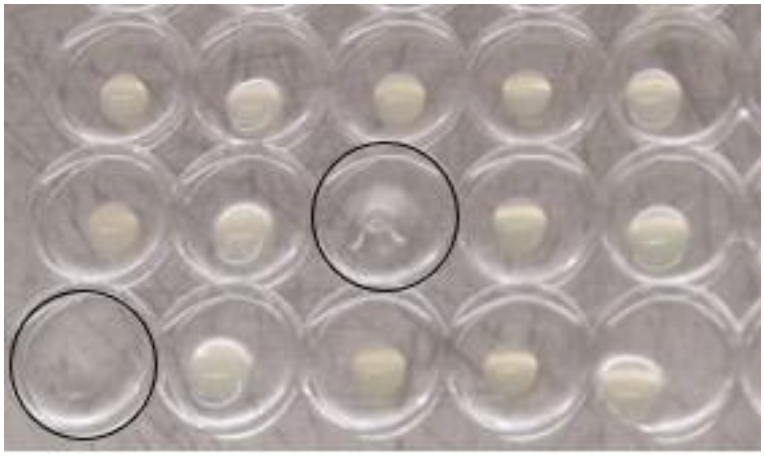

Figure 5: Screening of biosurfactant bacteria. Liquid cell culture was spotted on mineral oil. Encircled wells represent the drop collapse indicating the biosurfactant production.

2.3.4. Biofilm formation and biofilm coculture assay

The biofilm formation assay was performed in 96-well microtiter dishes made of polystyrene as previously described (O'Toole und Kolter 1998). Bacterial cultures that had been grown overnight in TSB were adjusted to an optical density (OD_{600}) of 0.05. After a 24-h incubation at 25 °C, planktonic cells were removed and biofilms were washed twice with NaCl 0.9% to remove nonadherent bacteria. Subsequently, a total of 200 µL 0.5% crystal violet (CV) solution was added to each well. After 30 min of incubation at room temperature, the CV solution was removed, and the well was rinsed twice with NaCl 0.9% to remove excess CV. Finally, the stained biofilm was solubilized in 200 µL of absolute ethanol and quantified by measuring OD_{595} of each well using Tecan microplate reader Infinite® 200 PRO (Tecan) (Figure 6). The biofilm formation was examined for each phyllosphere isolate, for single pathogens as well as for co-cultures that contained a phyllosphere bacteria isolate and a pathogen. The pathogens implemented in the assay were *E. faecalis, S. malthophilia* and *P. aeruginosa*. The interactions in the mixed biofilms (MBA) were defined as synergistic when the measured absorbance from the mixed biofilm was greater than that of the best single strain biofilm (BSA) producer of that mixture (Ren et al. 2015). In contrast the non-synergistic effect shows a less absorbance of the mixture compared to the single strains, i.e., (Abs_{595} MBA - Standard error) > (Abs_{59} BSA + Standard error) = Synergistic, while (Abs_{595} MBA + Standard error) < (Abs_{595} BSA - Standard error) = No synergistic (Ren et al. 2015).

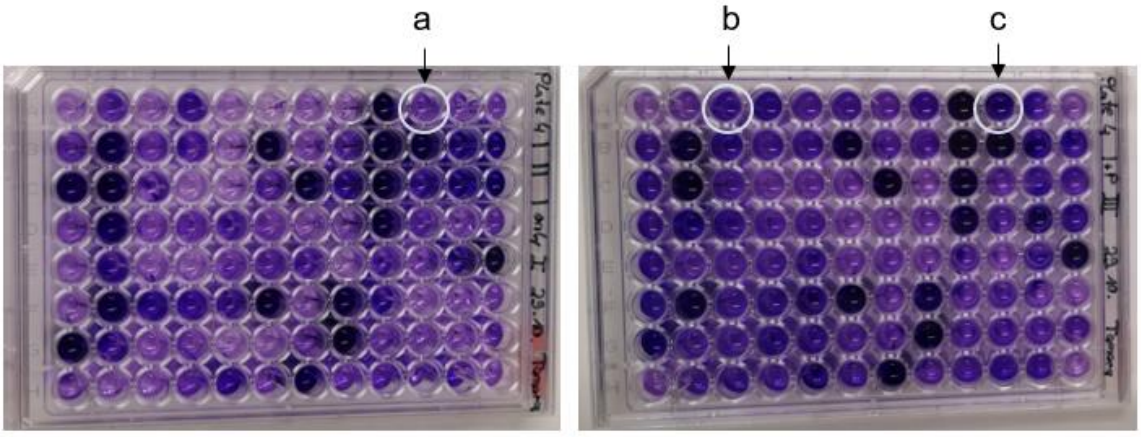

Figure 6: Biofilm staining with crystal violet and subsequent quantification by measuring OD_{595}. Left: Biofilm formation of single isolates; Right: Biofilm formation of the plant isolates coinoculated with Enterococcus faecalis. a: plant isolate only; b: Enterococcus faecalis only; c: synergistic effect of plant isolates and Enterococcus faecalis.

2.3.5. Antibiotic resistance profiling

Bacterial isolates were screened against 11 different antibiotics as listed in Table S 7. Agar plate-based assay was performed as previously described by Walsh und Duffy 2013. In brief, Mueller Hinton agar (MHA) plates containing a specific antibiotic at 20 µg/ml were inoculated with approximately 3 µL bacterial cultures using a multipoint inoculator and were incubated at 25 °C. MHA plates without antibiotic were used as controls. The inoculated plates were observed daily for three days. The experiments were performed in three replicates. Bacteria that showed visible growth after 4 days incubation on agar plate containing antibiotic were defined as resistant bacteria (Figure 7) (Walsh und Duffy 2013; D'Costa et al. 2006).

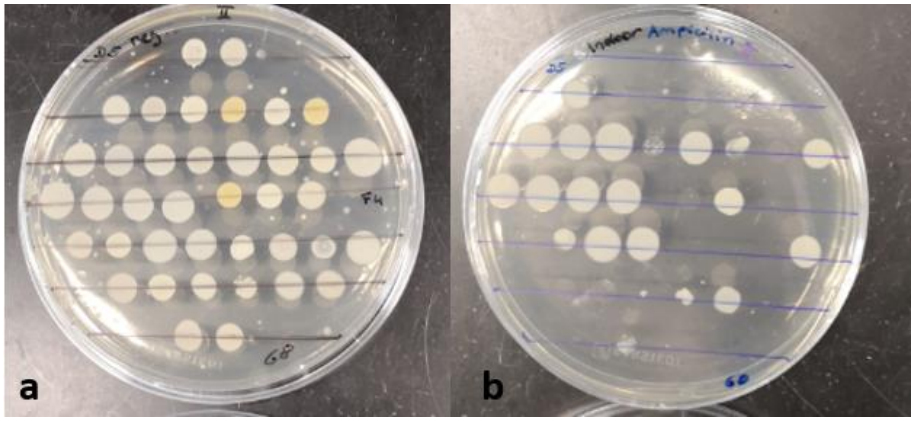

Figure 7: Antibiotic resistance test. Bacterial isolates were spotted on Mueller Hinton agar plates (MHA). a: MHA without antibiotic; b: MHA with 20 µg/ml Ampicillin.

2.3.6. Identification of culturable bacteria based on 16S rRNA gene sequencing

Representative bacteria from each assay were identified based on the sequence of the 16S rRNA gene. Genomic DNA was extracted using the MasterPure™ Complete DNA and RNA Purification Kit (Lucigen Corporation, USA) according to manufacturer's instructions with additional mechanical cell disruption step with glass beads in a FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA; 30 s, 6.5 m s^{-1}). Polymerase chain reactions (PCRs) was performed in Whatman Biometra® Tpersonal (Biometra 141 GmbH, Göttingen, Germany) according to the reaction mix in Table 1 and the program in Table 2.

Table 1: PCR reaction mix for bacteria identification based on 16S rRNA. Total volume 20 µL.

Reagent	Volume [µL]
Taq-&GO Ready-to-Use PCR Mix (MP Biomedicals, Thermo Fischer Scientific)	4
Primer 27F (10 µM)	1
Primer 1492R (10 µM)	1
Ultrapure water	13
DNA template	1

Table 2: PCR program for thermocycler for bacteria identification based on 16S rRNA.

	Temperature	Time	
Initial DNA denaturation	95 °C	5 min	
DNA denaturation	95 °C	20 s	30x
Primer annealing	54 °C	15 s	
Elongation	72 °C	30 s	
Final extension	72 °C	10 min	

The amplified 16S rRNA genes were Sanger sequenced at LGC Genomics, Berlin, Germany. After manually quality filtering to remove ambiguous sequences using BioEdit (Hall 2001), the sequences were compared against those of known origin using the Basic Local Alignment Search Tool (BLAST, (Boratyn et al. 2013)) and the GenBank database (http://www.ncbi.nlm.nih.gov) (Database resources of the National Center for Biotechnology Information 2013).

III. Results

3.1. Microbial community profiling in the plant phyllosphere

After quality filtering and removal of non-target sequences, a total of 174161 high quality reads were retained in the bacterial 16S rRNA gene fragment amplicon library with 427 to 33685 reads per sample and assigned to bacterial 1066 ASV (Table S 6). One sample was removed due to low number of bacterial reads. In the fungal ITS amplicon library, a total of 291841 high quality reads were obtained with 1865 to 23898 reads per sample and assigned to 1261 fungal ASVs. In the archaeal 16S rRNA gene fragment amplicon library, a total of 641196 high quality reads from fungal libraries were retained with 1841 to 51500 reads per sample and assigned to 358 archaeal ASVs (Table S 6).

3.1.1. Identification of microbial taxa from the phyllosphere of indoor plants

Alphaproteobacteria, *Gammaproteobacteria* and *Bacteroidia* were the dominant bacterial classes in *M. acuminata* and together accounted for 78% of total reads. In contrast *Bacilli*, *Gammaproteobacteria* and *Actinobacteria* were the dominant classes in *C. comosum* and accounted for 70.7% of total reads (Figure 8). When each of the two plant species community was assessed in more detail, specific differences in proportions of bacterial groups were evident. *Alphaproteobacteria* occurrence was relatively higher in *M. acuminata* from commercial store (54.6%) compared to the other sampling sites (36.1 – 38.4%) whereas *Gammaproteobacteria* showed the opposite trend. A relatively higher *Actinobacteria* abundance was observed in *C. comosum* from botanical garden (39.6%) compared to the other sampling sites (12.1-13.2%) whereas *Gammaproteobacteria* and *Bacilli* showed the opposite pattern.

