The Plant Microbiome

Hurghada, Egypt - 2018

International Symposium - The Plant Microbiome: Exploration of Plant-Microbe Interactions for Improving Agricultural Productivity

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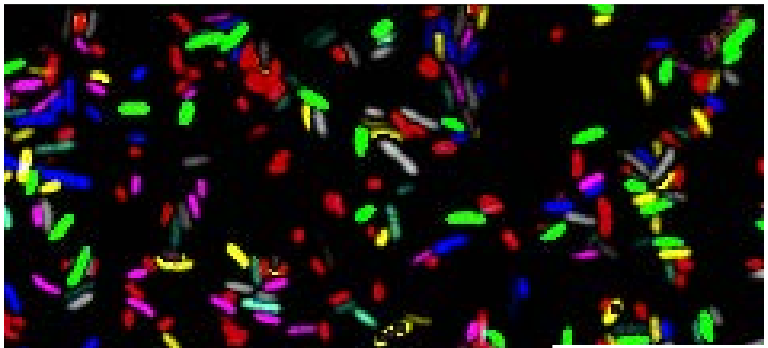

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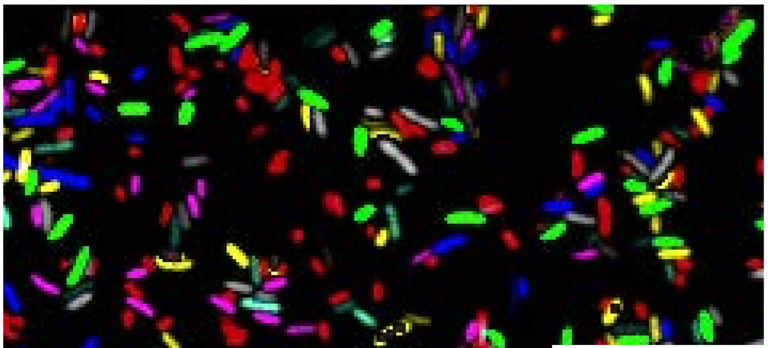

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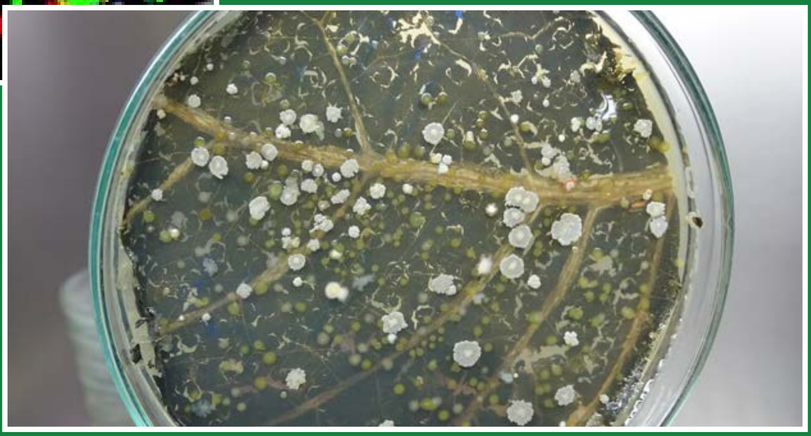

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The Cover Photographs:
***Left**: E. coli expressing seven different fluorescent proteins using sequential confocal laser scanning microscopy for the observation of bacterial communities at the single-cell resolution (courtesy of Mitja Remus-Emsermann)*
***Right**: In situ growth of CFUs colonies of endophytes on the surfaces of plant leaves, as plant-only-based culture media (courtesy of Rahma Nemr)*

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We all wish you a successful symposium and pleasant stay at El Gouna, Hurghada- Egypt.

The Organizing Committee

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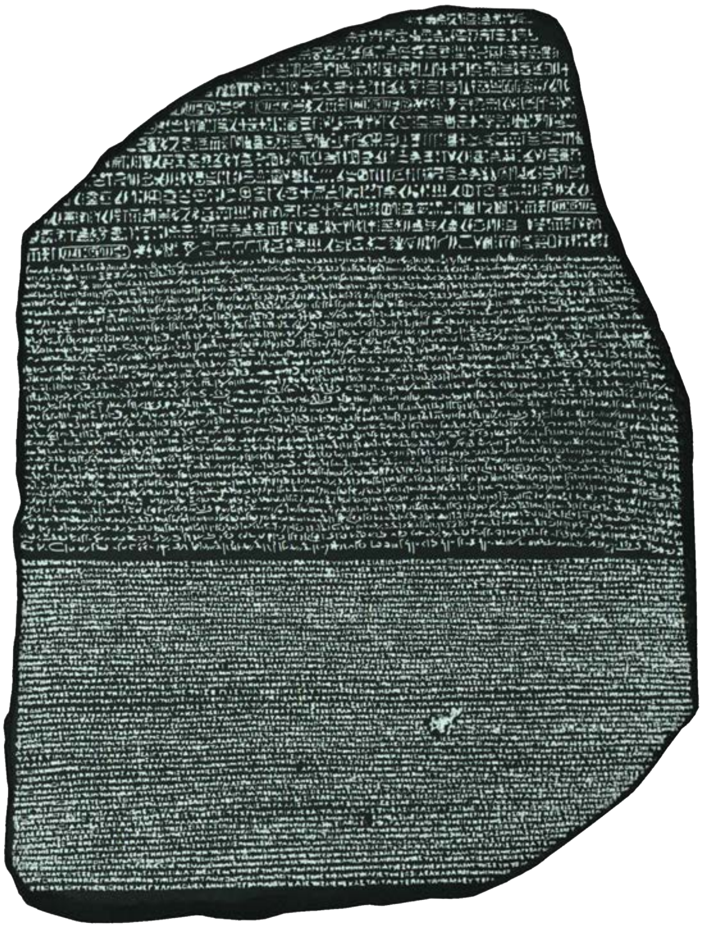

Rosetta Stone: Deciphered the codes of ancient Egyptian civilization

Welcome & Introduction

We are pleased to extend greetings and warm welcome to all of the participants of pgpmicrobiome2018 symposium.

The symposium will address many important issues and convene up to 150 scholars and graduate students from around the world.

Welcome to all who are gathering in El Gouna, Hurghada-Egypt to discuss research endeavors and share knowledge regarding the plant microbiome: current challenges and future perspectives with specific highlights on recent developments in culture-independent and -dependent techniques, the diversity of bacteria and fungi in plant compartments, eco-physiological conditions, and impact of biotic and abiotic stresses on composition and function of the plant microbiome and challenging future agriculture productivity. In addition to comprehensive sessions on PhD, post-doctoral, and research funding opportunities offered for junior researchers and graduate students by the German and Japanese organizations.

A warm word of gratitude goes to the sponsors who made it possible to organize the symposium. We acknowledge the principal support of Alexander von Humboldt Foundation to approve the symposium within the frame of "AvH-Kolleg series". In addition to the cooperative support of the German Research Foundation-DFG, German Academic Exchange Service-DAAD, the Technical University of Berlin-Camps El Gouna, the Japan Society for the Promotion of Science- JSPS, the Leibniz Institute of Vegetable and Ornamental Crops-IGZ, and Cairo University and its Journal of Advanced Research-JAR.

Egypt, the land of civilization and peace, the dawn of conscience welcomes you to enjoy its marvelous ancient history, generous hospitality, and nature beauty.

We all wish you the best for a fruitful and an enjoyable event.

The Organizing Committee

The beauty of ancient Egypt welcomes you

The Symposium Programme

Sunday, Nov. 18: Arrival Day

All day	Arrival at Hurghada airport and hotel transfer
16:00	Registration (Arena Inn Hotel)
20:00	Get together Dinner

Monday, Nov. 19: Day 1

08:00	Registration continued (TU Berlin El Gouna Campus)
09:00	Welcome: Representatives of the sponsors (CU, IGZ, AvH, DFG, DAAD, STDF, JSPS)
09:30	Opening keynote address: The plant microbiome, current challenges and future perspectives (*Angela Sessitsch*)

Session 1: Recent developments in culture-independent metagenomic techniques for environmental microbiomes, with potential implications on plant microbiome research (*Anton Hartmann and Gabriele Berg*)

10:00	Technical challenges and opportunities in microbiome research (*Florian Fricke*)
10:30	**Tea break**
11:00	*In silico* prediction of the potential and the risk of microbial inoculants for sustainable crop production (*Sascha Patz*)
11:30	How can bioinformatics help us to characterize pollution-cleaning soil microbiomes (*Mohamed El-Hadidi*)

Session 2: Recent progress in culture-dependent techniques towards the application of culturomics to the plant microbiome research (*Barbara Reinhold-Hurek and Jihyun Kim*)

12:00	Phytoplasma cultivation: Problems and solutions (*Assunta Bertaccini*)
12:30	Culturing the plant microbiome: The era of artificial media is about to end and the age of plant-only-based culture media had dawned (*Nabil Hegazi*)
13:00	Leaf surface microbial ecology at the bacterial individual resolution (*Mitja Remus-Emsermann*)
13:30	*In situ*-based cultivation method reveals high portion of plant beneficial bacteria in the rhizosphere of wheat plants (*Triticum aestivum* L.) grown in Chilean Andisol (*Jacquelinne Acuña*)
14:00	**Lunch**

Session 3: The plant microbiome: Diversity of fungi in plant compartments/spheres (*Philipp Franken and Kornelia Smalla*)

15:00	Functional diversity of root colonizing fungi (*Philipp Franken*)
15:30	Interaction of *Medicago truncatula* with arbuscular mycorrhizal fungi and pathogens- function of jasmonates and early responses (*Bettina Hause*)
16:00	*Kosakonia radicincitans* DSM 16656^{T} has a significant impact on the tomato microbiome (*Matthias Becker*)
16:30	Effect of coinoculation of *Rhizoglomus irregulare*, and hyphae attached phosphate solubilizing bacteria on *Solanum lycopersicum* (*Shubhangi Sharma*)
16:50	Integrating microorganisms in *Lithospermum erythrorhizon* plant production systems to enhance production of the secondary metabolite shikonin (*Alicia Varela Alonso*)
17:10	Growth enhancement of wheat plant by *Nigrospora sphaerica* and *Curvularia lunata* endophytic fungi isolated from *Melia azedarach* L. (*Samir Abdelgaleil*)
17:30	Evolution of land plants and their pathogens (*Abdelfattah Badr*)
17:50	**Tea break and poster session 1**
19:00	**Dinner**

Tuesday, Nov. 20: Day 2

Session 4: The plant microbiome: Diversity of bacteria in plant compartments/spheres (*Angela Sessitsch and Silke Ruppel*)

08:30	Assessment strategies of structural and functional diversities of plant-associated bacterial communities (*Anton Hartmann*)
09:00	Microbial communities at the root-soil interface (*Barbara Reinhold-Hurek*)
09:30	Sow the seeds - for a sustainable agriculture (*Gabriele Berg*)
10:00	**Tea break**
10:30	The rhizosphere microbiome - key for plant health and growth (*Kornelia Smalla*)
11:00	Legumes strategically control their microbial environment (*Tomomi Nakagawa*)
11:30	A keystone species in the rhizosphere microbiome of tomato resistant to bacterial wilt (*Jihyun Kim*)
12:00	**Lunch**

Session 5: Eco-physiological conditions orchestrating composition and function of the plant microbiome and its phyto-metabolome (*Assunta Bertaccini and Takuro Shinano*)

13:00	The plant microbiome as a resource for oxidative biocatalysts (*Wolfgang Liebl*)
13:30	Bacterial quorum sensing molecules as possible way to protect crop plants against plant and human pathogens (*Adam Schikora*)
14:00	Plant specialized metabolites in the rhizosphere: Dynamics and functions in biological communication (*Akifumi Sugiyama*)
14:30	Approach to investigate the root and microorganism interaction by ‘Rhizomics’ (*Takuro Shinano*)
15:00	Whole-cell mass spectrometric analysis for rapid classification of culturable microbes (*Katja Witzel*)
15:30	The plant growth-promoting bacteria *Kosakonia radicincitans* improves fruit yield and quality of *Solanum lycopersicum* (*Beatrice Berger*)
16:00	Osmoadaptation and hydroxyectoine accumulation enhance endophytism of encapsulated *Kosakonia radicincitans* in radish plants (*Mauricio Cruz Barrera*)
16:20	**Tea break and poster session 2**
18:30	**Dinner**

Wednesday, Nov. 21: Day 3

Session 6: Water quality and availability, and environmental stresses shape the plant microbiome and challenge future agriculture productivity (*Wolfgang Liebl and Kauser Malik*)

08:30	Challenges and possibilities for water re-use in irrigation optimization of technologies of waste water treatment as a potential resource for future agriculture in semi- /arid deserts (*Matthias Barjenbruch*)
09:00	Halophyte microbiome: Revealing its diversity, functions and applications (*Katarzyna Hrynkiewicz*)
09:30	Comparison of microbiome of halophyte (*Salsola stocksii*) and non-halophyte (*Triticum aestivum*) using culture-independent approaches (*Kauser Malik*)
10:00	Influence of biotic and abiotic stresses on tomato endophytes (*Bukhardt Flemer*)
10:30	Microbial diversity in the rhizosphere of meso-, halo- and xerophytes (*Samina Mehnaz*)
10:50	**Tea break**
11:20	Bambara groundnut-bacteria interaction; source of food security (*Olubukola Babalola*)
11:40	Bacterial endophytes for improved plant growth under nitrogen and water limited conditions (*Islam Abd El Daim*)

Session 7: Information sessions on PhD, post-doctoral and research funding opportunities (*Nabil Hegazi and Silke Ruppel*)

12:00	**Lunch**
13:00	Alexander von Humboldt Stiftung (AvH) sponsorship and programmes: *Jonas Jungbluth, Moemen Hanafy, Ebaa El-Hosary, Mohamed Abdalla, Hamada Ali*
14:00	Japan Society for the Promotion of Science (JSPS) programmes and Alumni in Egypt (JSPSAAE): *Naoko Fukami, Ibrahim Tantawy, Gad El-Qady, Walaa Saad, Neven Salah*
15:00	The Federal Ministry of Education and Research (BMBF)-Germany, research and research funding opportunities (*Susane Ruppert-Elias*)
15:20	- Deutsche Akademische Austauschdienst (DAAD) funding mechanisms, and pursuing PhD and Postdocs study in Germany. Pursuing your PhD and Posdoc at the Technical University of Munich (TUM) (*Heba Afifi*) - The Egyptian-German Day 2018-4: Current progress in student activities of Cairo University within the frame of cooperation with DAAD, AvH, BMBF/STDF, IGZ (*Mohamed Fayez*)
16:00	TU Berlin El Gouna campus tour and on-going activities
16:30	**Tea break**
17:00	Official closure, conclusions and prizes for oral and poster presentations of junior researchers (*Nabil Hegazi, Silke Ruppel and Anton Hartmann*)
19:00	**Gala dinner**

The Scribe V Dynasty

Welcome: Representatives of the sponsors

Alexander von Humboldt Foundation - AvH (*Dr. Thomas Hesse*)

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TU Berlin, and TU Berlin Campus El Gouna (*Prof. Matthias Barjenbruch*)

German Academic Exchange Service - DAAD (*Miss Heba Afifi*)

Japan Society for the Promotion of Science - JSPS (*Prof. Naoko Fukami*)

Leibniz Institute of Vegetable and Ornamental Crops - IGZ (*Dr. Silke Ruppel*)

Faculty of Agriculture, Cairo University (*Prof. Amr Mostafa*)

Opening keynote address

Angela Sessitsch

The plant microbiome – current challenges and future perspectives

Angela Sessitsch, Günter Brader, Friederike Trognitz, Birgit Mitter

Angela Sessitsch, angela.sessitsch@ait.ac.at,

AIT Austrian Institute of Technology GmbH, Center for Health & Bioresources, Bioresources Unit, 3430 Konrad-Lorenz-Straße 24, 3430 Tulln, Austria

The plant microbiome is considered as an accessory genome for plant providing complementary functions to their host such as nutrient mobilization and acquisition or functions to better cope with biotic and abiotic stresses. A number of microorganisms has been identified and selected for application as biofertilizer, plant strengthener or biopesticide and benefits seen in lab and greenhouse trials are highly promising. Nevertheless, field application remains a challenge. Reasons for this include the extreme complexity of plant-associated microbiota with which an inoculant strain has to compete or the poor availability of suitable delivery approaches for microbial inoculants. Furthermore, still mechanistic understanding on plant-microbe interactions or on holobiont interactions is missing. Ecological understanding, science-drive product development and smart delivery approaches are likely to improve plant microbiome applications and will pave the way to the integration of microbiome functions in smart agricultural systems.

Session 1

Recent developments in culture-independent metagenomic techniques for environmental microbiomes, with potential implications on plant microbiome research

Chairpersons: Anton Hartmann and Gabriele Berg

TECHNICAL CHALLENGES AND OPPORTUNITIES IN MICROBIOME RESEARCH

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Microbiome research continues to create tremendous excitement in human, animal and plant sciences, which is reflected in the growing body of scientific and popular literature. Various disease and disorders have been linked to host-associated or environmental microbiota features, such as microbial diversity or increased/decreased relative abundances of specific bacterial groups. However, most findings so far have been descriptive, proposing microbiota correlations with ecological or clinical parameters without demonstrating causal relationships or generating new mechanistic insights. For example, the disturbed state of the human microbiome during disease is often vaguely described as 'dysbiosis' but specific quantifiable and targetable parameters to predict dysbiosis are lacking. In this context, the field has been facing increasing criticism for its perceived overenthusiasm and limited progress into diagnostic or therapeutic application. Still, microbiome research holds tremendous potential for improving our medical, biological and ecological understanding, which is likely to substantially advance clinical and other applications in the future.

Here we provide an overview of some of the most important technical, bioinformatic and conceptual challenges and opportunities of microbiome research, including the discussion of methods for nucleic acid isolation, 16S rRNA gene amplification, targeted marker gene and metagenome shotgun sequencing; issues of sample contamination, protocol standardization and reproducibility; open (meta-)data and transparency; and new approaches for living microbiota analysis and quantitative microbiota profiling.

IN SILICO PREDICTION OF THE POTENTIAL AND THE RISK OF MICROBIAL INOCULANTS FOR SUSTAINABLE CROP PRODUCTION

Patz, S[1]*; Bağcı C[1]; Becker, M[2]; Berger, B[3]; Ruppel, S[2]; Huson, DH[1].
1 Center for Bioinformatics, University of Tübingen, Sand 14, 72076 Tübingen, Germany (sascha.patz@uni-tuebingen.de)
2 Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany
3 Institute for National and International Plant Health, Julius Kühn-Institute - Federal Research Centre for Cultivated Plants, Germany

Rising demands for sustainable crop production and the impact of climate change require new technologies to guarantee food security and quality. Beyond plant breeding strategies, there is much interest in exploring the potential of the microbiome to improve crop productivity and stress tolerance. Considerable efforts have been devoted to microbiome sequencing in order to detect beneficial host-microbe interactions. However, the underlying genetic mechanisms have to be understood to distinguish between distinct microbial lifestyles, ranging from mutualistic to pathogenic, not rarely facultative. Here we provide an *in silico* approach that predicts beneficial plant growth-promoting (PGP) traits and the risk of virulence by analyzing orthologous genes and syntenic gene clusters across phyla, which exceeds pan-genome capabilities.

Keywords: bioinformatics, orthologous syntenic gene clusters, plant growth-promotion, virulence, comparative genomics across phyla, metagenome

A number of annotation systems and databases are available to describe the functional diversity of a single genome up to multiple metagenomes. Among them are, in particular, the database of Clusters of Orthologous Groups of proteins (COGs), Gene Ontology (GO), Rapid Annotation of microbial genomes using (SEED-) Subsystem Technology (RAST) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). While virulence factors (VFs) form an own entity, PGP traits (PGPs; e.g. pathways, genes) are not explicitly represented together in such systems.

Following the structure of KEGG, we provide a hierarchical classification including the most functional categories belonging to the term "plant growth-promotion" (PGP) and visualized it as a multi-labeled tree (Fig. 1).

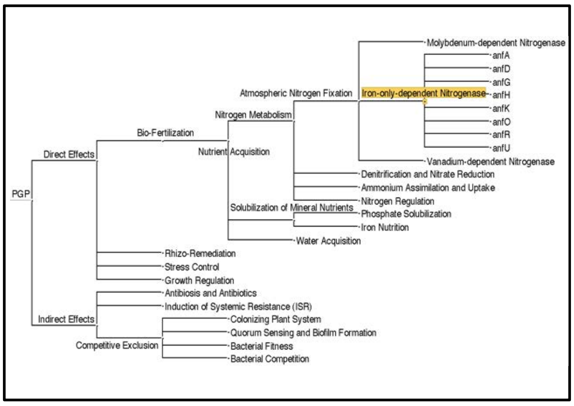


Fig1. Part of the hierarchical multi-labeled tree for the functional category "PGP" and its sub-classification toward the proteins / genes at the leaf nodes (MEGAN6). Leaves shown represent the atmospheric nitrogen binding alternative iron-only-dependent nitrogenase gene cluster (*anf*).

Internal nodes represent orthologous groups of common function, whereas leaves represent, proteins or genes. Not surprisingly, the PGP and VF classifications overlap due to common mechanisms used for e.g. attachment to and migration into hosts.

Clustering the co-occurrence and abundance of orthologous genes and syntenic gene clusters of VFs and PGPs across ~3000 genomes spanning all phyla, across single clades of beneficial and pathogenic strains, unveils specific "finger prints" (Fig. 2). We plan to use these patterns to generate prediction models for virulence and beneficial potential of strains within an entire microbiome. Mapping metagenomes and metadata, containing information about host specificity, against both trees will lead to a better understanding of host-microbe interaction.

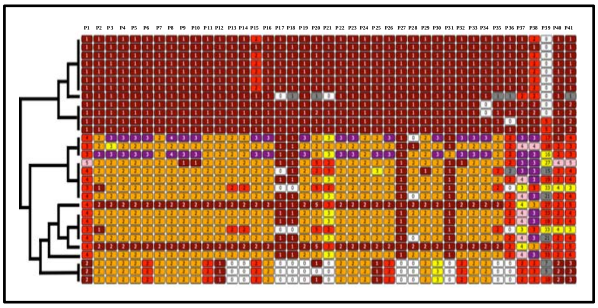


Fig2. Hierarchical clustering with heatmap of the co-occurrence and abundance profile of proteins (P1-41) coded as single genes or within syntenic gene clusters (SGCs) of VFs and/or PGPs for selected strains (rows), usable to differentiate between virulent and beneficial bacterial strains. (dark red: belonging to 1 SGC, orange: to 2 SGCs, purple: to 3 SGCs, light colors red, yellow and pink indicate an additional occurrence in the genome apart from SGCs, gray: not part of a SGC, white: absent, integers indicate protein count).

HOW CAN BIOINFORMATICS HELP US TO CHARACTERIZE POLLUTION-CLEANING SOIL MICROBIOMES?

Mohamed El-Hadidi*, Pascal Weigold, Johannes Harter, Hans-Martin Krause, Andreas Kappler, Andreas Gattinger, Sebastian Behrens and Daniel H. Huson

Bioinformatics Research Group, Center for Informatics Sciences (CIS), Nile University, 26th of July Corridor, Sheikh Zayed City, Giza, Egypt. Email: melhadidi@nu.edu.eg

Recent advances in Next Generation Sequencing (NGS) technologies has boosted the field of metagenomics and metatranscriptomics significantly, which enabled researchers to sequence thousands of microbial genomes isolated directly from the soil. Careful choice of sequencing strategy (including shotgun and targeted sequencing) followed by bioinformatic analysis pipelines fine-tuning can help in revealing interesting biological phenomena associated with soil microbiomes.

In this survey, we have performed different studies based on whole genome shotgun, targeted metagenomics and targeted metatranscriptomics to study the microbial role in degrading pollution-causing compounds and gases in either microcosm or field experiments. This collective study illustrates different sequencing and bioinformatic analysis approach applied, explaining the rationale behind why it was chosen and how it was successful in explaining and providing insights into a wide range of biological questions concerning with soil metagenomics. In one study [1], we identified and quantified the relative abundance of genes associated with (de)halogenation in different soil depths based on whole genome shotgun sequencing approach. Moreover, in another study, targeted metagenomics sequencing was applied to identify microbial species carrying genes capable of reducing the toxic nitrous oxide and converting it to nitrogen in a microcosm experiment [2] and field experiment across a vegetation period of Zea mays L [3]. Additionally, it was shown that biochar amendment to soil increases the abundance of microbial species carrying genes for nitrous oxide reduction significantly (Fig. 1). While metatranscriptomics is a powerful tool to monitor the expression of genes across time, we implemented targeted metatranscriptomics approaches [4] to monitor the abundance of microbial species that actively expressing genes for nitrous oxide reduction (Fig. 2). Although we have used different sequencing strategies and bioinformatics analysis pipelines, MEGAN 6 software was used extensively as a core analysis component in all previously illustrated studies for taxonomic binning and functional classification.

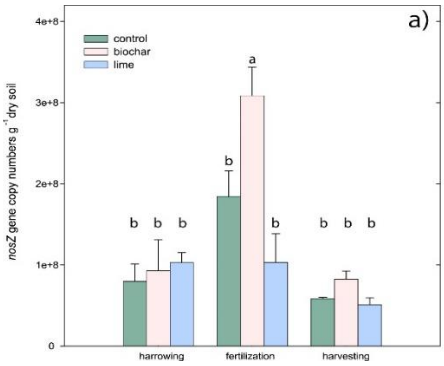


Figure 1: Increase in N_2O reducing genes (*nosZ* genes)

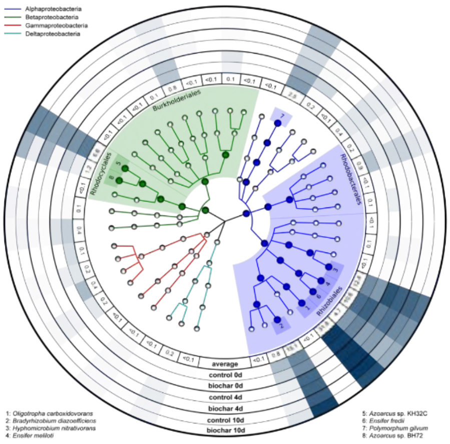
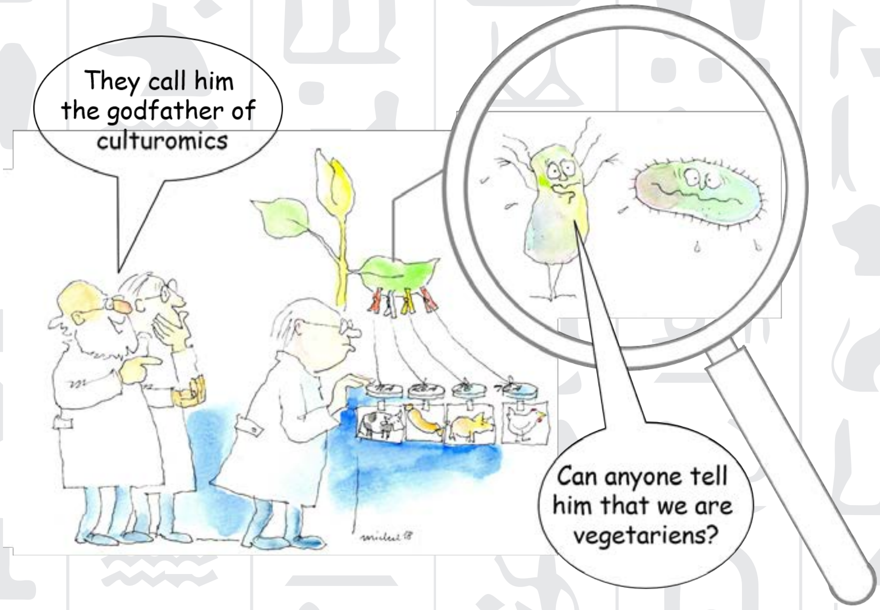


Figure 2: Taxonomic classification of bacterial species expressing *nosZ* N_2O reducing genes

References:
[1] Pascal Weigold et al. 2016. Scientific Reports 6, 28958.
[2] Johannes Harter et al. 2016. Science of The Total Environment 562, 379-390.
[3] Hans-Martin Krause et al. 2018. Soil Biology and Biochemistry. 119, 143-151.
[4] Johannes Harter et al. 2017. Scientific Reports 7 (1), 3338.