Dothideomycetes was the dominant fungal class and accounted for 64.2% of total reads (Figure 8). *Agaricomycetes* abundance was relatively higher in *M. acuminata* and *C. comosum* from commercial stores (35.7 and 17.4%, respectively) compared to the other sites (1.8-15.4%). *Nitrososphaeria* and *Methanomicrobia* dominated the archaeal community and represented 94.6% of total reads (Figure 8). There was a

relatively higher proportion of *Methanomicrobia* class in banana from commercial stores and *C. comosum* from private houses in comparison to the other samples.

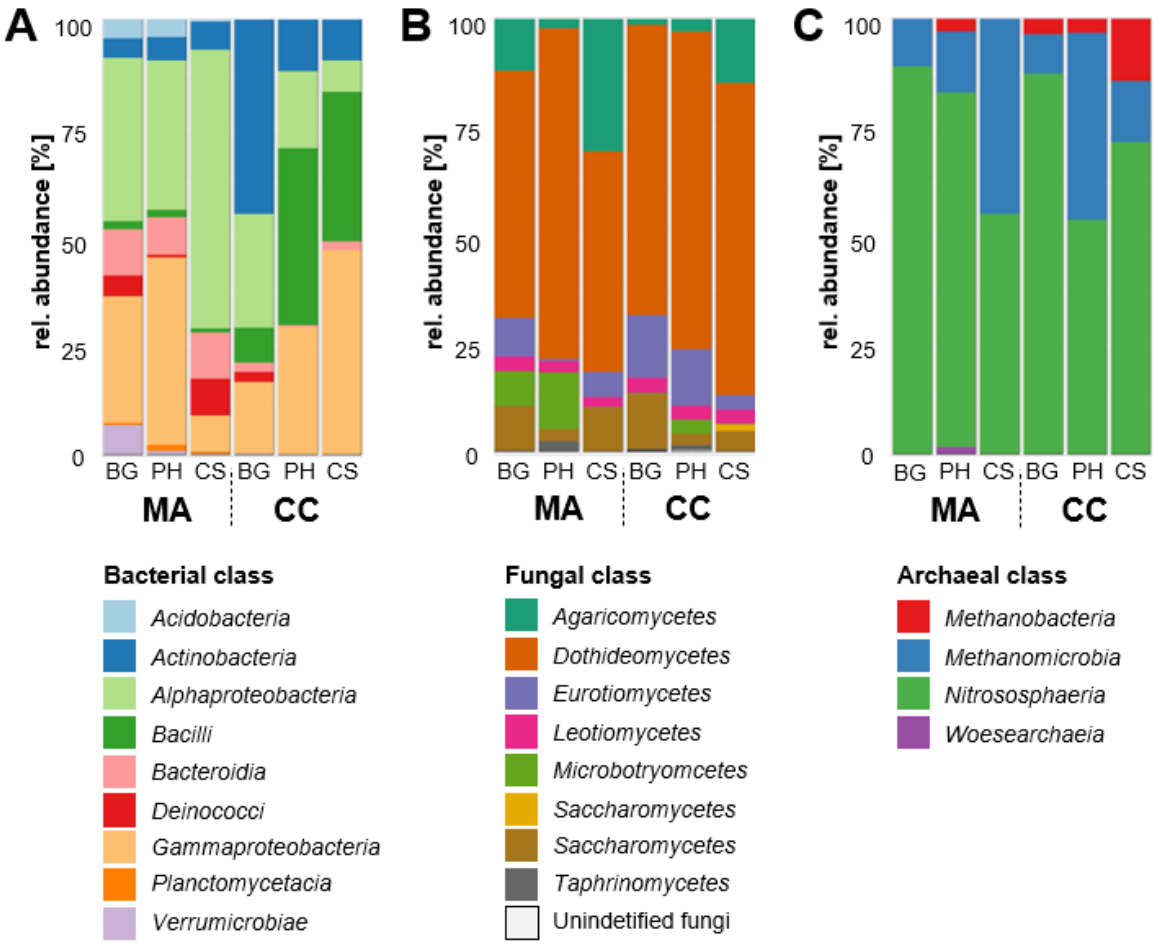

Figure 8: Relative abundance plot of the top 100 most abundant bacterial (A), fungal (B) and archaeal (C) ASV at class level, different plant species and sampling sites. BG: botanical garden; PH: private houses; CS: commercial stores. MA: M.acuminata; CC: C. comosum.

Bacillus and *Sphingomonas* were identified as the core member within the bacterial community in *C. comosum* with prevalent at least 75% of the total samples. Interestingly, *Sphingomonas* was also identified as the core phyllosphere member of *M. acuminata* together with *Rhizobium* with prevalent at least 80% of the total samples (Figure 9). This led to a deeper analysis of the two genera *Bacillus* and *Sphingomonas* regarding the secondary metabolites, as they likely play important roles.

The core fungal community was composed of unidentified *Capnoidales*, *Alternaria* and *Mycosphaerella* (Figure 9). Despite two taxa cannot be identified to genus level i.e. unidentified *Nitrososphaeraceae* and archaeon, genus *Methonosarcina* was defined as the core member of archaeal community and found in at least 75% of the total samples.

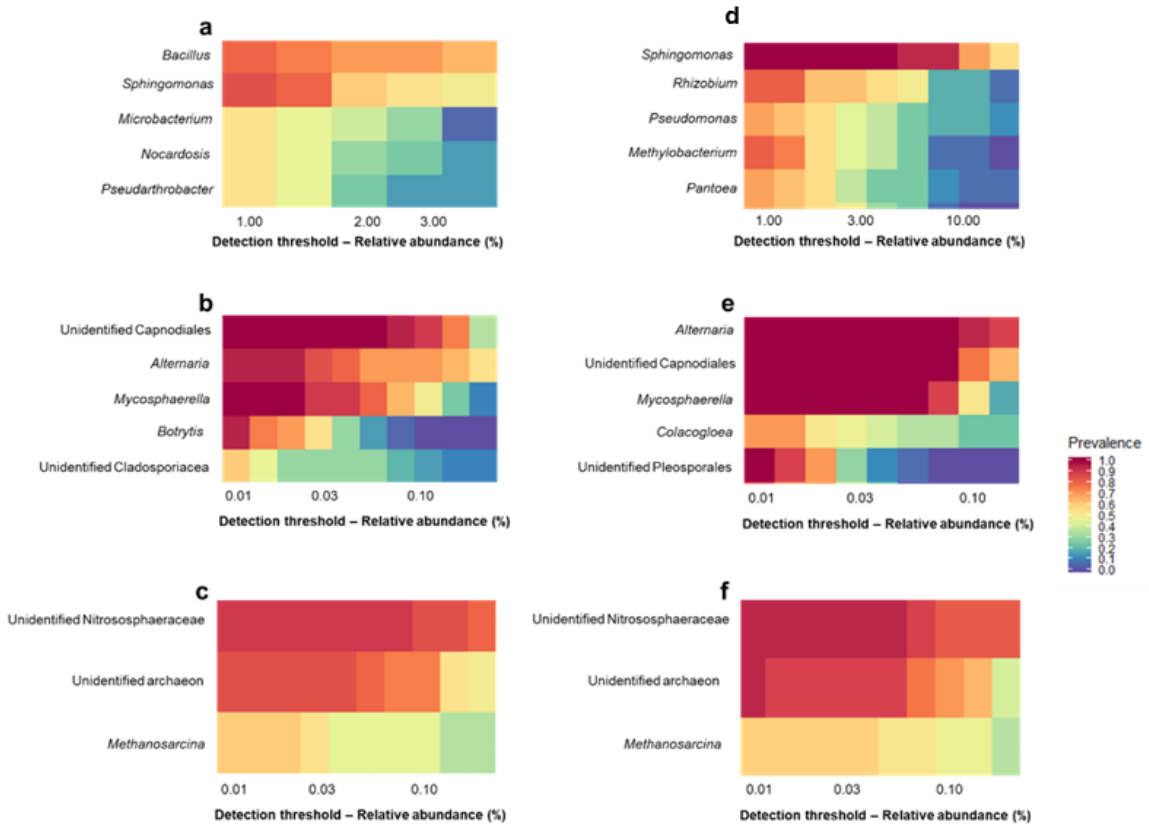

Figure 9: Bacterial, fungal and archaeal core microbiome at genus level of C. comosum (a -c, respectively) and M. acuminata (d-f).

3.1.2. Microbial diversity in different plant species and environments

With respect to plant species, a higher number of Shannon index (H) was observed for *M. acuminata* (H=3.4) compared to *C. comosum* (H=3.1). With respect to different environments, samples from private houses and the botanical garden had a relatively higher alpha bacterial diversity (H=3.4) compared to samples from commercial stores (H=2.6) as depicted in Figure 10.

However, Kruskal-Wallis test showed that plant species and environment did not influence bacterial richness (P=0.356 and P=0.123, respectively) (Table 3). Similar to bacterial fraction, although a higher number of fungal richness was observed from *M. acuminata* (H=2.9) compared to *C. comosum* (H=2.5), the differences were not statistically significant at the 95% confidence (Table 3). In contrast, the environment where the plant were grown was shown to influence fungal richness (P<0.001) (Table 3). Furthermore, samples from commercial stores significantly had the highest fungal richness (H=4.9) compared to samples from botanical garden (H=2.9) and private houses (H=2.3). A significantly higher value of Shannon index (H) was observed with the archaeal community for from *C. comosum* (H=2.7) compared to *M. acuminata* (H=2.4) (P=0.041) (Table 3, Figure 10). With respect to different environment, this factor did not influence archaeal richness (P=0.353) (Table 5).