Session 2

Recent progress in culture-dependent techniques towards the application of culturomics to the plant microbiome research

Chairpersons: Barbara Reinhold-Hurek and Jihyun Kim

PHYTOPLASMA CULTIVATION: PROBLEMS AND SOLUTIONS

Nicoletta Contaldo and Assunta Bertaccini

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Over fifty years had elapsed since Doi’s publication (1967) on the presence of mycoplasma-like organisms (MLO) now known as phytoplasmas in the sieve tubes of plants showing hormone imbalance and malformations. These phloem-limited bacteria are transmitted by plant sap-feeding insects and are classified based on molecular analysis techniques on the 16S ribosomal gene at the level of the '*Candidatus*' genus (Bertaccini et al., 2018). The few scattered evidences of cultivation possibility were recently confirmed by using media based on those successfully used for human and animal mycoplasma cultivation.

The support of complex artificial media allow to achieve the isolation and growth of phytoplasmas from infected plant material, both maintained in micropropagation and sampled in the fields (Contaldo et al., 2012, 2016). A method for the isolation, purification and biochemical characterization of selected phytoplasma strains was established (Figs. 1 and 2). Colony morphology, PCR and sequencing analyses confirmed the isolation of phytoplasmas from different ribosomal groups from different infected plant species.

Results of biochemical tests including urea and arginine hydrolysis, and glucose fermentation indicated that the isolated phytoplasmas under study were urea negative and glucose and arginine positive. Different combinations of antibiotics, carbon sources and NaCl concentrations were visually evaluated for the degree of turbidity of the liquid media, due to the presence of contaminating micro-organisms and / or symbionts, and by the ability to form phytoplasma-like colonies in agar. To confirm the results obtained from visual observation and optical microscopy, selected colonies with characteristic morphology were subjected to PCR analysis followed by RFLP and / or sequencing, which confirmed the phytoplasma presence and identity. The phytoplasma isolation and biological characterization are relevant for disease management and containment measures to contrast their epidemics.

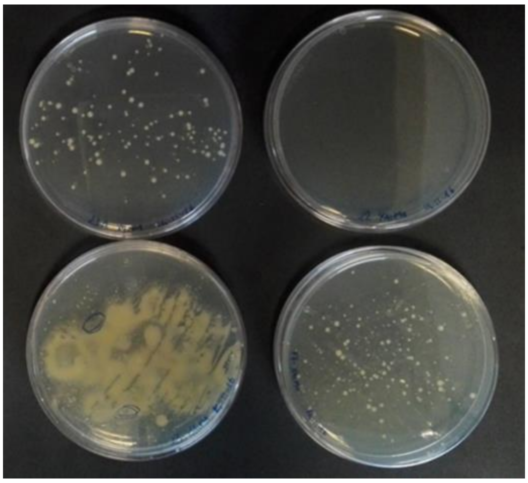

Figure 1. Isolation and purification steps from field collected plant samples symptomatic (1-4) asymptomatic (2-3).

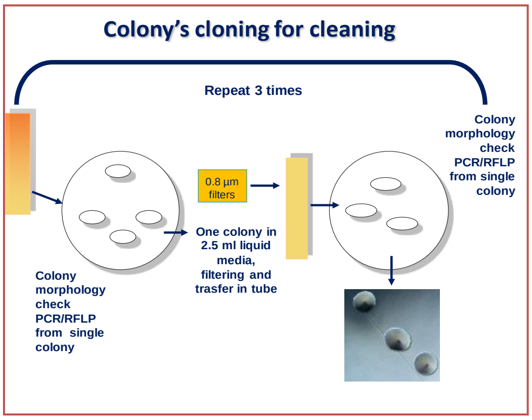

Figure 2. Schematic representation of phytoplasma purification procedure.

Bertaccini et al. 2018. Phytoplasmas: Plant Pathogenic Bacteria I. Springer, 1-30 pp.
Doi et al. 1967. Ann. Phytopath. Soc. Japan 33: 259
Contaldo et al. 2012. Phytopath. Mediterranea 51: 607
Contaldo et al. 2016. J. Microbiol. Methods 127: 105

CULTURING THE PLANT MICROBIOME: THE ERA OF ARTIFICIAL MEDIA IS ABOUT TO END AND THE AGE OF PLANT-ONLY-BASED CULTURE MEDIA HAD DAWNED

Hegazi NA*, Sarhan MS, Youssef HH, Hamza MA, Fayez M, and Ruppel S.
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Classical artificial/synthetic culture media appeared to seriously undermine *in vitro* cultivation of members of environmental microbiomes, and hinder their accessibility in pure cultures. **Metagenomic sequencing** revealed that the majority of microbes in nature have no representatives among the cultured organisms.

During the last two decades innovative culturing media and conditions were developed. It appeared that uncultivable microorganisms could be brought into pure cultures by: a) mimicking their natural environments to providing specific nutritional matrices with longer incubation; b) simulating **in situ microenvironments**; e.g. encapsulating cells in **gel microdroplets**, cultivating within **diffusion chambers** that later developed into **isolation chip (ichip)**; c, cultivating single-cells on **microfluidic streak plates.** Such new approaches are not limited to the plant microbiome, but further explored with the human gut microbiome, where the combinations of multiple culture media and culture conditions together with the extensive use of MALDI-TOF-MS were developed into what is known as high throughput **culturomics.**

It is still important to develop new cultivation techniques and protocols suitable for pure cultivation under *in situ* environmental conditions while manipulating simple operations. Therefore, we introduced the **plant-only-based culture media** in a number of preparations and applications to improve cultivability of the plant microbiome. Through a series of publications, we proved the challenge of the media and provided answers to the following specific questions, to be discussed throughout the presentation.

1. Can plant materials as such, without any amendments, support *in vitro* cultivability of representatives of rhizobacterial isolates? [1]
2. Will the plant-only-based culture media provide good biomass production of varying isolates of rhizobacteria? [2]
3. Do the plant-only-based culture media increase cultivability of *in situ* populations of rhizobacteria, with different community composition? [3]

4, 5. Will it be possible to make the proposed plant-only-based culture media more practical and easy to apply, and to what extent do the plant-fed rhizobacterial communities differ from the chemically-fed ones? [4]

6. Do the plant-only-teabag culture media extend cultivability and diversity to reveal the not-yet-cultured/hard-to-culture bacteria? [5]
7. Are the plant-only-based culture media competent in enriching uncultivable candidate bacterial divisions/phyla? [6]

In conclusion, the future use of plant-only-based culture media will support and extend the cultivability of the plant microbiome to reveal their unexplored communities of the uncultivable candidate bacterial divisions/phyla; revise the long-established information on the ecology/physiology of the plant microbiome, and help to combine metagenomics to metatranscriptomics, -metabolomics and -phenomics. It will also aid in applying culturomics approaches and high throughput cultivation strategies.

[1]Nour, et al. 2012. J. Ad. Res. 3, 35.; [2] Mourad et al. 2018. Microbes Environ. 33: 40; [3]Youssef, et al. 2016. J. Ad. Res. 7, 305.; [4]Sarhan, et al.2016. Physiol. Plant., 15,403; [5]Hegazi, et al. 2017. PLoS ONE 12: e0180424. DOI: 10.1371/journal.pone.0180424; [6] *Sarhan, et al. 2018.Microbes & Environ. in press.*

Nabil Hegazi acknowledges the continuous support of Alexander von Humboldt Stiftung and DAAD, and the close cooperation of Leibniz-Institute IGZ throughout the on-going research projects

LEAF SURFACE MICROBIAL ECOLOGY AT THE BACTERIAL INDIVIDUAL RESOLUTION

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Leaf surfaces are home to diverse bacterial communities. Within these communities, every individual cell perceives its unique environment and responds accordingly. In this presentation, I will assume the perspective of individual bacteria in an attempt to describe how the spatially heterogeneous leaf surface determines the fate of bacterial colonisers (Remus-Emsermann and Schlechter 2018).

Based on previous research that investigated the role of chance in successful colonisation of leaves (Remus-Emsermann et al. 2012; Remus-Emsermann et al. 2013) and the study of spatial explicit distributions of natural bacterial communities (Remus-Emsermann et al. 2014), we recently initiated a project to investigate the dynamics and assembly processes within leaf-colonising bacterial communities. To that end, we are using a gnotobiotic system and make use of the model plant *Arabidopsis thaliana* as a host for a representative collection of bacterial strains. The employed bacteria are fluorescently labelled to facilitate their identification on leaves using fluorescence microscopy (Schlechter et al. in preparation). In combination with *in silico* and *in vitro* experiments to determine the metabolic overlap between the bacterial strains, we are testing how resource demands impact on co-occurrence on leaves and artificial leaves. Preliminary results suggest that resource overlap is a major driving factor of bacterial aggregation and segregation on plant leaves (Figure 1).

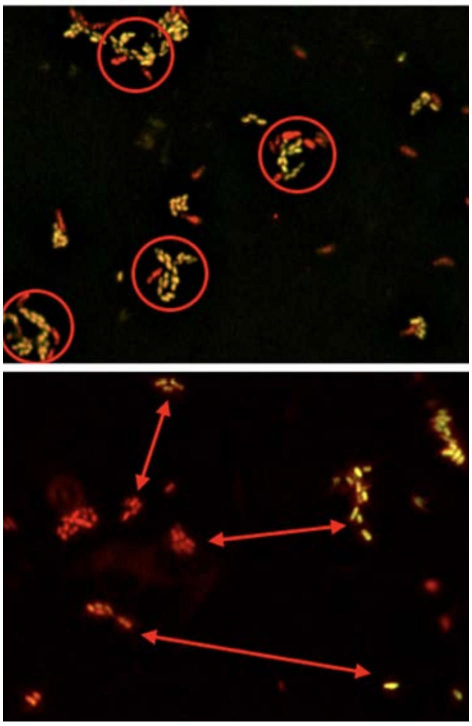

Figure 1 A) co-aggregation between a resource specialist and a resource generalist. B) Segregation between two resource generalists.

Remus-Emsermann and Schlechter 2018 New Phytologist
Remus-Emsermann et al. 2012 ISME J
Remus-Emsermann et al. 2013 Env Rep Rep, why not 2014, env. Micro?
Schlechter et al. in prep.

IN SITU-BASED CULTIVATION METHOD REVEALS HIGH PORTION OF PLANT BENEFICIAL BACTERIA IN THE RHIZOSPHERE OF WHEAT PLANTS (*Triticum aestivum* L.) GROWN IN CHILEAN ANDISOL

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Plant growth-promoting rhizobacteria (PGPR) are important for plant fitness and agriculture biotechnology. In this sense, PGPR isolation is based on standard cultivation which represent a minor portion (≤0.1%) of rhizobacteria in nature. On the last decade, *in situ* cultivation approaches (such as iChip application) have allowed the isolation and study of a wider diversity of environmental bacteria. Here, we built and used an iChip-like based microwell chamber (MC) to isolate bacteria from wheat rhizosphere (Figure 1). One g of rhizosphere soils were diluted (10^{-3}), inoculated into MC (agar 1.5%), placed in the rhizosphere of wheat seedlings in pots and then incubated for 2 months under greenhouse conditions. Samples from MC were randomly taken every week and transferred to NM-1 and LB agar plates.

A total of 206 isolates were recovery from MC and characterized by partial 16S rRNA gene sequencing. The most dominant isolates belong to Proteobacteria (70.4%), Firmicutes (24%), Actinobacteria (4%) and Bacteroidetes (1.5 %) phylla. At genus level, the majority of isolates were *Bacillus* and *Pseudomonas* (45) followed by *Stenotrophomonas* (35), *Delftia* (15), and *Geobacillus* (4). Among other plant-soil associated genera, one strain of *Acinetobacter*, *Agrobacterium*, *Bosea*, *Citrobacter*, *Flavobacterium*, *Lelliottia*, *Micrococcus*, *Serratia*, *Variovorax*, and *Xanthomonas* was also isolated (Figure 2).

According to megaBLAST, 82% of isolates (169) did not show significant similarity with plant or human pathogenic strains; 40% and 42% of isolates showed a high similarity with PGPR and biogeochemical cycle associated bacteria, respectively (Figure 3). The use of *in situ* cultivation in MC is an attractive strategy to isolate a higher diversity of rhizosphere to be studied their potential as PGPR for agriculture.

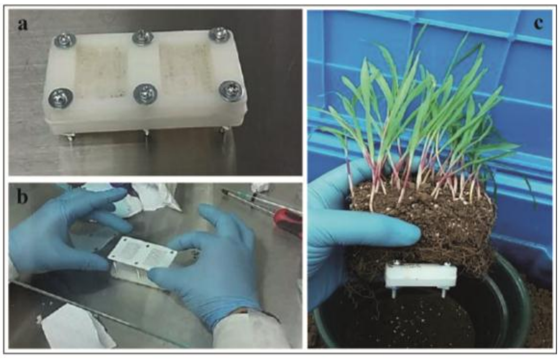

FIGURE 1 (a) Microwell chamber (MC) prototype; (b) MC inoculation with bacterial dilution from rhizosphere soil samples; (c) MC incubation in wheat plants rhizosphere.

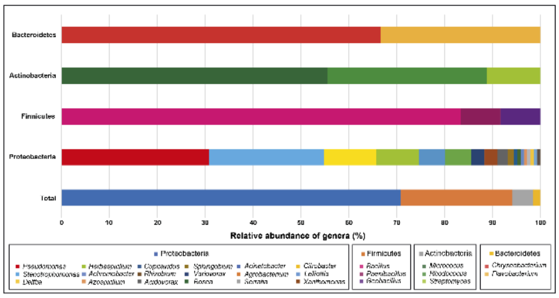

FIGURE 2 Taxonomic affiliation of isolates obtained from rhizosphere of wheat plants through *in situ* cultivation.

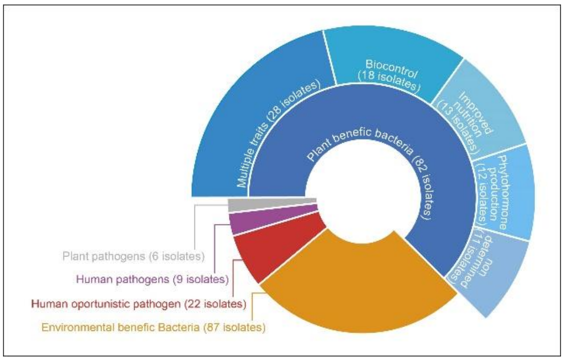

FIGURE 3 Characterization of *in situ* cultivated isolates according to NCBI megaBLAST tool.

Acknowledgements: FONDECYT (N° 11160112, 1160302, 1181050).

THE PLANT LEAF BY ITSELF IS AN EXCELLENT PAD-SUBSTRATE FOR *IN SITU* CULTIVATION OF THE PLANT MICROBIOME

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The ability to unveil previously uncultured members of environmental microbiomes will enhance our comprehensive understanding of microbial ecology and physiology, and explore new microbial resources. All recent methods of improving culturability are attempting to mimic naturally occurring conditions, e.g. low-substrate concentrations, addition of signaling compounds and *in situ* cultivation within diffusion chambers later developed into isolation chip (ichip). They all favor the growth of slower-growing *k*-strategy and oligotrophic species. It is still important to develop new cultivation techniques that are particularly suitable for pure cultivation under *in situ* environmental conditions while manipulating simple operations. For this purpose, we introduced the leaf-based culture media for *in situ* culturing members of the plant microbiome.

Basically, the plant leaf was used as a pad substrate to feed the over-laid inocula prepared for endophytes of different plant compartments, endo-rhizosphere and endo-phyllosphere The inocula are either directly applied to the sterilized leaf surface (LS) or filtered through membrane filters (MF) that were later laid onto the top of leaf surfaces (Fig. 1). Post a number of physical treatments, surfaces of the autoclaved leaves allowed exchange and access of multiple plant nutrients in their natural concentrations that supported excellent growth of introduced endophytes, very much quantitatively-comparable to standard culture media (R2A medium) (Fig. 1). Further, the leaf-based-culture media supported higher diversity of culturable populations that were extended to multiple genera of phyla *Alphaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria* not confined to only *Firmicutes* genera that dominated the growth on synthetic culture media of R2A (Fig. 2). It is evident that the leaf-based culture media furnish a milieu/ theatre of complex and diverse nutritional matrices suitable for the cultivation of plant-associated microorganisms – especially the endo-phyllosphere bacteria. The method by its simplicity is of promising future application towards the recovery of plant-niched microorganisms that are reluctant for cultivation because of inaccessibility and/or high concentrations of nutrients as well as those requiring syntrophic partners or interspecific or intraspecific interactions.

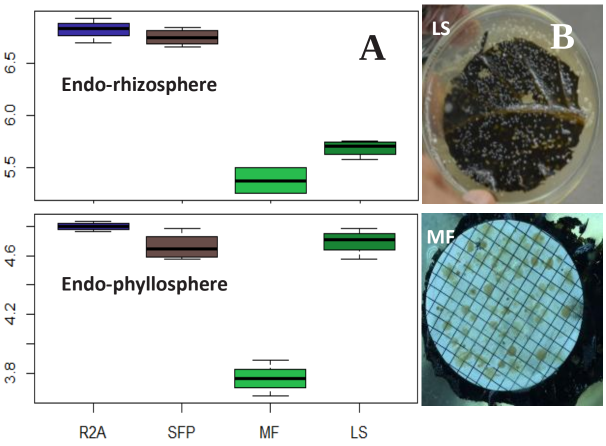

Fig1. A: ANOVA analysis of log CFUs counts recovered by the leaf-based culture media (LS, leaf-surface; MF, membrane filter) compared to the standard plant powder (SFP) and R2A culture media; B, colony development on either leaf culture media.

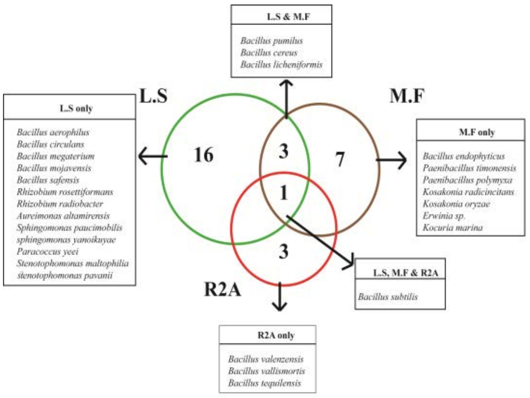

Fig2. Venn diagram at species level for all bacterial isolates recovered on various tested culture media (LS, MF, R2A) displaying unique and overlapping species.

Keywords: Plant microbiome, Culturability, in situ cultivation, Plant leaf
***We acknowledge the close cooperation and support of IGZ throughout the research project**

PLANT SYRUPS ARE APPROPRIATE SUBSTRATES TO PREPARE PLANT-ONLY-BASED CULTURE MEDIA TO IMPROVE CULTIVABILITY OF ENDOPHYTIC PLANT MICROBIOME

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The cultivable portion of microbiota (< 10 %) is not representative of their total phylogenetic diversity. It appeared that the classical chemically synthetic culture media generated low diversity and did not support members of the not yet cultured communities. We herein proved that syrups, as well as powder teabags, of clover and wheat were nutritionally rich enough to supporting the *in vitro* growth of a consortium of pure isolates of *Bacillus licheniformis*, *Klebsialla oxytoca* and *Pseudomonas putida.* The tested plant based media were as efficient as the chemically synthetic R2A, diluted NA and CCM culture media. They support good growth of confined and non-slimy colonies, eliminating the over-riding of µ-colonies developed with prolonged incubation time. 16S rRNA gene sequence indicated that all of the 62 secured isolates , representing all tested culture media, fell into 5 distinct phyla; *Firmicutes* were the dominant (51.6 %) followed by *Alphaproteobacteri*a and *Gammaproteobacteria* (19.4 % of either) (Fig. 1A). Furthermore, we provide, for the first time, data on the abundance of culturable *Chryseobacterium* sp., *Cronobacter* sp, *Enterobacter* sp, *Escherichia* sp, *Kosakonia* sp, *Rhizobium* sp and *Tsukamurella* sp on the tested plant based media, but not on the standard R2A culture medium (Fig. 1B). In general, the introduced culturing methodology is recommended to increase cultivability and resolution of plant microbiomes.

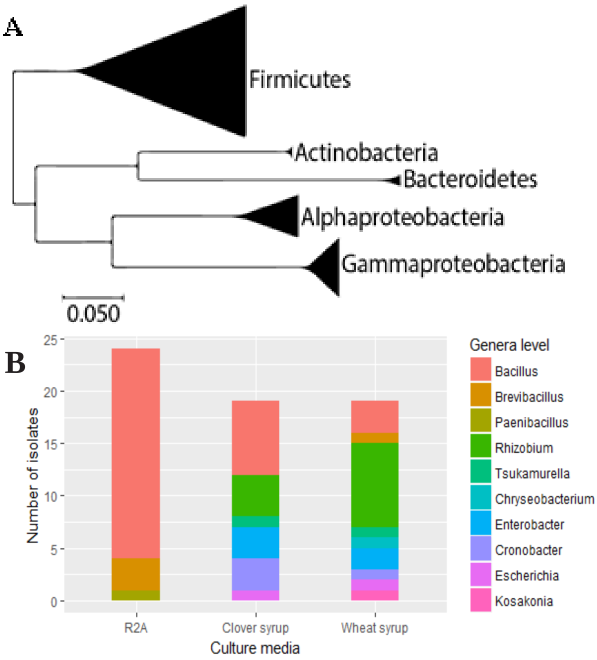

Figure 1. Distribution and taxonomic affiliation of 62 isolates based on 16S rRNA gene sequences. A, Maximum Likelihood phylogenetic tree of the major phyla associated to clover rhizosphere and phyllosphere; B, Relative abundance of isolates, obtained from various culture media at genus-level.

Keyword: Plant syrups, cultivability, plant microbiome, *Firmicutes*, *Alphaproteobacteri*a, *Gammaproteobacteria*,

***We acknowledge the** cooperation and support of IGZ throughout the research project.

Poster II-3

Plant Pellets: A Novel Strategy For Quick Preparation Of Plant-Only-Based Culture Media For Cultivation And Value-Added Biomass Production Of Rhizobacteria

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Keywords: Plant-only-based culture media; plant pellets; rhizobia; value-added biomass production

Developing culture media from cheap substrates with rich nutritional profiles is an important strategy towards the large-scale cultivation of rhizobacteria required to enhance crop productivity. We recently introduced plant-only-based culture media that sufficiently secured *in vitro* cultivation of a wide array of rhizobacteria. To facilitate the application of plant-only-based culture media for value-added biomass production of rhizobacteria, we have developed plant pellets (PP). They consisted of Egyptian clover powder (*Trifolium alexandrinum* L.) together with supplements of the agro-byproducts, glycerol and molasses. We assessed the potential of PP culture media, prepared from different concentrations of plant pellets (with or without containment in teabags), to *in vitro* grow and produce biomass of the model rhizobacterium, *Rhizobium leguminosarum*.

Based on measurements of optical density and dry weight, PP culture media did support good growth and biomass production of rhizobia similar to the recommended standard culture medium, Yeast-extract mannitol (YEM). Increasing the concentration of the PP and composite molasses significantly boosted the biomass levels of rhizobia. Notably, the teabagless preparations of PP culture media produced the highest level of biomass.

We conclude that PP are sufficient and efficient to feed and produce rhizobia biomass, labor-effective, and can be easily stored for continuous use. Assessing the ability of PP culture media prepared from different substrate combinations to cultivate various rhizobacteria will give insight on how plant pellets may be optimized for value-added biomass production.

Fig. 1. Developed plant pellets to feed rhizobacteria.

Table 1. Culture medium effect on dry weight (DW, g L^{-1}) and optical density (OD$_{600nm}$) of *R. leguminosarum* grown on all tested culture media after two weeks of incubation. Values are based on 2 biological and 3 technical replicates. "PP" denotes plant pellets; subscript "5 or 10% m" indicates the concentration of molasses used for PP development; subscript "ntb" indicates that the PP culture medium was prepared without the use of teabags.

Culture medium	**Max DW (g L^{-1})**	**Mean DW (g L^{-1})**	**Max OD$_{600nm}$**	**Mean OD$_{600nm}$**
YEM	4.25	3.29^{b} ± 0.35	0.81	0.47^{b} ± 0.07
PP$_{5\%m}$ 8 g L^{-1}	1.75	1.35^{d} ± 0.14	0.52	0.27^{c} ± 0.04
PP$_{5\%m}$ 16 g L^{-1}	2.14	1.67^{d} ± 0.15	0.77	0.25^{c} ± 0.06
PP$_{10\%m}$ 8 g L^{-1}	1.45	1.24^{d} ± 0.07	0.53	0.22^{c} ± 0.05
PP$_{10\%m}$ 16 g L^{-1}	2.91	2.32^{c} ± 0.17	1.11	0.52^{b} ± 0.11
PP$_{ntb10\%m}$ 8 g L^{-1}	5.29	4.34^{a} ± 0.36	1.29	0.58^{b} ± 0.12
PP$_{ntb10\%m}$ 16 g L^{-1}	5.60	4.23^{a} ± 0.38	2.29	0.78^{a} ± 0.19

*All PP culture media contain 8 ml L^{-1} glycerol.
*Different letters indicate different significance levels (ANOVA, $P \leq 0.05$).

*We acknowledge the close cooperation and support of Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany throughout the research project.