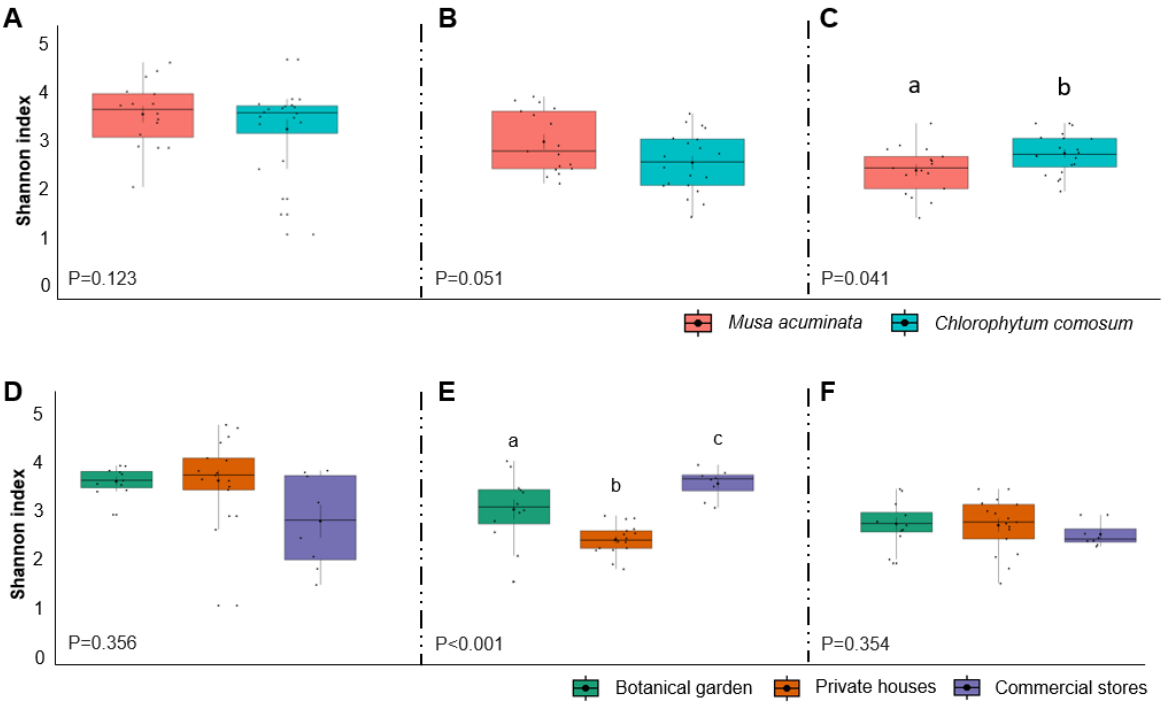

Figure 10: Phyllosphere associated bacterial (A,D), fungal (B,E), and archaeal (C,F) richness according to Shannon index for each plant species and environment. Different letter above the box indicate significant different according to Tukey's HSD at P<0.05.

Table 3: Effect of plant location and environment on the phyllosphere microbial richness according to Shannon index (alpha diversity).

Factor	Shannon index
Bacteria	
Plant species	0.356
Environment	0.123
Fungi	
Plant species	0.051
Environment	<0.001*
Archaea	
Plant species	0.041*
Environment	0.353

*Asterisks denote statistical significance of microbial richness based on Kruskal-Wallis test (p ≤0.05).

Plant species and environment where the plants were grown affected the structure of phyllosphere microbial communities. Nonmetric multidimensional scaling (NMDS) ordination showed that bacterial communities formed discrete clusters according to plant species as well as environment (Figure 11). All factors (plant species and environment) and their interactions significantly influenced the bacterial community structures (P<0.05). Plant species was the dominant factor that influenced the bacterial variation (13.7%) (Table 4) whereas environment and its interaction with plant species explained 12.5% and 9.3%, respectively. Pairwise comparison showed that each environment harboured a unique bacterial community structure ($P_{adjusted} < 0.05$).

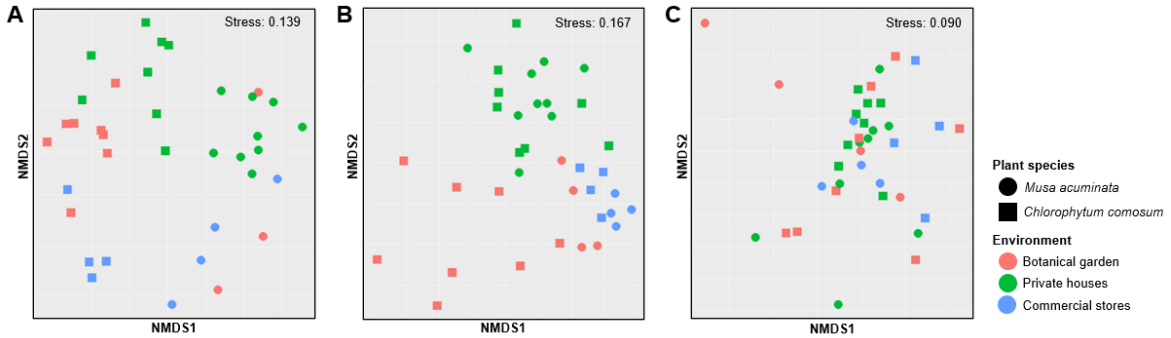

Figure 11: Nonmetric multidimensional scaling (MDS) plot showing bacterial (A), fungal (B) and archaeal (C) communities from different plant species and environment.

Similar to bacteria community, fungal community formed discrete clusters according to plant species as well as environment which can be seen in Figure 11. All factors (plant species and sampling site) and their interactions significantly influenced the

fungal community structures (P<0.05). However, environment was the major factor that affected the fungal community structure, which explained 25.6% of fungal variation, compared to plant species and its interaction, which explained 8.6% and 12.5% of fungal variation respectively. Pairwise comparison showed that each sampling site has unique fungal community structure ($P_{adjusted}$<0.05) (Table 4). In contrast to the bacterial and fungal components, there was no clear clustering from archaeal community based on the factors tested in this study (plant species and environment factor) (Figure 11). Although interaction between plant species and environment significantly influenced archaeal community structure (P=0.021), this factor only accounted for 8.5% of archaeal variation. A separate adonis analysis, performed by separating the environment into individual sampling sites and their interaction with plant species, showed an impact on the archaeal community structure (P=0.019 and P=0.005, respectively) and explained a total of 36.4% of the variation (Table 5) indicating a high variability between sampling sites.

Table 4: Effect of plant location and environment on the phyllosphere microbial community similarity (beta diversity).

Factor	Microbial community similarities	
	R^2 value	P value
Bacteria		
Plant species (P)	0.137	0.001*
Environment (E)	0.125	0.001*
P * E	0.093	0.001*
Fungi		
Plant species (P)	0.086	0.001*
Environment (E)	0.256	0.001*
P * E	0.125	0.001*
Archaea		
Plant species (P)	0.023	0.589
Environment (E)	0.062	0.224
P * E	0.085	0.021*

*Asterisks denote statistical significance of microbial richness based on adonis test (p ≤0.05).

Table 5: Separate Adonis analysis of archaeal community structure on the phyllosphere. Environment was separated into individual sampling sites.

Factor	Microbial community similarities	
	R^2 value	P value
Plant species (P)	0.023	0.480
Individual sampling site (S)	0.174	0.019*
P * S	0.190	0.005*

3.2. Metagenomics-based assessment of the ecological function and antimicrobial resistance profiling of phyllosphere bacterial community

Based on the results of the 16s rRNA fragment amplicons, this thesis focussed only on the function of bacterial components of the microbiome in more detail by utilizing metagenomic dataset as well as cultivation dependent methods as described later. This is due to bacterial communities of the microbiome are shown to be more similar regardless the sampling site compared to the fungal and archaeal community. Moreover, comprehensive analysis that encompass both archaeal, fungal and bacterial component would entail more work than could be reasonably accomplished in a Master thesis. For a better understanding of the microbial functions in the microbiome, the focus in the metagenomics was placed on genes that are related to secondary metabolites and on *Bacillus* and *Sphingomonas* taxa, as these taxa were defined as the core microbiome and likely play important roles for host physiology.

3.2.1. Ecological function of the phyllosphere bacterial microbiome of *Chlorophytum comosum and Musa acuminata*

As plant microbiomes are known to harbour diverse and highly specialized secondary metabolites, particular attention was placed on genes with COG functions related to secondary metabolite biosynthesis, transport and catabolism

(COG category: Q). A higher relative abundance of this function was observed in the phyllosphere metagenome of *C. comosum* (2.4%) compared to *M. acuminata* (1.5%). Among genes that were classified to COG Q categories, we detected a high number of reads assigned to peptide synthetase (7.2%) and cytochrome P450 (6.8%) in the *C. comosum* metagenome (Table S 9). The latter was also found in the *M. acuminata* metagenome in a relatively lower proportion (Table S 11). Biosynthetic genes belonging to non-ribosomal peptide synthetases were also observed from both metagenomes (2.5 and 2.2% in *C. comosum* and *M. acuminata*, respectively). A numerous read that classified to Tol biopolymer transport system (4.1%) was detected in *M. acuminata* but its prevalence was relatively low in *C. comosum* metagenome (0.05%). In addition, multicopper oxidase was also detected in both of metagenomes (3.5 and 1.5% in *C. comosum* and *M. acuminata*, respectively).

Two bacterial lineages, *Bacillus* and *Sphingomonas* were subjected to deepening analyses in order to screen for secondary metabolites-related genes as these taxa were members of the bacterial community of *C. comosum* and *M. acuminata* respectively (Figure 9). *Bacillus* accounted for 4.8% of total bacterial reads in *C. comosum* metagenomic dataset whereas *Sphingomonas* accounted for 22.7% of total bacterial reads in *M. acuminata* metagenomic dataset. Interestingly *Sphingomonas* was also found in a high abundance from the *C. comosum* metagenomic dataset while *Bacillus* only represented a very small fraction (0.03%) in the *M. acuminata* metagenomic dataset. *Bacillus* harboured genes associated to polyketide synthase (Table S 10). Several genes which are involved in the process of siderophore production including *iucC*, *iucd*, *asb* were detected in *Bacillus* contigs (Najimi et al. 2008; Lam et al. 2018a). The ABC transport system which facilitates the export for substances like antibiotics and siderophores could also be found in the *Bacillus* genera as well as in *Sphingomonas* (Mir et al. 2006). Moreover, the metagenomic data of *Sphingomonas* indicated a presence of Tol biopolymer transport system (Table S 12).