CLAY BEADS AS A LOW-TECH AND LOW-COST METHOD FOR LONG-TERM MICROBIAL PRESERVATION

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The maintenance of any living bacterial or fungal culture is not only a time-consuming issue but also needs constant concern lest valuable strains pass out. Standard microbial preservation methodologies, including lyophilization, cryopreservation, liquid-drying, and spray-drying, require advanced facilities and skills that may not be readily available for ordinary microbiology laboratories. Therefore, developing procedures that facilitate/accelerate this essential task are of great value.

Here, we introduce a practical and cheap methodology for long-term bacterial preservation, employing the simplest equipped laboratory facilities (Fig. 1). We used the conventional Egyptian clay-beads (used in classical pottery handcraft) for preserving and long-term maintenance of microorganisms. The efficiency of clay beads in bacterial preservation was assessed at three different storage temperatures (freezing at -20°C, cooling at 4°C and ambient) by measuring bacterial cell viability (CFUs counts) over time up to 42 month. In general, the strains *Bacillus circulans* (8E) and *Klebsiella oxytoca* (En11/2) survived at all the tested three different storage conditions (freezing, cooling, and ambient) for more than 10 months (Fig. 1 and 2). *Bacillus circulans* exhibited longer survivability, with less reduction in viable cell counts, at all three storage conditions throughout the storage period, while *Klebsiella oxytoca* maintained its viability better with freezing and cooling storage, compared with ambient temperature. Both strains remained pure without any detected contamination during storage period.

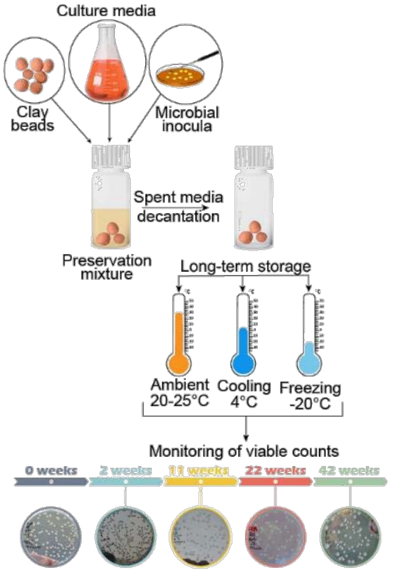

Fig. 1. Workflow of proposed and experimented microbial preservation in clay beads method.

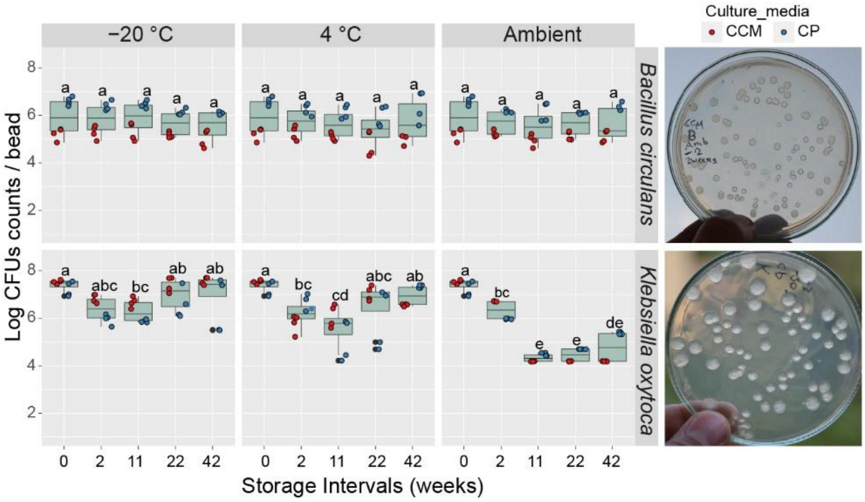

Fig. 2. Box plots of log viable counts (CFUs) of *B. circulans* and *K. oxytoca* recovered from clay beads kept, for up to 42 weeks, at different storage temperatures (-20°C, 4°C, 20-25 °C). Color code indicates two different culture media [standard culture media (CCM) and plant-only-based culture media (CP)] used initially to obtain biomass of tested microbes. Different letters indicate significant differences based on Tukey’s HSD ($P < 0.05$, n=8). The far right panel shows examples of CFUs counts.

PLANT-BASED CULTURE MEDIA DEVELOPED FOR IMPROVING CULTIVABILITY OF THE PLANT MICROBIOME ASSOCIATED TO THE HEAVY METAL-TOLERANT *Arthrocnemum macrostachyum*

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The land area adjacent to Manzala Lake is currently subjected to changes due to the intensive efforts made to overcome the problem of heavy metals pollution. This necessitates that a great attention should be given to record the biodiversity of natural tolerant microbiota having the ability of bioremediation.

To mimic the natural environment of the tested microbiome, the juice of the heavy metal-tolerant *Arthrocnemum macrostachyum* was used for culture media preparation and culturing the rhizosphere microbiome. Representative plant samples of the heavy metal-stressed environment around the lake, as well as relatively un-stressed ones, were tested for the *in vitro* cultivation and in situ recovery of the heavy metal-tolerant microbiome. Beside the chemically synthetic nutrient agar, the experimental design comprised using plant juice as such or diluted with the heavy metal-polluted water.

Colonies of rhizospheric microorganisms nicely developed on surface-inoculated agar plates prepared from crude and diluted juices. The great diversity and richness of associated culturable bacteria on lake water diluted-plant juice indicated their high tolerance to the heavy metals prevailing in the lake water (Fig1). Seventy-four isolates were secured and purified and examined for Al, Cr, Cu and Zn resistance. The majority of isolates did successfully withstand concentrations of 36.0, 16.0, 0.1 and 0.3 mM of Al, Cr, Cu, and Zn respectively. The most resistant seven isolates were further identified adopting the API microtube system which indicated that isolates belonged to: *Raoultella ornithinolytica, Providencia rettgeri, Burkholderia cepacian, Bacillus circulans and Bacillus lentus*. This particular group of isolates is good candidates, as bio-remediators, to restrict the level of heavy metal pollution of the lake as well as similar environments.

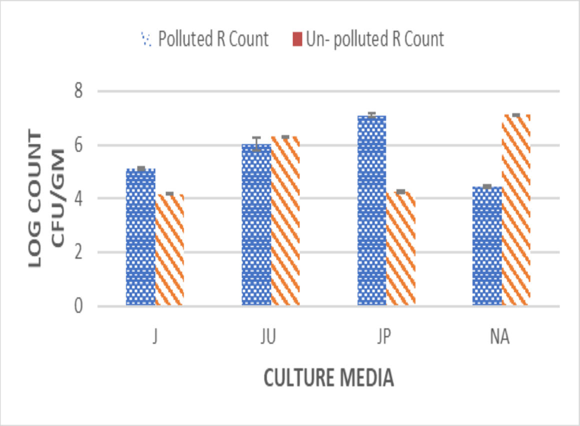

Fig. 1. Total cultuivable rhizospheric bacteria (CFUs) of *Arthrocnemum macrostachyum* developed on the plant juice culture media (J, juice diluted with distilled water; JU, juice diluted with un-polluted water; JP, juice diluted with polluted water) compared to the Nutrient agar (NA) medium.

WATER HYACINTH (*Eichhornia crassipes*) JUICES AND DEHYDRATED POWDER TEA BAGS ARE EFFECTIVE SUBSTRATES SUPPORTING GROWTH AND CULTURABILITY OF RHIZOBACTERIA

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Abstract— Traditional microbiological methods of cultivation recover 1-10 % of the total bacterial species from the different environments remaining the others "unculturable" in the laboratory. The majority of unculturables that do not grow on standard media are expected to play critical role in cycling of nutrients, synthesizing novel natural products and impacting the surrounding organisms and environment. Juice of the water hyacinth (*Eichhorina crassipes*), either crude or its successive dilutions (1:1, 1:10, 1:30, 1:50 v/v) supported the in vitro development of Bacillus megaterium, *Bacillus subtilis*, *Azotobacter chroococcum* and *Rhizobium leguminosarum* biovar. *phaseoli* with doubling times (23.1 – 63.0 min.) comparable, if not shorter, to those calculated using the standard chemically-synthetic culture media (nutrient, N-deficient mannitol and yeast extract agar media; 48.0 – 64.8 min.). Rhizospheric microorganisms of legume (broad bean, lentil) and non-legume (wheat, maize) plants nicely developed on surface-inoculated agar plates prepared from crude and diluted juices of the macrophyte. Tea bags packed with the dehydrated powders (5 and 10 g l-1) of the plant supported the in situ recoverability of total rhizobacteria in population densities (3 x107 - > 108 cfu.g-1) comparable, if not exceeded, to those enumerated on the recommended culture media. Morpho-physiological identification of a number of isolates developed on the plant juice and tea bag culture media revealed that they are not akin to those cultured on the chemically-synthetic culture media, possibly representing a portion of recommended media - unculturables. Hence, molecular tools are indispensable to discover those which have not yet been cultured. These new isolates might be of special prominence in relation to agricultural, industrial and medical aspects.

Keywords: Plant juices, tea bags, rhizobacteria, unculturables, water hyacinth.

CULTURING WITH NUTRIENT-FREE CULTURE MEDIA: ECHOING THE PLANT INOCULUM EFFECT INTO PLANT-ONLY-BASED CULTURE MEDIA

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Unculturable populations of the plant microbiome account for more than 90%, being still under shadow. Diluting culture media is among the several approaches to increase culturability; 1/10 diluted R2A and 1/100 strength nutrient agar successfully brought unculturable members of *Proteobacteria* and *Bacteroidetes* into cultures (Ishii *et al.*, 2017; Joo *et al.*, 2017). Such approaches are mimicking the nutritional matrices of natural environments, giving a chance for oligotrophs to emerge and favouring members of k-strategists over those of r-strategists.

Here, we investigated the sunflower phyllosphere microbiota through cultivation on almost nutrient-free culture media, i.e. water agar (WA), and plant-only-based culture media prepared from plant juices either autoclaved and diluted 1:20 (SAJ_{20}) or filtered and diluted 1:200 (SFJ_{200}), compared to the standard artificial R2A culture media (diluted 1:100, $R2A_{100}$). The rationale is to evaluate self-feeding of the existing phyllosphere microbiota exclusively on the traces of plant nutrients present in the inoculum of plant dilutions prepared for CFUs counting on surface-inoculated agar plates.

All tested culture media, including the nutrient-free water agar, gave comparable CFUs counts in the range of Log 3.2-3.9 g^{-1} leaf. Higher presence of microcolonies (µCFUs) was reported on the water agar as well as the very diluted plant-juice culture media, compared with diluted R2A. More than 100 single µ-colonies, developed on water agar and diluted sunflower juice, were re-generated/subcultured on both sunflower juice culture media and R2A. A total of 32 µ-colonies that grew only on sunflower juice but not R2A were further analysed and taxonomically identified by 16S rRNA gene sequencing. Alignment of successful 16S rRNA gene sequences showed the predominance (>90-100%) of 8 genera of the difficult-to-grow phyla of *Actinobacteria*, compared to the very low incidence of the big phyla of *Proteobacteria* and *Firmicutes* (Fig. 1). The most common genus (>60%) was *Curtobacterium*.

Our results pose the ultimate use of plant-only-based culture media containing very-diluted plant nutrients, available either in the plant inoculum and/or extensively-diluted plant juices, for the cultivation of the plant microbiome. It is a genuine and practical approach to mine for fastidious members of the plant microbiome and other environmental microbiomes, and to explore their composition/diversity.

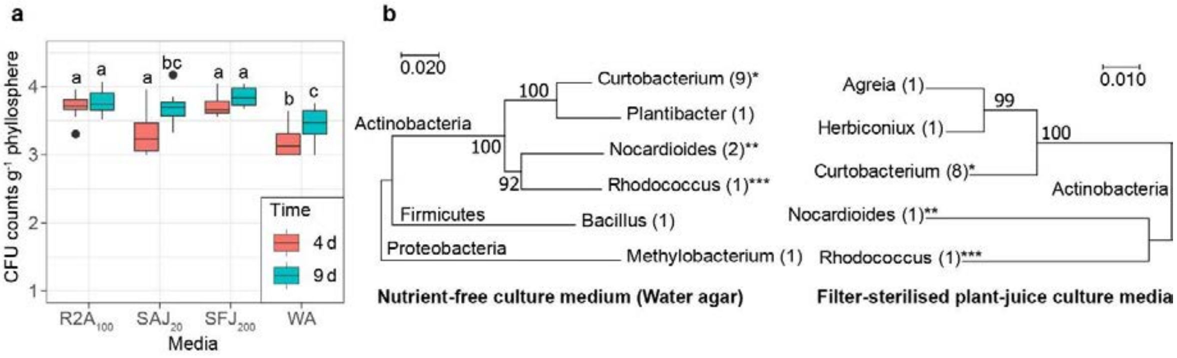

Fig. 1. CFUs counts and taxonomic affiliation of the picked µ-colonies. **a**, two-way ANOVA of CFUs counts developed on all tested culture media; **b,** Neighbour-Joining phylogenetic tree displaying the commonalities among the isolates of WA and SFJ_{200}, based on 16S rRNA gene sequencing, using bootstrap test with 1000 replicates. The evolutionary distances were computed using the Jukes-Cantor method. The analysis involved 27 nucleotide sequences for both groups.