3.2.2. Antibiotic resistance in the phyllosphere metagenome of *Chlorophytum* comosum *and Musa acuminata*

Within the phyllosphere metagenomes of *C. comosum* and *M. acuminata*, a relatively low number of reads (0.006-0.012%) from the phyllosphere metagenome of *C. comosum* and *M. acuminate* was assigned to 170 and 149 ARGs respectively. Those ARGs were classified into 23 and 22 antibiotic classes. Taxonomic classification showed that a high proportion of ARGs originated from *Proteobacteria*. *Gammaproteobacteria* accounted for up to 30% of ARG carriers in the analysed plant species (Figure 12). Other prevalent carriers were identified as *Actinobacteria*, *Alphaproteobacteria*, and *Betaprotebacteria*. Together they accounted for 58% in *C. comosum* and for 62% in *M. acuminata* (Figure 12).

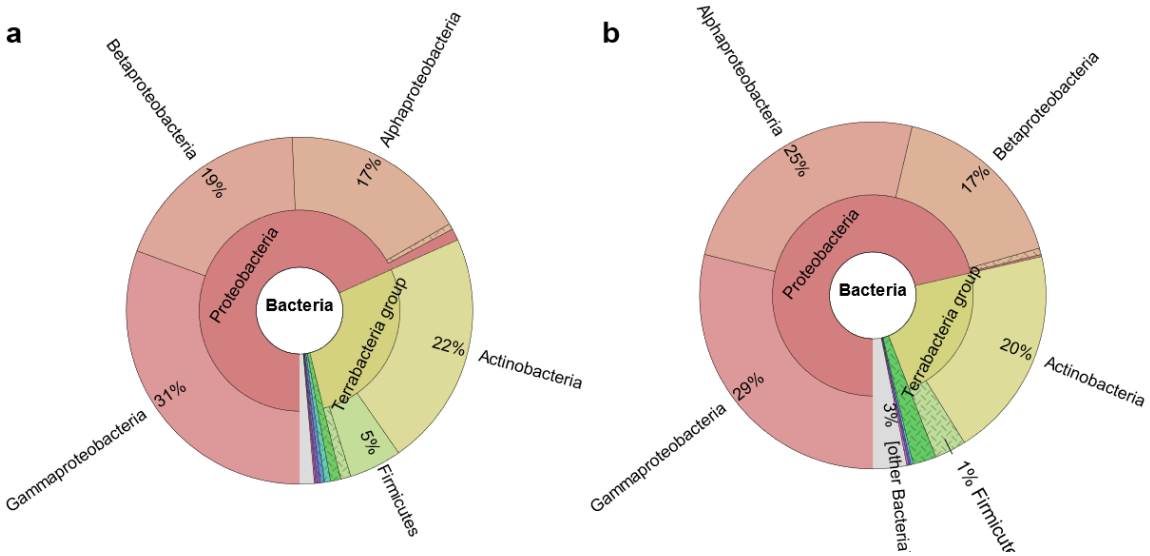

Figure 12: Taxonomic classification of the assigned ARGs showed that a high proportion originated from Proteobacteria. a: C. comosum; b: M.acuminata.

Multidrug and macrolide-lincosamide-stretogramin (MLS) were the most predominant antibiotic resistance gene class which accounted 59.1% and 54.9% of total detected ARGs in the phyllosphere metagenome of *C. comosum* and *M. acuminata* respectively (Table S 13). A relatively higher number of genes in the phyllosphere metagenome of *C. comosum* and *M. acuminata* (73 and 61 ARGs, respectively), were classified into multidrug classes compared to other classes. In the multidrug class the predominant *acrB* gene is part of the AcrAB-TolC multidrug efflux protein complex. The most abundant gene of the MLS class was *macB* with occurrences of 70.9% in *C. comosum* and 60% in *M. acuminata* in this class. The

β- lactam resistance was also predominant with a relative abundance of 6.9 % in *C. comosum* and 7.6 % in *M. acuminata* metagenome (Figure 13).

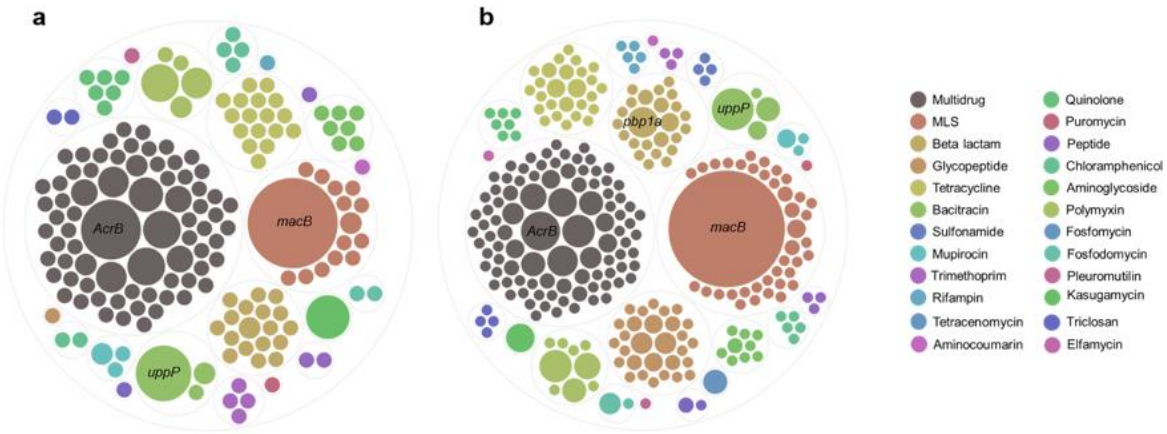

Figure 13: ARGs grouped by antibiotic resistance gene classes. Each bubble represents the different genes and their abundance is reflected by bubble size. a: C. comosum; b: M.acuminata.

3.3. Assessment of functional activity and antimicrobial resistance profiling of cultivable phyllosphere bacteria

Culture dependent assays were conducted with samples from the botanical garden to limit the number of isolates and to allow a better comparison of putative function with metagenomic data. In total, 389 isolates (n= 264 from *C. comosum* and n= 125 from *M. acuminata)* were collected and used for functionality assays.

3.3.1. Antagonistic activity against opportunistic human pathogens

In general, more tested isolates from *C. comosum* showed inhibitions against the human opportunistic pathogens (Figure 14). From a total of 264 isolates, 50 (18.9%) inhibited at least one model pathogen, whereas the half of them (25) showed inhibition against five different opportunistic pathogens. The numbers of isolates that inhibited *P. aeruginosa* (n=40, 15.2%), *E. faecalis* (n=39, 14.7%), *S. maltophilia* (n=34, 12.9%) *S. haemolyticus* (n=33, 12.5%) and *E. coli* (n=26, 9.8%) had almost the same numbers. There were no tested isolates with inhibition against *A. baumanii.* A total of 125 bacteria were isolated from *M. acuminata* and 13 of them (10.4 %) had an inhibitory effect against at least one pathogen. Most inhibition was observed against *S. haemolyticus* (n=10, 8%) followed by *P. aeruginosa* (n=6, 4.8%), *E. faecalis* (n=4, 3.2%), *E. coli* and *S. maltophilia* (n=3, 2.4%) and *A. baumanii* (n=2, 1.5%).

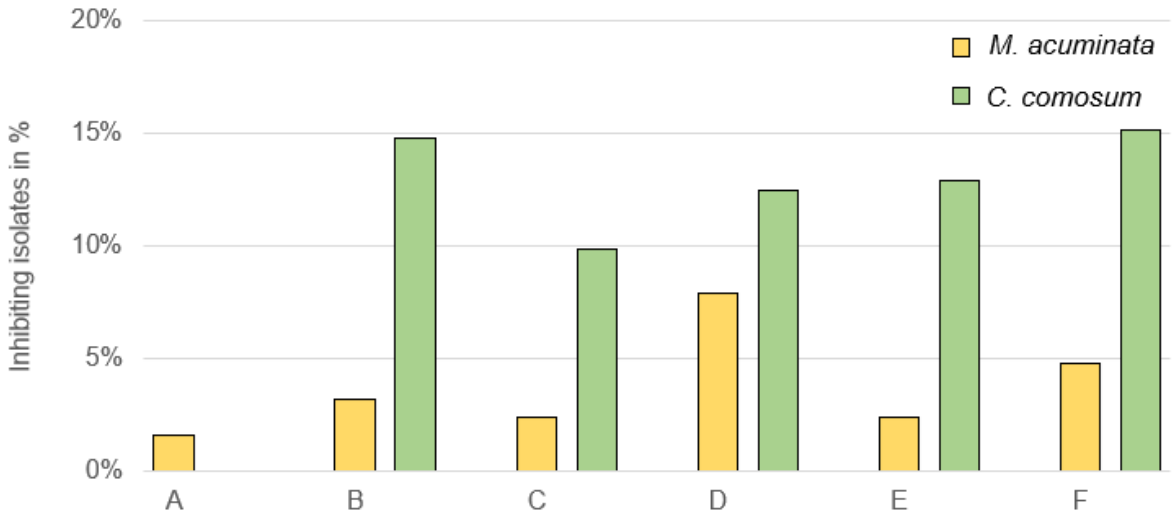

Figure 14: Number of isolates in % that inhibit human opportunistic pathogens. A: A. baumanii; B: E. faecalis; C: E. coli; D: S. haemolyticus; E: S. malthophilia; F: P. aeruginosa.

3.3.2. Screening of biosurfactant producing bacteria

The qualitative screening for biosurfactant-producing bacteria indicated a correlation between biosurfactant production and pathogen inhibition. For the *M. acuminata* isolates, five out of 125 isolates showed positive results in the drop collapse assay. Interestingly four of them also showed inhibitory activities against opportunistic pathogens. A similar trend was observed for *C. comosum*, where 33

of 264 isolates were able to produce biosurfactants and 31 of them also showed an inhibitory effect against the opportunistic model pathogens.