References: Ishii *et al.* 2017. *Int. J. Syst. Evol. Microbiol.*67:1841-8; Joo *et al.* 2017. *Int. J. Syst. Evol. Microbiol.*67:532-6

Session 3

The plant microbiome: Diversity of fungi in plant compartments/spheres

Chairpersons: Philipp Franken and Kornelia Smalla

FUNCTIONAL DIVERSITY OF ROOT COLONIZING FUNGI

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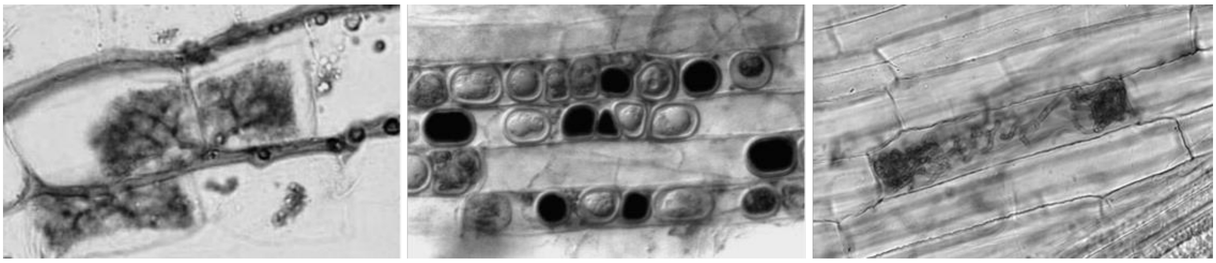
Plant roots are colonized by a numerous different types of microbes, whereof fungi have some unique features which make their interactions special. Most importantly, fungi show hyphal growth and these hyphae connect the surrounding of the plant root with the intradical space. In addition, fungal mycelia connect all roots in a given ecosystem and support the exchange of nutrients and signals among plants even of different species.

Among root-colonizing fungi, three major groups have been shown to be present in nearly all ecosystems. All fungi of the Glomeromycotina (Zygomycota) form arbuscular mycorrhizas; mutualistic symbioses found in 80% of all land plants. Sebacinales (Basidiomycota) includes fungi forming different types of mycorrhizal symbioses, but members of this order can be found as non-mycorrhizal endophytes in nearly all investigated roots. Dark Septate Endophytes is a form group among the Ascomycota. It has been thought that they are restricted to harsh environments, but nowadays it is clear that they colonize also roots of crops in anthropogenic ecosystems. In order to understand their contribution to plant performances and their potential use in plant production systems, it is necessary to elucidate the differences and similarities in their functions.

Functional diversity has been shown for these three groups of fungi concerning their contribution to plant nutrition from different types of resources. This is based on their diverse abilities to feed on organic matter, to solubilise inorganic fixed forms of mineral nutrients or to transport nutrients along their hyphae with different velocities.

Root-colonizing fungi are often applied to protect plants against biotic and abiotic stressors. Different results were obtained which could be also based on functional diversity. Here, we will summarize the current knowledge about the different mechanisms of the three groups of fungi concerning their abilities to antagonise pathogens, to locally and systemically induce resistance responses of the plant and to increase their antioxidative potential.



Structures of root colonizing fungi from left to right: Arbuscule of *Rhizoglomus irregulare* (Glomeromycotina), chlamydospores of *Serendipita indica* (Sebacinales), microsclerotium of *Cadophora spec.* (Dark Septate Endophytes)

INTERACTION OF *Medicago truncatula* WITH ARBUSCULAR MYCORRHIZAL FUNGI AND PATHOGENS – FUNCTION OF JASMONATES AND EARLY RESPONSES

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Phytohormones, among them jasmonic acid (JA) and its derivatives, are known to play a role in responses of plants to various stresses, but also in developmental processes[1]. Prominent examples for functions of JA are given by the plant interactions with beneficial microorganisms, such as arbuscular mycorrhizal (AM) fungi, but also interactions with nitrogen-fixing rhizobacteria are believed to be regulated by JAs. To get deeper insights, functional analyses by transgenic approaches were performed to investigate the role of JAs during the interaction between *Medicago truncatula* with an AM fungus, a nitrogen-fixing rhizobacterium or a pathogen. Transgenic roots exhibiting diminished JA levels showed a significant delay in AM, but a role of JA in the formation of nodules could not be demonstrated[2,3]. As wounding of plants leads to endogenous rise of JA, we addressed the question, whether wounding of leaves affects the biotic interactions of *M. truncatula* roots. Repeated wounding led to increased AM, whereas nodulation was not affected. In contrast, infection with the pathogen was reduced, which might be caused by JA-induced defense mechanisms, whereas positive effects of leaf wounding on AM point to JAs as positive regulators of AM formation[4,5].

In addition, we are interested in understanding the first steps of recognition and interaction of plants towards its close environment. We analyzed the very early reaction of *M. truncatula* towards an AM fungus or a pathogenic oomycete by root secretome and transcriptome analyses. Analysis of secondary metabolites in root exudates by untargeted LC-MS revealed major differences between exudate patterns of plant roots in contact with both microorganisms. Exploiting microarray analysis, a differentially expressed gene was identified that was upregulated after infection with the pathogen and encodes a putative sesquiterpene synthase (MtTPS10). Heterologous expression of *MtTPS10* in yeast led to production of a blend of sesquiterpenes and sesquiterpene alcohols with a major peak identified as himalachol. Moreover, plants carrying a *Tnt1*-insertion in the *MtTPS10* gene (*Mttps10*) lacked the emission of sesquiterpenes upon infection. Compared to wild type plants, *Mttps10* plants showed higher susceptibility to pathogen infection. Our data suggest that production of sesquiterpenes in roots by MtTPS10 plays a previously unrecognized role in defense response of *M. truncatula*.

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Kosakonia radicincitans DSM 16656^T HAS A SIGNIFICANT IMPACT ON THE TOMATO MICROBIOME

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The recent worldwide discovery of plant growth-promoting (PGP) *Kosakonia radicincitans* in a large variety of crop plants suggests that this species confers significant influence on plants, both in terms of yield increase and product quality improvement. We compared the genome of type strain DSM 16656 to other fully sequenced genomes of *K. radicincitans* and closely related bacterial taxa, and found that all to date known members of *K. radicincitans* exhibit genomic features that are absent from other bacteria (1). One of these features is an additional type VI secretion system (T6SS). T6SSs are supposed to be important for optimal fitness during plant colonization either for coping with the plant response or with the resident microbiota (2). To test our hypothesis that the three distinct type T6SSs enable *K. radicincitans* to exert significant influence on other bacteria competing for the same ecological niche, we performed a PhyloChip analysis. Two weeks after inoculating DSM 16656 into leaves and roots of young tomato plants the bacterial and archaeal community composition was significantly affected.

Knowing about the potential of DSM 16656 to “manipulate” the plant microbiome, we are now studying the interaction of *K. radicincitans* DSM 16656 and plant-specific microbial keystone species, as this will provide important insight into the stability of plant-associated microbial hubs (3).

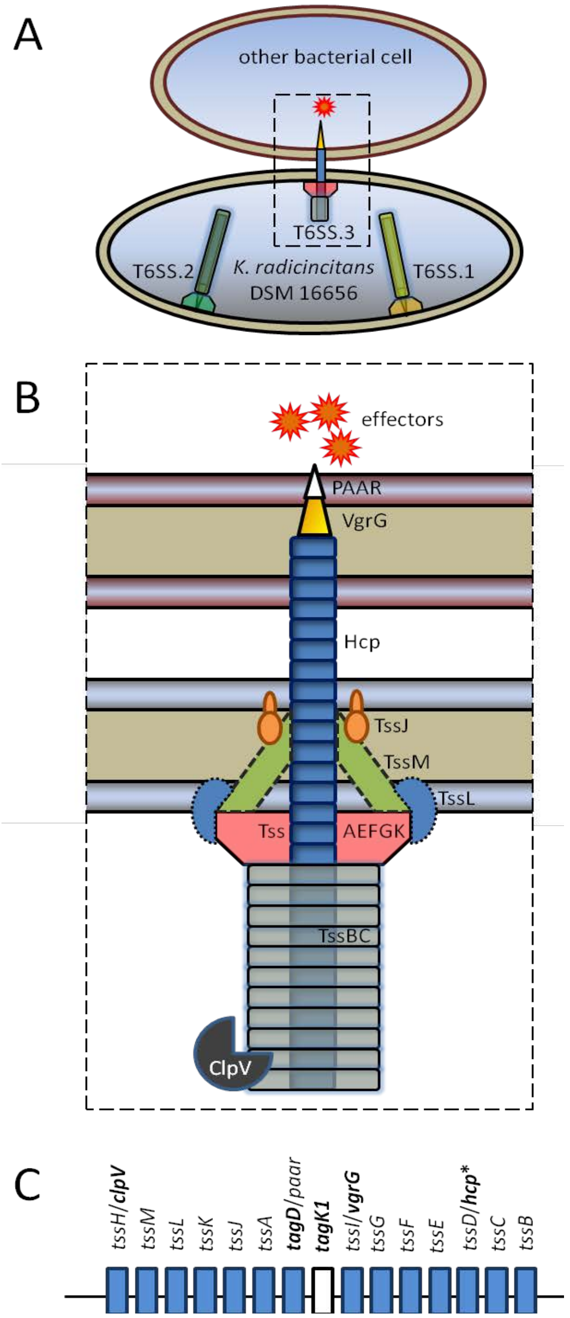

Figure 1: Type VI secretion systems (T6SSs) of *K. radicincitans* DSM 16656. (A) DSM 16656 carries three T6SS gene clusters; (B) General composition of T6SSs; (C) *K. radicincitans*-specific gene cluster T6SS.3.

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EFFECT OF COINOCULATION OF *Rhizoglomus irregulare*, AND HYPHAE ATTACHED PHOSPHATE SOLUBILIZING BACTERIA ON *Solanum lycopersicum*

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Sustainable agricultural practices are needed to improve plant yield and to solve the global crises of optimal food production in the coming years without further detrimental impact on the environment. Arbuscular mycorrhizal fungi share a symbiotic relationship with majority of terrestrial plants, playing a key role in improving availability of nutrients and water uptake of plants. AM fungi are barely able to solubilize phosphate in significant amounts, but can aid in the transfer of P from the soil to the plants. It is therefore important studying the interaction between P-solubilizing bacteria and AM fungi, to unravel if the bacteria can improve P nutrition of plants by AMF. It is further important to decipher these interactions in order to exploit the full potential of these microorganisms as bioinoculants. One approach for enhancing the effects of such bioinoculants could be co-formulations.

The aim of the present study is to isolate phosphate solubilizing bacteria strongly attached to the hyphae of *Rhizoglomus irregulare* using a two compartment pot system (a root compartment and a hyphal compartment), which were separated by a 30µm nylon mesh through which AMF hyphae could pass but not the plant roots. *Allium ampeloprasum* (Leek) was used as the host plant inoculated with *R. irregularre*.

A total of 128 bacteria were isolated, out of which 12 showed stable phosphate solubilizing activity. Finally, three bacteria belonging to the Pseudomonas family, namely PSB1, PSB11 and PSB18 showed highest potential for inorganic and organic phosphate mobilization.

The three bacteria were further evaluated for their functional characteristics, for interaction with the AM fungus and for their impact as single or co-inoculations on plant growth promoting traits. We tested the effect of co-inoculants of the bacterial fungal consortia on *Solanum lycopersicum* and found that plants inoculated with the combination of fungus and bacteria had significantly higher root biomass and improved P uptake compared to the single inoculations.

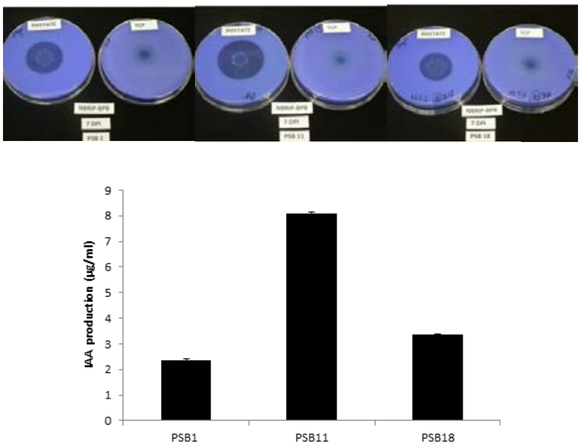

Fig 1: (a) Phosphate solubilization (b) IAA production by PSB1, PSB11 and PSB18

Fig 2: Effect of co-inoculation of *Rhizoglomus irregulare* and PSB's on the growth of tomato plants and the increase in the root biomass.

Oral III-5

INTEGRATING MICROORGANISMS IN *Lithospermum erythrorhizon* PLANT PRODUCTION SYSTEMS TO ENHANCE PRODUCTION OF THE SECONDARY METABOLITE SHIKONIN

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Arbuscular mycorrhizal fungi and endophytic bacteria and fungi have shown yield enhancing and growth promoting effects in agricultural systems (Mitter *et al*. 2013). There is growing evidence that plant-associated microorganisms have a great impact on the chemical composition of their host plant (Brader *et al*. 2014). Also increases in production of bioactive ingredients of medicinal plants have been observed (Kilam *et al*. 2017).

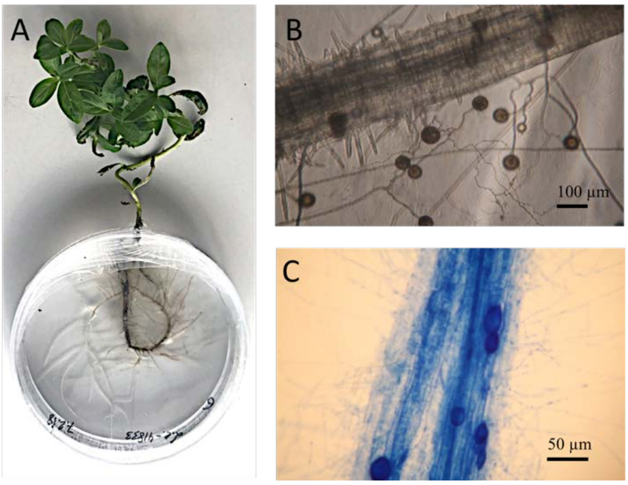

FIGURE 1. *Lithospermum erythrorhizon* can be mycorrhized by *Rhizophagus irregularis* strain MUCL 41833. (A) Plant and fungus are co-cultivated in a half-closed arbuscular mycorrhizal plant *in vitro* culture system. (B) Spores germinate and contact the roots 4 weeks after inoculation and (C) colonize the roots 8 weeks after inoculation.

The aim of this project is to improve the production of shikonin in the medicinal plant *Lithospermum erythrorhizon* by application of microorganisms. The naphthoquinone shikonin is a secondary metabolite of medicinal interest with antimicrobial activity. Shikonin production is known to be induced by fungal elicitors, methyl jasmonate, and ethylene. Arbuscular mycorrhizal fungi are known to modulate jasmonate mediated signalling. This effect will be tested for its efficiency to promote shikonin production under *in vitro*, glasshouse and field conditions.

In vitro cultures of *L. erythrorhizon* were successfully established and mycorrhized (FIGURE 1). Further *in vitro* studies are ongoing to quantify the induction of shikonin production by *Rhizophagus irregularis* strain MUCL 41833. Additional 11 mycorrhiza species and strains are tested for their effect on secondary metabolite production in a greenhouse trial.

GROWTH ENHANCEMENT OF WHEAT PLANT BY *NIGROSPORA SPHAERICA* AND *CURVULARIA LUNATA* ENDOPHYTIC FUNGI ISOLATED FROM *MELIA AZEDARACH* L.

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Melia azedarach L (Meliaceae), an exotic medicinal plant transported to Egypt from Asia, was chosen to isolate endophytic fungi from its leaves. The endophytic isolates were identified on the basis of morphological characters and molecular identification using18S rDNA. The isolates are available under GenBank accession numbers; MF113055 as ***Nigrospora sphaerica*** and MF113056 as ***Curvularia lunata***.

Numbers of laboratory tests were conducted to find out their potential to produce important plant growth hormones as Indole acetic acid (IAA) and solubilizing phosphorus. The tests indicated the ability of both endophytes to secret (IAA) but only ***N. sphaerica*** could solubilize phosphate. A pot experiment was conducted to evaluate endophytic isolates ability to enhance the growth of wheat (***Triticum aestivum***) plants. The endophytic fungi were dried and mixed with soil at 50, 100, 200 mg/ kg and sterilized wheat seeds were sown. Both endophytic fungi enhanced plant growth but ***N. spherica*** (MF113055) significantly increased shoot and root lengths, and fresh and dry weights in concentration dependent manner. ***Curvularia lunata*** (MF113056) was re-isolated from wheat leaves at the end of the experiment so it is a true endophyte had the ability to re-infect disinfected plants and can spread systemically from roots to leaves.

EVOLUTION OF LAND PLANTS AND THEIR PATHOGENS

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Current system of classification of life forms on earth recognizes acellular (viruses) and cellular forms. Cellular forms are divided into prokaryotes (bacteria) and eukaryotes. Eukaryotes include protests, as a basal group, from which, algae, plants, fungi and animals were evolved at different times in the long history of life on earth. From palaeo-botanical and morphological evidence and the analysis of DNA-based phylogenies, the first land plants (liverwort–like forms) were evolved from charophycean algae about 475 million years ago. Although fungi branches with animals in the tree of life, fungi are much older than plants and plant–fungal interactions are considered to be as old as the evolutionary period of higher plants (1). Even the colonization of land by plants is believed to be with the help of fungal partners and these associations date back to, the time period in which higher plants evolved. Fossils of fungi dated to that time resemble examples of putative parasitic plant–fungus associations (similar fungi probably were long established parasites of aquatic algae), but evidence of saprophytism is rare until much later (2). Beneficial plant–fungal interactions provide stability to both partners, but harmful interactions may result in host destabilization. Mycorrhizas are specialized soil fungi associated with plant roots in a symbiotic relationship (3).

Co-evolution is a reciprocally induced evolutionary process of two or more species in response to each other and the ecosystem. Co-evolution had been common in nature and many complex organisms require coevolved interactions to survive and reproduce, examples include mutualistic interactions such as plants and their pollinators, rhizopia and legumes, mycorrhizae roots and parasitic interactions such as plants and their pathogenic fungi. The complexity of co-evolution between plants and associated fungi has maintained a wide gap between the assumptions of formal models and the observed genetics and population dynamics. In particular, past work has typically assumed that only one or perhaps two genetic loci are involved in the co-evolution interaction but multi-locus genetics model involving effects of immigration by occasionally introducing a randomly chosen genotype was also proposed (4). More recent work, proposed a horizontal gene transfer (HGT) which facilitated the evolution of plant parasitic mechanisms that represent specific acquisitions to life as a plant parasite. The predicted functions of the HGT candidates may be aiding entry to plant cells, allowing efficient nutrient acquisition and leading to microbial proliferation in plant tissue (5). However, plant–fungal evolutionary interactions are by far more complex than previously thought, with not only a single fungal interactor, but rather a whole plant-associated microbiome (5). Much derails of the role of fungi as major interactors with plants, remains to be explored, especially regarding associations of bio-trophic fungal pathogens. In the era of the ‘omics’ and super resolution of information, we should take advantage of the associated genomics, transcriptomics, metagenomics and advanced microscopy tools to open up new avenues for exploring plant–microbe interactions.

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AM FUNGAL SYMBIOSIS WITH OKRA PLANTS AS AFFECTED BY SOIL MOISTURE CONDITIONS

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CO: Contributed equally to this work.

Aims This study investigated the species community and abundance of arbuscular mycorrhizal fungi (AMF) originating from an irrigated okra-field near Khartoum (Sudan) as affected by soil drought conditions in symbiosis with okra (*Abelmoschus esculentus* L.) plants.

Methods Prior to use in the glasshouse experiment, AMF strains from the field soil sample were first propagated on sorghum (*Sorghum bicolor* L.) and then, using the pot substrate and sorghum root fragments, applied to inoculate okra plants. After 4 weeks of growth the water content in the pot substrate was either maintained at 20% (W) or reduced to 10% (D). Plant biomass and nutrient uptake were determined and the presence of AMF strains (OTUs) in the roots and the pot substrate were analysed using next generation sequencing.

Results The AMF sequencing analysis revealed the highest abundances for representatives of the order *Glomerales* and *Diversisporales* and the total number of detected OTUs was similar for both the treatments W and D. While some AMF strains were similarly abundant irrespective of the treatment, we found a clear shift of abundance of distinct AMF strains in roots and substrate samples in the drought treatment. I.e., more sequences were read for *R. arabicus* at the expense of *Dominikia* sp. in treatment D compared with W. Plant total biomass was significantly reduced to 50%, also P concentration and total content of P in shoots were significantly lower in treatment D compared with W. In contrast higher shoot concentrations were found for N, K and Fe, while the concentration of Zn was sufficient in the shoot of both treatments.

Conclusions AMF strains were differently able to enforce their abundance in roots and rhizosphere of okra plants under dry substrate conditions. The availability of P but not of other elements was clearly limited in the substrate due to the drought treatment.

Image1. Root and soil sampling from field (Khartoum) (A), Microscopic image of AMF colonization in root samples (B), propagation of AMF from the field soil samples using sorghum plants (C) Okra plants from greenhouse experiment (D).

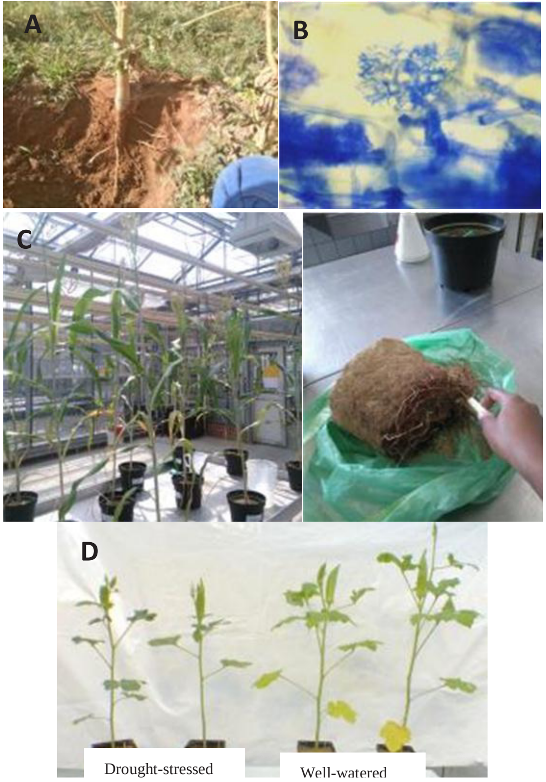

Figure2. Okra plant dry biomass (g) and AMF root colonization under well-watered and water-stressed conditions.

	Treatment		*P*-value
	W	D	
Total plant dw (g per plant)	23.6 b ± 1.6	10.1 a ± 0.6	**<0.001**
Shoot dw (g per plant)	19.6 b ± 2.5	8.5 a ± 0.7	**<0.001**
Root dw (g per plant)	4.0 b ± 0.8	1.6 a ± 0.4	**<0.001**
AMF colonised root length (%)	91.5 b ± 3.1	81.1 a ± 2.9	**<0.001**

INTERACTION BETWEEN PLANTS AND MICROORGANISMS: ANTIMICROBIAL POTENTIAL OF ESSENTIAL OILS AND SESQUITERPENES ISOLATED FROM EGYPTIAN PLANTS

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The essential oils have been isolated from 23 Egyptian plants by hydrodistillation using a Clevenger-type apparatus. The chemical compositions of the oils were analyzed using gas chromatography-mass spectrometry. In the first experiment, eight essential oils showed ***in vitro*** antifungal activity against ten phytopathogenic fungi with ***Mentha microphylla*** oil being the most effective one. When tested for the control of gray mold and soft rot in strawberry, the essential oils exhibited a strong reduction of disease incidences. In the second experiment, 18 essential oils were tested for their effects on mycelial growth inhibition and spore germination of fungi. Some of the tested oils caused remarkable inhibition of mycelial growth and spore germination of examined fungi. On the other hand, the isolated oils showed variable degree of antibacteabril activity against ***Agrobacterium tumefaciens*** and ***Erwinia carotovora*** var. ***carotovora***.

The chemistry and biological activity of two Egyptian plants, ***Magnolia grandiflora*** L. and ***Ambrosia maritima*** L. were investigated. Repeated column chromatography followed by crystallization led to isolate two sesquiterpene lactones (costunolide (**1**) and parthenolide(**2**)) from ***M. grandiflora*** and five pseudoguaianolide sesquiterpenes (neoambrosin (**4**), damsinic acid (**5**), damsin (**6**), ambrosin (**7**), and hymenin (**8**)) from ***A. maritima***. The structure (Fig.1) of the isolated compounds was identified by extensive spectroscopic analysis of ^{1}H NMR, ^{13}C NMR, MS and UV.

Among the isolated compounds (**1-3**), parthenolide (**2**) showed the highest antibacterial activity against food borne and plant pathogenic bacteria. In antifungal assay on plant pathogenic fungi, costunoilde (**1**) showed the strongest activity against three fungi, ***Nigrospora*** sp**.**, ***Rhizocotonia solani*** and ***Helminthosporium*** sp., with EC_{50} values of 0.48, 2.92 and 2.96 mg/L.

Compounds (**4-8**) inhibited the mycelial radial growth and spore germination of ***Botrytis cinerea*** and ***Fusarium oxysporum***. On the other hand, neoambrosin (**4**) showed the highest antibacterial activity among the tested sesquiterpenes with MIC values of 150 and 90 mg/L against ***A. tumefaciens*** and ***E. carotovora***, respectively.

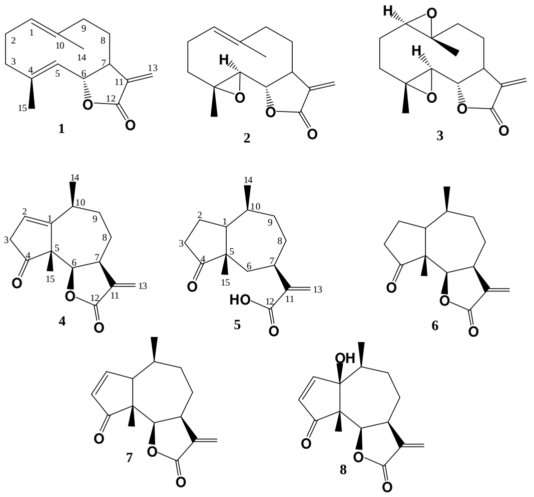

Figure 1. The Chemical structures of sesquiterpenes isolated from Egyptian plants.