3.3.3. Screening for biofilm formation and biofilm co-culture assays

The biofilm formation potential was examined for all plant-associated isolates as well as for the human opportunistic pathogens *E. faecalis*, *S. maltophilia* and *P. aeruginosa* and mixed bacterial consortia. A significant reduction in biofilm formation was observed in co-cultures of indoor plant isolates with *E. faecalis* where the non-synergistic effects were found from 91.2% (n=114) of *M. acuminata* isolates and 97.2% (n=256) *C. comosum* total isolates. A similar trend was observed in *S. maltophilia*, where the non-synergistic values for both plants were also relatively high (88% - n=110 for *M. acuminata* and 87.9% - n=232 for *C. comosum*) (Figure 15). In total, 65.6% (n=82) of 125 cultivated isolates from *M. acuminata* and 29.1% (n=77) of the 264 bacteria originated from *C. comosum* showed synergistic interactions with *P. aeruginosa.*

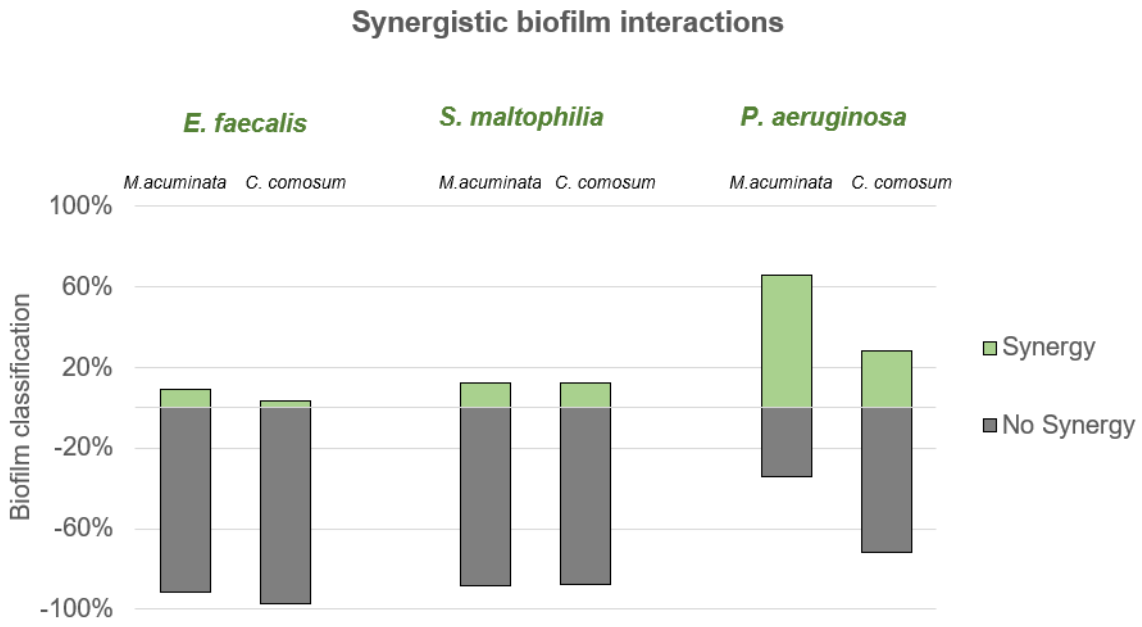

Figure 15: Synergistic interactions between co-cultured human opportunistic pathogens and indoor plant associated bacteria.

3.3.4. Antibiotic resistance profiling

The bacterial culture collections (n=264 from *C. comosum* and n=125 from *M. acuminata)* were screened for resistance against 11 different antibiotics as listed in Supplementary Table S 7. Twenty-nine isolates from *M. acuminata* and 67 from *C. comosum*, did not show any resistances against the employed antibiotics (Figure 16). However, 75% of the cultivated bacteria were resistant to at least one antibiotic. The most common resistances in *M. acuminata* were against Trimethoprim (56%), Ampicillin (54.4%) and Penicillin G (50.4%) and in *C. comosum* against Ampicillin (53.4%), Penicillin G (48.9%) and Erythromycin (45.0%) (Figure 17).

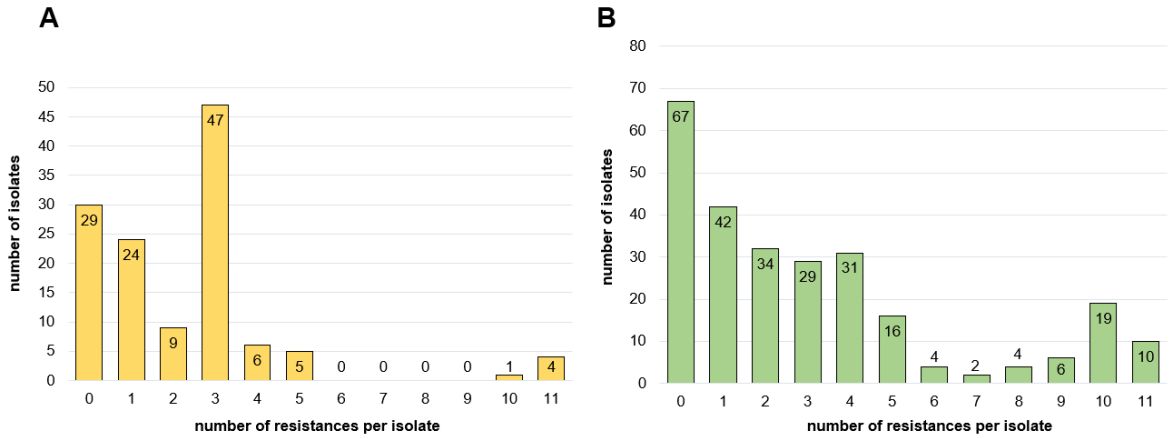

Figure 16: Number of isolates with corresponding number of antibiotic resistances. Absolute number of bacteria was 389. A total of 125 and 264 isolates were cultivated from Musa acuminata (A) and Chlorophytum comosum (B) respectively.

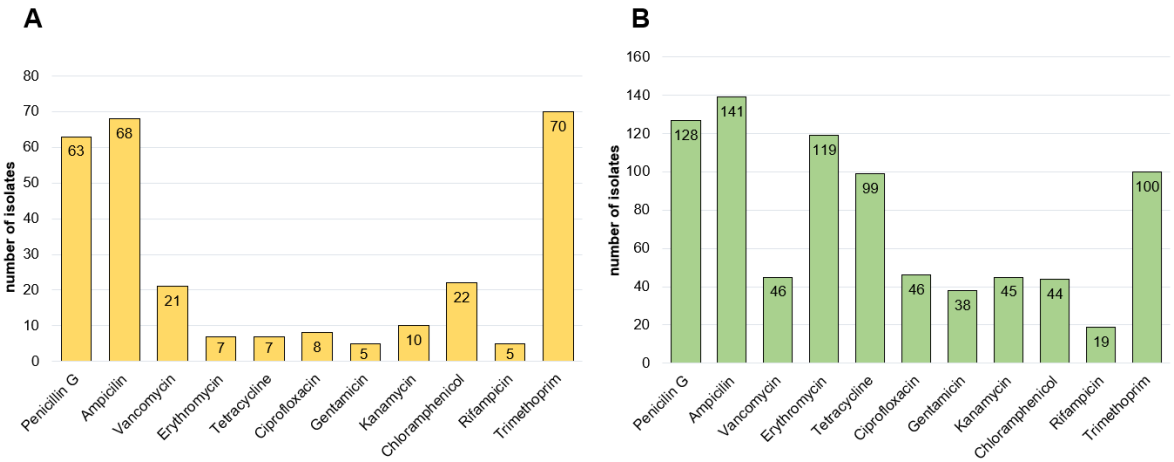

Figure 17: Number of isolates that grown under media with antibiotics. Absolute number of bacteria was 389. A total of 125 and 264 isolates were cultivated from Musa acuminata (A) and Chlorophytum comosum (B) respectively.

3.3.5. Identification of culturable bacteria based on 16S rRNA gene

In total, 37 representative bacteria from each assay were identified based on the sequence of the 16S rRNA gene (Table S 8). Bacteria were selected that had inhibited at least one pathogen (n=21) and those that had shown at least 10 antibiotic resistances (n=16). The most abundant genus obtained from the sequencing data was *Bacillus*. 31 of the total 37 isolates, i.e. 84%, were assigned to this genus and included both antibiotic-resistant species and the 21 bacteria that had previously been selected for their pathogen inhibition. The remaining isolates that were not assigned to *Bacillus* were identified as *Staphylococcus*, *Micrococcus* and *Paenibacillus* and these showed a high number of antibiotic resistances in the culture assays. This result confirms the findings of the amplicon sequencing and supports that *Bacillus* represents a dominant taxon with beneficial effects on human health by competing opportunistic pathogens.

IV. Discussion

The positive effects of indoor plants from a psychological point of view are undisputed, but plant-associated bacteria and their potential to support human health have so far been neglected (Mahnert et al. 2015). The phyllosphere of indoor plants represents a very extensive microbial habitat which is affected by internal and external different bacteria, fungi, yeasts and algae (Lindow und Brandl 2003). In the present study, the structure and ecological function of selected indoor plant microbiomes from different plant species and origins were characterized. This work revealed that the two model plants *C. comosum* and *M. acuminata*, harboured core microbial members irrespective of the environment in which they were grown. Moreover, cultivable bacteria from both plant communities counteracted opportunistic human pathogens. The overall findings of this study provide evidence for the positive implications of indoor plants on human health by inhibiting important human pathogens.

The results of this thesis confirmed that the phyllosphere is generally dominated by *Alpha-* and *Gammaproteobacteria* as well as *Actinobacteria* and *Firmicutes* (Kembel et al. 2014; Rastogi et al. 2013). The core microbiome displays *Bacillus* in *C. comosum* and *Sphingomonas* in *M. acuminata* with a high relative abundance. These taxa were also mentioned in previous studies as prominent inhabitants of the phyllosphere in other plant species (Rastogi et al. 2013; Kim et al. 1998). *Dothideomycetes* were the most dominant fungal class and belong to *Ascomycetes* which are often found in the phyllosphere (Jumpponen und Jones 2009; Kembel und Mueller 2014; Qian et al. 2018). *Dothideomycetes* occur all over the world and, because of their saprotrophic abilities, are very important for the ecosystem functioning as they break down plant degradation products. *Capnodiale*s, which belong to the *Dothideomycetes*, are known to be resistant to harsh conditions such as high UV radiation or reduced nutrient availability and thus have advantageous properties for the colonization of the leaf surfaces (Qian et al. 2018). *Nitrososphaeria* was the most dominant community and belongs to the *Thaumarchaeota*, a group that is also commonly found in the phyllosphere (Taffner et al. 2019). Their role in plant is so far unknown; however, foregoing studies

demonstrated that this taxon plays an important role in the carbon cycle in soil (Li et al. 2019; Tang et al. 2019).

The colonization of microorganisms on leaves depends on the plant species and the environment where the plants were grown. The phenotypic characteristics are controlled by the genotype and imply its importance for the microbiome. Previous studies have already shown that varieties of the same plant species differ in the bacterial population (Lindow und Brandl 2003; Whipps et al. 2008). In this approach, the plant species was identified as the dominant factor influencing the bacterial community. Thus this study also supports previous findings where the plant species was shown to affect the bacterial and fungal community structure of indoor plants which is likely accountable to differences in leaf morphology and host metabolism (Mahnert et al. 2018b). The environment has also been shown to influence the bacterial community, and this suggests that the bacterial occupants in the surrounding are also decisive to colonize the phyllosphere. However, the environment influenced the fungal community even more. The leaf surface is generally a hostile environment for microbial colonizers as it is exposed to rapid fluctuation of temperature and humidity (Lindow und Brandl 2003) which differs among built environments. Previously it was shown that the exchange with the outside air is an important factor in the colonization of fungi on plants (Adams et al. 2013) and that they are often found on leaf surfaces as spores that can easily spread in the air (Lindow und Brandl 2003). The archaeal colonization was even more specific to the sampling site and confirmed earlier findings that this can also depend heavily on external influences such as open windows or even house residents (Pakpour et al. 2016). The results provide insights into the structure and function of indoor plant associated bacterial communities. In general, the bacterial population showed substantially less variation between different built environments in comparison to the fungal and archaeal community of the phyllosphere. For this reason, ecological function studies and complementary metagenomic analyses were conducted on the bacterial fraction.

In the metagenomic data, a high prevalence of genes related to secondary metabolites was detected from the bacterial fraction. The multienzyme complex of non-ribosomal synthetases catalyses the production of numerous natural products which exhibit a broad range of biological functions including antimicrobial, phytotoxic

and antiviral activities (Le Govic et al. 2019) and can be found in both, *C. comosum* and *M. acuminata.* Within the bacterial collection, we identified a high prevalence of biosurfactant-producing *Bacillus* that were able to inhibit growth of the tested opportunistic human pathogens. The production of biosurfactants from lipopeptides is a result of nonribosomal biosynthesis and common for *Bacillus* species (Płaza et al. 2015). There were also several genes found which are involved in the process of siderophore production including *iucC*, *iucd*, *asb* in *Bacillus* (Najimi et al. 2008; Lam et al. 2018b) and are commonly used by antagonistic bacteria to compete against pathogenic bacteria.

Despite overlapping results between shotgun metagenomic sequencing and cultivation experiments, significant differences between the individual methods were observed. *Sphingomonas* was not identified in the culture, although high abundances were observed in the amplicon and metagenome analysis of *M. acuminata*. The cultivation of microbes depends on several factors, such as specific nutrients, oxygen demand, temperature, pH, or missing growth factors from other organisms in a community (Vartoukian et al. 2010). Conventional culture media are used for laboratory cultivation but can never fully resemble the natural habitat of the microorganisms. Bacteria that can best adapt to these conditions grow faster and can inhibit others, although they are not the dominant or ecologically most important representatives in the natural environment (Vester et al. 2015).

This study further explored how indoor plant associated bacteria interaction affected mixed biofilm production when they were combined with model pathogens. The potential indirect beneficial effects of plant associated bacteria on human health as counterparts against pathogens were observed. About 19% of the culturable isolates from *C. comosum* and 10% of isolates from *M. acuminata* showed inhibitions against at least one of the six tested human opportunistic pathogens (*A. baumanii*, *E. faecalis*, *E. coli*, *S. haemolyticus*, *S. malthophilia* and *P. aeruginosa*). This study suggests that biosurfactant-producing bacteria can also inhibit and suppress the growth of certain pathogens because the screening for such bacteria indicated a correlation between biosurfactant production and pathogen inhibition. Another way to persist is biofilm production, which can improve the fitness of the bacterial population in more extreme environments and is therefore the most common type of bacterial life. Previous outcomes (Burmølle et al. 2006; Raghupathi

et al. 2017) suggested that an individual strain may need to adapt to the presence of other strains and results in a synergy or non-synergy effect. In general, it is believed that bacteria that colonize the same habitat show more synergistic effects and increased biofilm formation, indicating an adaptive response to long-term coexistence (Madsen et al. 2016). In contrast, a decrease in biofilm formation and stronger defence strategies are observed in co-cultured bacteria species that normally do not live in the same environment and have not had time for evolutionary adaptation (Madsen et al. 2016). In this study, due to the different natural environments of the plant-associated isolates and the human opportunistic pathogens, rather non-synergistic effects were assumed. With respect to the phyllosphere isolates that were tested against the human opportunistic pathogens *E. faecalis* and *S. maltophilia* non-synergistic effects were largely observed, which indicates their potential to reduce the biofilm formation of human pathogens. In contrast to these expected results, *P. aeruginosa* showed mostly synergistic effects in the mixed biofilms. The positive compatibility between *Pseudomonas* and *Bacillus* regarding biofilm formation has reported (Ansari und Ahmad 2019). This might be explainable by the high prevalence of members of the genus *Pseudomonas* in plant-associated habitats (Jasim et al. 2014; Sitaraman 2015). Therefore, as they may have adapted to each other, synergistic effects were observed in this approach.

Our resistome analysis demonstrated that indoor plant associated bacteria possess a range of antimicrobial resistance features. A relatively low number of reads (0.006-0.012%) from the phyllosphere metagenome could be assigned to 170 and 149 ARGs respectively compared to other studies (Cernava et al. 2019). It has been shown in the metagenomics that a large portion of the antibiotic resistance genes can be assigned to the *Proteobacteria*, but when the methods are combined, the genus *Bacillus*, which belongs to the *Firmicutes*, appears to be an important member of the microbiome of *C. comosum.* The metagenomic data showed multidrug and macrolide-lincosamide-stretogramin (MLS) as the most predominant antibiotic resistance gene class. In the multidrug class the predominant *acrB* gene is part of a multidrug efflux protein complex which enables resistance to a broad range of antibiotics (Hobbs et al. 2012). The most abundant gene of the MLS class, namely *macB*, is also found in many Gram-negative bacteria and represents a noncanonic ABC-type transporter that interacts with the membrane channel TolC to

export macrolide antibiotics (Xu et al. 2009) which may explain a high prevalence of isolates that were resistant against Erythromycin (Greene et al. 2018). These insights also correlated with the results of the cultured isolates from the phyllosphere, which were included in this work. Approximately 75% of the cultivable isolates from both plants showed resistances against antibiotics, whereas 10.9% of the *C. comosum* isolates and 4% of the *M. acuminata* isolates showed inhibition against 10 or 11 antibiotics. Many studies have shown that most rhizosphere isolates are multi-drug resistant and cover almost all classes of antibiotics (Walsh und Duffy 2013; D'Costa et al. 2006). In general, it was found that the coding genes for multidrug efflux pumps are highly conserved and thus represent an evolutionary old and general mechanism, hence it is also suspected that this is a natural defence strategy (Martinez et al. 2009). This is supported by the finding that these multidrug efflux pumps are relevant for bacterial metabolism as they are responsible for the detoxification of intracellular metabolites and the intercellular trafficking (Martinez et al. 2009). Overall, we could show that indoor plant-associated bacteria can act as natural ARG reservoirs. However, the total number of ARGs is relatively low and thus likely possess a reduced risk to be transferred to human pathogens, which are mostly antagonized by the natural phyllosphere community.

V. Conclusion

This thesis provides key insights into the function of microbes on the leaf surface of the widespread indoor plants *C. comosum* and *M. acuminata* and their potential effects on human health. Since indoor microbial diversity is rather low, the hypothesis was that house plants with their balanced microbiome are able to improve the indoor microbiome and support human health. The phyllosphere microbiomes were shown to be mostly influenced by plant species and the built environment where the plant was grown in. The bacterial community from built environment samples showed a higher congruence. This study also revealed that indoor plant bacterial communities harbour numerous antimicrobial features and possess antagonistic properties against human opportunistic pathogens.

As it becomes more and more important to find new solutions how to reduce infections with multi-resistant opportunistic pathogens especially in clinical settings,

the concept of a balanced and diverse indoor microbiome can be crucial. Plant-associated, beneficial microbes can help to suppress these pathogens, but their natural resistome with numerous different antibiotic resistances must also be considered.

VI. References

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VII. Appendix

Table S 1: Details of indoor plant sampling sites for amplicon analysis. Site locations: Botanical garden (Austria; 47°04'55.3"N 15°27'29.4"E); Private house (Austria; approx. 46°31'39.9"N 14°18'01.2"E and 47°03'21.9"N 15°26'56.5"E) and commercial stores (Austria; 47°02'01.5"N 15°25'23.7"E and 47°02'02.3"N 15°25'14.0"E).

Sample ID	Plant species	Site location	Description
CC-bg-1-1	*Chlorophytum comosum*	Botanical garden-Graz	I-1
CC-bg-1-2	*Chlorophytum comosum*	Botanical garden-Graz	I-2
CC-bg-1-3	*Chlorophytum comosum*	Botanical garden-Graz	I-3
CC-bg-2-1	*Chlorophytum comosum*	Botanical garden-Graz	I-4
CC-bg-2-2	*Chlorophytum comosum*	Botanical garden-Graz	I-5
CC-bg-2-3	*Chlorophytum comosum*	Botanical garden-Graz	I-6
CC-bg-3-1	*Chlorophytum comosum*	Botanical garden-Graz	I-7
CC-bg-3-3	*Chlorophytum comosum*	Botanical garden-Graz	I-8
CC-pg-1	*Chlorophytum comosum*	Private house - Graz	I-13
CC-pg-2	*Chlorophytum comosum*	Private house - Graz	I-14
CC-pf-1	*Chlorophytum comosum*	Private house - Ferlach	I-15
CC-pf-2	*Chlorophytum comosum*	Private house - Ferlach	I-16
CC-pf-3	*Chlorophytum comosum*	Private house - Ferlach	I-17
CC-pf-4	*Chlorophytum comosum*	Private house - Ferlach	I-18
CC-pk-1	*Chlorophytum comosum*	Private house - Kärnten	I-19
CC-pk-2	*Chlorophytum comosum*	Private house - Kärnten	I-20
CC-c1-1	*Chlorophytum comosum*	Commercial shop 1 - Graz	I-30
CC-c1-2	*Chlorophytum comosum*	Commercial shop 1 - Graz	I-31
CC-c2-1	*Chlorophytum comosum*	Commercial shop 2 - Graz	I-34
CC-c2-2-1	*Chlorophytum comosum*	Commercial shop 2 - Graz	I-35
MA-bg-1-1	*Musa acuminata*	Botanical garden-Graz	I-9
MA-bg-1-2	*Musa acuminata*	Botanical garden-Graz	I-10
MA-bg-2-1	*Musa acuminata*	Botanical garden-Graz	I-11
MA-bg-2-2	*Musa acuminata*	Botanical garden-Graz	I-12
MA-pg-1-1	*Musa acuminata*	Private house - Graz	I-21
MA-pg-1-2	*Musa acuminata*	Private house - Graz	I-22
MA-pg-2-1	*Musa acuminata*	Private house - Graz	I-23
MA-pg-2-2	*Musa acuminata*	Private house - Graz	I-24
MA-pg-2-3	*Musa acuminata*	Private house - Graz	I-25
MA-pf-1	*Musa acuminata*	Private house - Ferlach	I-26
MA-pf-2	*Musa acuminata*	Private house - Ferlach	I-27
MA-pk-1	*Musa acuminata*	Private house - Kärnten	I-28
MA-pk-2	*Musa acuminata*	Private house - Kärnten	I-29
MA-c1-1	*Musa acuminata*	Commercial shop 1 - Graz	I-32
MA-c1-2	*Musa acuminata*	Commercial shop 1 - Graz	I-33
MA-c2-1	*Musa acuminata*	Commercial shop 2 - Graz	I-36
MA-c2-21	*Musa acuminata*	Commercial shop 2 - Graz	I-37

Table S 2: Golay barcode constructs for amplicon analyses.

Marker gene	Number of samples	Forward primer Golay	Reverse primer Golay
16S Bacteria (515-926)	38 (with negative)	golay_**bc_01** - **bc_38**	**golay_bc_131_rev_pad**
ITS1 Fungi (ITS1-ITS2)	38 (with negative)	golay_**bc_01** - **bc_38**	**golay_bc_133_rev_pad**
16S Archae (349-519)	38 (with negative)	golay_**bc_01** - **bc_38**	**golay_bc_135_rev_pad**

Table S 3: Primer pad and linker sequence used for amplification with Golay barcodes.

Marker gene	Primer	Primer pad	Linker
16S Bacteria	515f_primer_pad	TATGGTAATT	GT
	926r_primer_pad	AGTCAGCCAG	GG
ITS1 Fungi	ITS1f_primer_pad	TATGGTAATT	GT
	ITS2r_primer-pad	AGTCAGCCAG	GG
16S Archaea	349f_primer_pad	TATGGTAATT	GT
	519r_primer_pad	AGTCAGCCAG	GG

Table S 4: Reaction mix for first PCR. Amplification of marker gene.

Reagent	**Volume [µL]**
Taq-&GO Ready-to-Use PCR Mix (MP Biomedicals, Thermo Fischer Scientific)	2
Primer_f (10 µM)	0.1
Primer_r (10 µM)	0.1
Ultrapure water	6.8
DNA template	1

Table S 5: Reaction mix for second PCR. Barcoding.

Reagent	**Volume [µL]**
Taq-&GO Ready-to-Use PCR Mix (MP Biomedicals, Thermo Fischer Scientific)	6
BC_f (10 µM)	1.2
BC_r (10 µM)	1.2
Ultrapure water	19.6
DNA template	2

Table S 6: Read statistics of raw and filtered (no non-target sequences) quality sequences of the amplicon dataset.

Sample ID	Bacteria			Fungi			Archaea		
	Raw quality sequences	Filtered quality sequences	% non-target sequences	Raw quality sequences	Filtered quality sequences	% non-target sequences	Raw quality sequences	Filtered quality sequences	% non-target sequences
I1	22,920	5,567	75.7	23,386	9,137	60.9	32,649	32,649	0.0
I3	13,439	6,047	55.0	13,273	4,332	67.4	22,665	22,665	0.0
I5	5,545	787	85.8	5,924	4,261	28.1	15,552	15,552	0.0
I6	6,693	2,994	55.3	8,428	5,651	32.9	11,480	11,480	0.0
I8	13,005	1,881	85.5	12,771	6,651	47.9	12,971	12,962	0.1
I11	168,805	3,960	97.7	10,590	7,564	28.6	24,477	24,381	0.4
I13	3,648	1,547	57.6	6,546	4,942	24.5	11,316	11,316	0.0
I14	10,522	5,874	44.2	13,296	10,680	19.7	18,084	18,084	0.0
I15	11,357	2,885	74.6	14,977	11,753	21.5	24,540	24,540	0.0
I16	3,546	1,203	66.1	6,510	4,558	30.0	13,286	13,286	0.0
I17	6,093	2,024	66.8	8,136	5,704	29.9	17,343	17,340	0.0
I18	8,527	2,896	66.0	14,906	11,201	24.9	13,581	13,581	0.0
I19	10,622	4,022	62.1	12,637	7,608	39.8	16,373	16,373	0.0
I20	6,414	5,054	21.2	8,566	4,635	45.9	13,304	13,304	0.0
I21	58,367	1,657	97.2	10,982	5,605	49.0	21,577	21,534	0.2
I22	59,371	3,682	93.8	9,576	5,001	47.8	21,476	21,476	0.0
I23	128,904	4,272	96.7	4,867	2,711	44.3	2,976	2,823	5.1
I24	91,587	1,637	98.2	12,443	5,827	53.2	23,399	23,236	0.7
I25	256,642	3,778	98.5	15,272	6,841	55.2	12,652	11,608	8.3
I26	291,632	8,536	97.1	12,375	4,787	61.3	27,912	27,879	0.1
I27	171,744	7,931	95.4	9,081	3,940	56.6	14,686	14,679	0.0
I28	149,624	6,428	95.7	15,909	8,354	47.5	19,003	18,993	0.1
I29	157,086	33,685	78.6	9,357	1,865	80.1	6,819	6,791	0.4
I30	10,709	6,596	38.4	13,350	5,772	56.8	17,646	17,613	0.2

I31	5,284	2,522	52.3	8,990	5,889	34.5	21,113	21,090	0.1
I32	60,126	6,005	90.0	7,559	4,178	44.7	17,330	17,218	0.6
I33	251,084	16,302	93.5	8,961	5,853	34.7	34,026	33,964	0.2
I34	5,028	1,915	61.9	8,341	5,265	36.9	12,803	12,508	2.3
I35	10,647	3,757	64.7	13,608	7,602	44.1	32,573	32,508	0.2
I36	10,117	679	93.3	14,240	10,483	26.4	26,064	21,412	17.8
I37	194,168	8,010	95.9	13,456	9,015	33.0	51,634	51,500	0.3
I39	3,505	1,237	64.7	25,681	11,206	56.4	10,094	10,094	0.0
I40	12,138	4,293	64.6	43,172	23,898	44.6	9,116	9,116	0.0
I41	9,594	3,178	66.9	29,497	15,125	48.7	5,128	5,128	0.0
I42	5,915	893	84.9	25,155	15,000	40.4	6,485	6,485	0.0
I43	9,358	427	95.4	31,455	20,598	34.5	1,841	1,841	0.0
I44	NA	NA	NA	17,587	8,349	52.5	4,187	4,187	0.0

Table S 7: Antibiotics used in the plate assays.

Antibiotic	Antibiotic class	Manufactures	Spectrum
Benzylpenicillin (Penicillin G)	β-lactam	Roth, Germany	Gram +
Ampicillin	β-lactam	Roth, Germany	Gram +/ -
Vancomycin	Glycopeptides	Sigma-Aldrich, Missouri, USA	Gram +
Erythromycin	Macrolides	Roth, Germany	Gram +/ -
Tetracycline	Tetracyclines	Merck, Germany	Gram +/ -
Ciprofloxacin	Fluoroquinolones	Sigma-Aldrich, Missouri, USA	Gram -
Gentamycin	Aminoglycosides	Roth, Germany	Gram +/ -
Kanamycin	Aminoglycosides	Roth, Germany	Gram +/ -
Chloramphenicol	Other	Roth, Germany	Gram +/ -
Rifampicin	Other	Duchefa Biochemie, Netherlands	Gram +/ -
Trimethoprim	Other	Sigma-Aldrich, Missouri, USA	Gram +/ -

Table S 8: Sequence results of representative cultivable isolates with a short overview of the results from the plate tests. MA = M. acimunata, CC = C. comosum. B = E. faecalis, E = S. maltophilia, F = P. aeruginosa.

		Sequence result	No. Of antibiotic resistances	Pathogen Inhibition	Biosurfactant production	Biofilm -Synergy B	E	F
1	*MA*	*Bacillus paranthracis*	3	1				YES
2	*MA*	*Bacillus megaterium*	0	1				YES
3	*MA*	*Bacillus velezensis*	0	4	YES			YES
4	*MA*	*Bacillus subtilis*	1	3	YES			YES
5	*MA*	*Bacillus subtilis*	1	4	YES			YES
6	*MA*	*Bacillus altitudinis*	1	1				
7	*MA*	*Bacillus cereus*	3	2				
8	*MA*	*Bacillus cereus*	1	3				
9	*CC*	*Bacillus megaterium*	2	1				
10	*CC*	*Bacillus amyloliquefaciens*	2	5	YES			YES
11	*CC*	*Bacillus velezensis*	0	5	YES		YES	YES
12	*CC*	*Bacillus amyloliquefaciens*	1	5	YES			YES
13	*CC*	*Bacillus simplex*	1	1				
14	*CC*	*Paenibacillus xylanexedens*	0	1				
15	*CC*	*Bacillus siamensis*	0	5	YES			YES
16	*CC*	*Bacillus amyloliquefaciens*	0	5	YES	YES		YES
17	*CC*	*Bacillus velezensis*	2	5				YES
18	*CC*	*Bacillus velezensis*	2	4	YES			YES
19	*CC*	*Bacillus cereus*	3	1				
20	*CC*	*Bacillus velezensis*	1	5	YES		YES	YES
21	*CC*	*Bacillus velezensis*	0	4	YES		YES	
22	*MA*	*Bacillus subtilis*	11	0				YES
23	*MA*	*Staphyllococcus sp.*	11	0				YES
24	*CC*	*Bacillus gibsonii*	10	0			YES	
25	*CC*	*Micrococcus sp.*	10	0				
26	*CC*	*Bacillus sp.*	10	0				
27	*CC*	*Paenibacillus sp.*	10	0				
28	*CC*	*Bacillus velezensis*	11	0				
32	*CC*	*Bacillus mycoides*	11	0				
33	*CC*	*Paenibacillus sp.*	11	0				
34	*CC*	*Bacillus cereus*	10	1				
35	*CC*	*Bacillus paranthracis*	10	0				
37	*CC*	*Bacillus velezensis*	10	0			YES	
39	*CC*	*Bacillus idriensis*	10	0			YES	
40	*CC*	*Bacillus paranthracis*	11	0				
42	*MA*	*Staphylococcus epidermidis*	11	0				
47	*CC*	*Bacillus wiedmannii*	11	0				

Table S 9: Genes with COG functions related to secondary metabolites biosynthesis, transport, and catabolism from C. comosum.

Genes	No of hits	RA (%)
Peptide synthetase	795	7.16
cytochrome P450	750	6.76
Multicopper oxidase	389	3.51
non-ribosomal peptide synthetase	276	2.49
Virulence factor Mce family protein	264	2.38
IucA / IucC family	257	2.32
FtsX-like permease family	249	2.24
TIGRFAM outer membrane adhesin like protein	243	2.19
MlaD protein	235	2.12
Haemolysin-type calcium-binding repeat (2 copies)	221	1.99

Table S 10: COG category Q functions from the genus Bacillus in C. comosum.

Genus	Function	No of hits	RA (%)
Bacillus	COG2508 Regulator of polyketide synthase expression	28	0.25
Bacillus	IucA / IucC family	27	0.24
Bacillus	Flavin containing amine oxidoreductase	27	0.24
Bacillus	L-lysine 6-monooxygenase (NADPH-requiring)	22	0.20
Bacillus	Isochorismatase family	22	0.20
Bacillus	Transcriptional regulator	18	0.16
Bacillus	Phytanoyl-CoA dioxygenase (PhyH)	15	0.14
Bacillus	O-methyltransferase	12	0.11
Bacillus	Siderophore biosynthesis protein	11	0.10
Bacillus	COG1233 Phytoene dehydrogenase and related proteins	11	0.10
Bacillus	COG1228 Imidazolonepropionase and related amidohydrolases	10	0.09
Bacillus	protein, possibly involved in aromatic compounds catabolism	9	0.08
Bacillus	Catalyzes the oxidation of uric acid to 5- hydroxyisourate, which is further processed to form (S)-allantoin	8	0.07
Bacillus	Methyltransferase	6	0.05
Bacillus	Tellurite resistance protein TehB	6	0.05
Bacillus	calcium- and calmodulin-responsive adenylate cyclase activity	6	0.05
Bacillus	Amidohydrolase family	6	0.05
Bacillus	Catalyzes the first step in the D-alanylation of lipoteichoic acid (LTA), the activation of D-alanine and its transfer onto the D-alanyl carrier protein (Dcp) DltC. In an ATP- dependent two-step reaction, forms a high energy D-alanyl-AMP intermediate, followed by transfer of the D-alanyl residue as a thiol ester to the phosphopantheinyl prosthetic group of the Dcp. D-alanylation of LTA plays an important role in modulating the properties of the cell wall in Gram-positive bacteria, influencing the net charge of the cell wall	6	0.05
Bacillus	COG0179 2-keto-4-pentenoate hydratase 2-oxohepta-3-ene-1,7-dioic acid hydratase (catechol pathway)	6	0.05
Bacillus	COG0412 Dienelactone hydrolase and related enzymes	5	0.05
Bacillus	ubiE/COQ5 methyltransferase family	5	0.05
Bacillus	Methyltransferase domain	4	0.04
Bacillus	FtsX-like permease family	4	0.04
Bacillus	dithiol-disulfide isomerase involved in polyketide biosynthesis	3	0.03
Bacillus	Esterase PHB depolymerase	3	0.03
Bacillus	Protein of unknown function (DUF2848)	1	0.01
Bacillus	Putative SAM-dependent methyltransferase	1	0.01

Table S 11: Genes with COG functions related to secondary metabolites biosynthesis, transport, and catabolism from M. acuminata.

Gene	No of hits	RA (%)
Fumarylacetoacetate (FAA) hydrolase family	363	7.0
Periplasmic component of the Tol biopolymer transport system	212	4.1
Phosphopantetheine attachment site	210	4.0
KR domain protein	159	3.1
COG2931, RTX toxins and related Ca2 -binding proteins	132	2.5
Amino acid adenylation domain	123	2.4
non-ribosomal peptide synthetase	112	2.2
Amidohydrolase family	109	2.1
Flavin containing amine oxidoreductase	99	1.9
Isochorismatase family	97	1.9

Table S 12: COG category Q functions from the genus Sphingomonas in M. acuminata.

Genus	Function	No of hits	RA (%)
Sphingomonas	Periplasmic component of the Tol biopolymer transport system	212	4.07
Sphingomonas	Phosphopantetheine attachment site	122	2.34
Sphingomonas	Phytoene dehydrogenase	80	1.54
Sphingomonas	COG0179 2-keto-4-pentenoate hydratase 2-oxohepta-3-ene-1,7-dioic acid hydratase (catechol pathway)	56	1.08
Sphingomonas	Amidohydrolase family	36	0.69
Sphingomonas	Copper-binding protein	14	0.27
Sphingomonas	Cupin domain	13	0.25
Sphingomonas	Cytochrome p450	13	0.25
Sphingomonas	2-keto-4-pentenoate hydratase 2-oxohepta-3-ene-1,7-dioic acid hydratase	12	0.23
Sphingomonas	Isochorismatase family	9	0.17
Sphingomonas	Methyltransferase type 11	8	0.15
Sphingomonas	ABC-type transport system involved in lysophospholipase L1 biosynthesis, permease component	8	0.15
Sphingomonas	Methyltransferase domain	8	0.15
Sphingomonas	Catalyzes the conversion of maleate to fumarate	6	0.12
Sphingomonas	COG1228 Imidazolonepropionase and related amidohydrolases	5	0.10
Sphingomonas	Methionine biosynthesis protein MetW	4	0.08
Sphingomonas	N-acetyltransferase	3	0.06
Sphingomonas	Domain of unknown function (DUF4396)	3	0.06
Sphingomonas	Methyltransferase small domain	3	0.06
Sphingomonas	dienelactone hydrolase	2	0.04
Sphingomonas	ABC-type transport system involved in resistance to organic solvents, permease component	2	0.04
Sphingomonas	Thioesterase superfamily	1	0.02
Sphingomonas	protein, possibly involved in aromatic compounds catabolism	1	0.02

Table S 13: Occurrence of antibiotic resistances in C.comosum (ACC) and M.acuminata (BMA).

Class	Relative abundance (%)	
	ACC	BMA
aminocoumarin	0,4	0,4
aminoglycoside	2,6	3,1
bacitracin	5,8	5,8
beta_lactam	6,9	7,6
bleomycin	0,4	0,0
chloramphenicol	0,7	0,9
fosfomycin	0,4	1,3
fosmidomycin	0,4	1,3
glycopeptide	0,4	1,8
kasugamycin	2,6	0,9
macrolide-lincosamide-streptogramin	17,5	13,4
multidrug	41,6	41,5
mupirocin	1,5	2,2
peptide	0,7	0,9
pleuromutilin	0,4	0,4
polymyxin	4,7	5,4
puromycin	0,4	0,4
quinolone	2,2	2,7
rifampin	1,5	0,4
sulfonamide	0,4	0,9
tetracycline	6,2	6,3
triclosan	0,7	0,9
trimethoprim	1,5	0,9

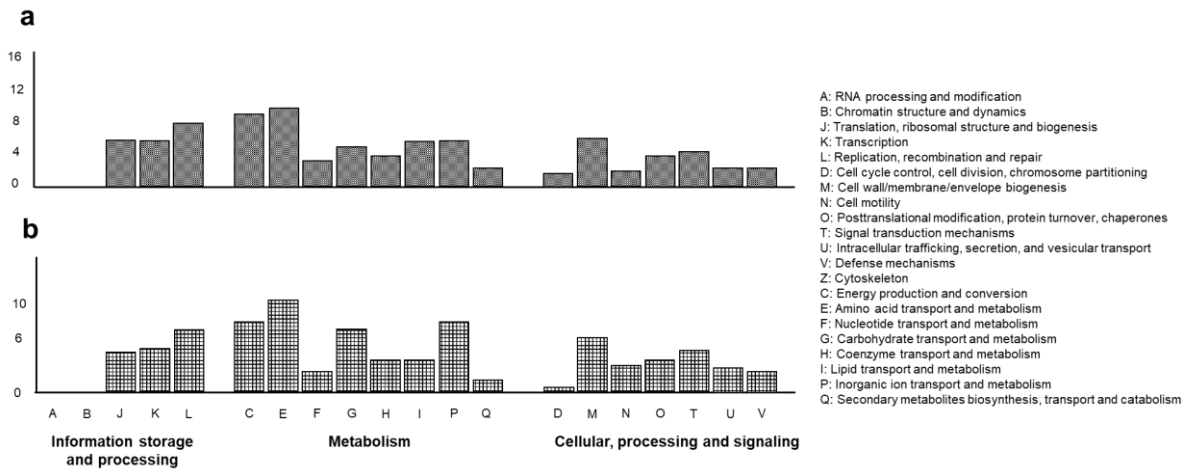

Figure S 1: Clusters of Orthologous Groups of proteins (COGs).

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