STUDIES ON THE EFFECTS OF FUNGAL PATHOGENS ON NUTRITIONAL CONTENTS OF WATER MELON *(Citrullus lanatus* L.) IN JALINGO, TARABA STATE, NIGERIA

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ABSTRACT

Studies of fungi associated with the post-harvest fruit rot of Water Melon (*Citrullus lanatus* L.) and their effect on the sugar content was conducted in Yola through random sampling technique in three (3) markets of Yola North and Yola South area of Adamawa State (Jimeta Modern Market, Yola Market and Kasuwar Gwari). The sample size of 201 Water Melon was taken and five species of fungi were found to be associated with the fruit rots of Water Melon they are; *Aspergillus niger*, *Aspergillus parasiticus, Saccharomyces cerevisae, Aspergillus Fumigatus* and *Rhizopus Stolonifer*. The most commonly isolated organisms was *Aspergillus Parasiticus* (40%) followed by *Aspergillus fumigatus* and *Aspergillus niger (20%), Saccharomyces cerevisae* and *Rhizopus Stolonifer (10%)*. Analysis of sugar content level from Healthy (6.01%), Fresh (3.81%) and Disease Water Melon fruit (3.12%) indicate significant decrease in sugar content levels in the disease fruit than in the healthy and fresh fruits it shows that the fungal organisms are utilizing the sugar present in the fruit causing a massive decrease of the fruit sugar content. Statistically ($P < 0.05$) the results were significant with analysis of variance.

Keywords: Fungal Pathogens, *Citrullus lanatus*, Nutritional Content, Jalingo

Session 4

The plant microbiome: Diversity of bacteria in plant compartments/spheres

Chairpersons: Angela Sessitsch and Silke Ruppel

ASSESSMENT STRATEGIES OF STRUCTURAL AND FUNCTIONAL DIVERSITIES OF PLANT-ASSOCIATED BACTERIAL COMMUNITIES

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Bacteria associated with plants are of great importance, because of their relevance for an optimized plant development and yield, as well as health balance of plants during their whole growth cycle. In particular, specific bacteria, called plant growth promoting bacteria (PGPB), can provide plants with improved resistance towards abiotic and biotic stress situations. However, it is still a big challenge to assess plant-associated bacterial communities, including PGPBs, in a comprehensive way in the rhizosphere as well as surfaces and endo-spheres of roots, shoots, seeds and even pollen. The utmost challenge is to identify specific functional roles through which these bacteria interact with the plant hosts and contribute to its improved performance concerning nutrition and pathogen resistance. In this paper, examples of model and field studies will be reported. The important roles of new methodological developments for a detailed understanding of the plant microbiome in cultivation-based and in molecular and –omic investigations are also to be highlighted. In depth understanding of the bacteria-plant interactions can also be derived from direct *in situ* localization approaches, involving specific molecular or immuno-labelling protocols for target bacteria of interest. In combination with this newly accessible information about the presence and activity of specific bacterial populations, more focused and specifically adapted isolation attempts of bacteria with possible plant growth promoting potential from specific plant spheres can yield bacteria for agro-biotechnological applications.

More than 100 years ago, Lorenz Hiltner postulated the rhizosphere concept, highlighting the importance of microbe-plant interactions by the demonstration of high colonization densities of microbes in the rhizosphere, including the root surface, the surrounding soil and even root interior (1). Microbes residing in different plant micro-habitats can be localized nowadays with very high resolution in a quantifiable manner using e. g. high resolving laser scanning microscopic and image processing tools (2). Beyond the characterization of the bacterial communities from plant habitats based on DNA (metagenome), extracted RNA and meta-transcriptomics allows to decipher bacterial functions / genes expressed in plants (3, 4). Profound knowledge about beneficial PGPB-interactions and communication with their plant hosts and the plant microbiomes will be very valuable for the recently proposed phytobiome concept towards integrated sustainable agricultural management (5).

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MICROBIAL COMMUNITIES AT THE ROOT-SOIL INTERFACE

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Soil quality and fertility are essential components for bio-economic productivity. Roots have a profound impact on rhizosphere soil and consequentially on soil characteristics and microbiomes. Catch crops can be an option to preserve or even improve soil productivity because of their effect on soil fertility and health. A long-term field experiment of catch cropping (CATCHY) was established in two different locations in Northern and Southern Germany. Single catch crops (white mustard, Egyptian clover, phacelia and bristle oat), catch crop mixtures (a mixture of the above and a commercial mixture) and main crops (wheat and maize) have been grown. To investigate how catch crops can affect the microbial diversity and particularly the microbial nitrogen cycling communities, we are studying first the short-term effect of different catch crop mixtures on the microbiomes associated with soils and roots. We compared these microbiomes with wheat plants, representing the microbial community before a catch crop treatment. Roots, rhizosphere and bulk soils were collected from representative samples of wheat plants, fallow treatment (without roots), and three catch crop variants. The microbial population structure was studied using rRNA and *nifH* amplicon sequencing of DNA extracts obtained from root, rhizosphere and bulk soils of wheat plants, catch crops, and the following main crop maize. The OTU-based analysis showed that the main driver for community composition appeared to be the location, likely due to soil differences. However, microbial communities differed with plant species and could thus lead to different functional traits after catch crop cultivation. Plants enriched a specific microbiome in rhizosphere soil and roots, selecting for particular bacteria and excluding others, leading to specific communities at the soil-root interface in contrast to bulk soil. Data on the maize microbial community suggest that different catch cropping techniques may take effect on the main crop already after one cropping cycle.

Reinhold-Hurek et al., 2015. Annu. Rev. Phytopathol. 53: 403

SOW THE SEEDS - FOR A SUSTAINABLE AGRICULTURE

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The plant microbiome is crucial for growth and health (1). While the microbiome in the rhizo- and phyllosphere is well studied in this context, seeds were often considered as reservoir for pathogens or as free of microorganisms. However, recent studies reveal an unexpected microbial diversity and abundance within seeds. Moreover, they showed a vertical transmission of a core microbiome (1, 2). Soil type, climate, geography and plant genotype were identified as main drivers of the seed microbiota (2.4). Within millennia of domestication, crops and their seeds underwent traceably many different adaptive trends, allowing rapid speciation and divergence that lead to phenotypic and genotypic distinction to their wild ancestors. During those dynamics, also the microbiomes have secretly co-evolved with the host plants (2). Interestingly, bacterial endophytes represent the main symbiotic component within seeds. In seeds of Alpine plants, which we studied to understand the native seed-microbe interaction, they form a beneficial network with archaea. In contrast, fungi represent an antagonistic component (unpublished results). In addition, domestication and intensive agricultural management changed the seed microbiota. This resulted in diversity loss, which has consequences for *one* health issues (5). To restore microbial diversity, bacterial seed treatments can be designed. They can be developed based on the rich diversity of seeds of wild ancestors or other native plants. Those seed biologicals can be harnessed for sustainable agricultural approaches by improving stress tolerance and resilience of modern crops (3).

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THE RHIZOSPHERE MICROBIOME - KEY FOR PLANT HEALTH AND GROWTH

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The rhizosphere microbiome - referring to bacteria, archaea, fungi, and oomycetes is assumed to play a key role for plant health and growth but also for soil properties and various ecosystem services. Recently, we could show the influence of agricultural management on the rhizosphere microbiome of lettuce and barley as well as on plant growth and health via expression studies of plant defence related genes. Improving plant growth and health by means of soil amendments (biofumigation) or inoculants could be further strategies to influence plant growth and health directly or via microbiome and metabolome shifts. A better understanding of the rhizosphere microbiome and its interactions with the soil and plant is therefore key to a sustainable plant production and will ensure food safety.

The last decade brought about remarkable methodological progress for rhizosphere microbiome studies through new cultivation-independent methods (DNA/RNA- or microscopy-based analysis) but also in other disciplines of rhizosphere research (e.g. physics, chemistry, hydrology). Despite the enormous progress made in the availability of novel approaches that allow a cultivation-independent study of the rhizosphere microbiome, our present understanding of the factors that modulate the rhizosphere (microbiome, water availability, nutrient cycling, soil properties) is, however, still very fragmented and limited. Therefore a new DFG financed priority program SPP2089 was recently launched aiming at unraveling the spatio-temporal pattern in the rhizosphere that brings together the different aspects, processes and scales of rhizosphere research under a common framework in order to utilize this knowledge for improving our understanding of resilience of agricultural soils.

LEGUMES STRATEGICALLY CONTROL THEIR MICROBIAL ENVIRONMENT.

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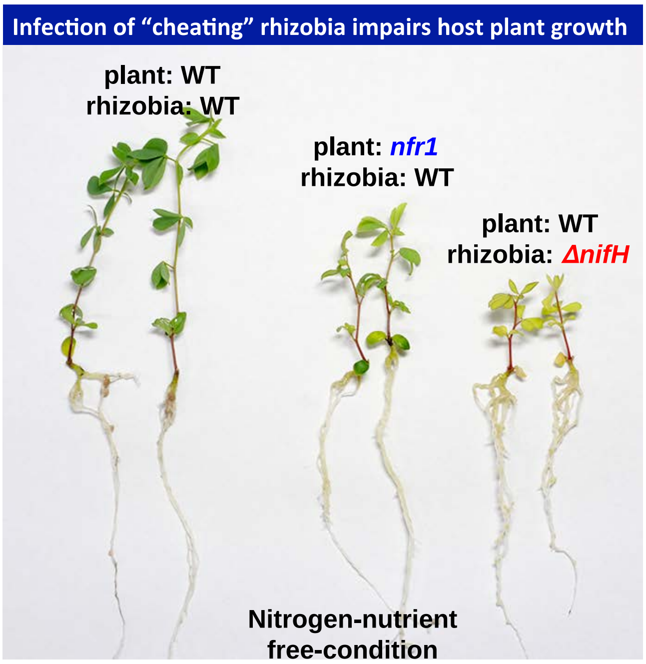
Plants are living with many kinds and amounts of microbes often including pathogenic and/or beneficial microbes in natural environment. Recent studies indicated that both plants and surrounding microbial community, termed 'microbiota', influence each other on their growth and survival. Friendly micobiota improves the host plant growth and agricultural productivity whereas hostile microbiota impairs it. Rhizobia are representative "friendly" soil microbes, which infect inside root cells of leguminous plants and fix atmospheric nitrogen to ammonia and supply it to host plants in exchange for a supply of photosynthate. Owing to the symbiosis, legumes can grow vigorously even under nitrogen-nutrient deficient condition.

Host legumes recognize their symbiotic partners by Nod factors (NFs), symbiotic signal molecules secreted from rhizobia. However, NFs cannot guarantee the rhizobial nitrogen fixation because the genes involved in NF-synthesis or -secretion are independent from nitrogen fixation activity. How host legumes manage a risk of partner's cheating is largely unclear. I will discuss the host mechanism for this problem based on recent findings.



A KEYSTONE SPECIES IN THE RHIZOSPHERE MICROBIOME OF TOMATO RESISTANT TO BACTERIAL WILT

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Bacterial wilt is a severe plant disease caused by the soil-borne bacterium *Ralstonia solanacearum*. Although plant disease resistance is known to be mediated by its own immune system, plant-associated microorganisms may play an important role. We initiated a whole metagenomic analysis of the rhizosphere communities of two tomato cultivars, Hawaii 7996 and Moneymaker, that are either resistant or susceptible to bacterial wilt, respectively. Taxonomic analysis of the 16S rDNA reads, which have been extracted from the whole metagenome data, revealed that the proportion of *Flavobacteriia* is higher in the rhizosphere of Hawaii 7996 than in the rhizosphere of Moneymaker, whereas the proportion of *Betaproteobacteria* is higher in Moneymaker than in Hawaii 7996. Through phylogenetic binning, we were able to reconstruct the genome of a novel uncultured *Flavobacteriaceae* bacterium, designated TRM1, from the metagenomic sequences of Hawaii 7996. Based on the genome information, we successfully isolated the corresponding bacterium that contributes to the disease resistance. Our study illuminates that microbiome structures of the rhizospheres are distinct between the two cultivars and underscores the pivotal role that the native microbiome plays in protecting plants from infection.

[Financial support from the Strategic Initiative for Microbiomes in Agriculture and Food, the National Research Foundation (NRF-2014M3C9A33068822 and NRF-2011-0017670), the Next-Generation BioGreen 21 Program (PJ008201), and BK21 PLUS]

MONITORING THE MICROBIAL QUALITY AND OF FIELD-GROWN FENUGREEK (*TRIGONELLA FOENUM GRAECUM L.*) INCLUDING PATHOGENIC *E.COLI* UNDER EGYPTIAN FARMING CONDITIONS [a]

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Fenugreek (*Trigonella foenum graecum L.*) is a traditional Egyptian legume crop, widely used as sprouts with meals for high nutritional value while seeds are used as a spice, condiment and in the production of pharmaceuticals. As vegetables, it may be microbiologically- contaminated, at various stages of production. There are several types of *E. coli* strains that may cause human gastro-intestinal illness. Ruminants are regarded as the natural reservoirs of enterohemorrhagic *E. coli* (EHEC) strain O157:H7 which excreted in large numbers in surrounding environments. Due to the low infective dose and severity of hemorrhagic disease, *E. coli* O157:H7 is considered as emergence pathogen.

During 2015/2016, the agricultural environment of fenugreek fields in Upper Egypt (Menia and Fayoum governorates) were examined for microbial quality and possible contamination with pathogenic *E.coli* (*E.coli* 0157:H7 and 0104:H4 strains). In terms of indicators of pollution, total bacterial counts at 20°C and 35 °C, total coliforms TC, faecal coliforms FC and % of FC to TC, the plant-soil environment of Fayoum was of higher microbiological quality compared to Menia. The field irrigation waters showed FC density lower than 10 cells/ml, and their HBC values were less than 10^5 CFU ml^{-1}, a level that is acceptable by WHO for irrigation water sources. A total of 100 isolates was obtained from original and enriched samples of both environments and subjected to biochemical tests beside the ability to grow and ferment sorbitol in CT-SMAC medium. All belonged to Proteobacteria, Gammaproteobacteria, Enterobacteriales, Enterobacteriacea, and representing the following genera: *Enterobacter* spp. (71) *Klebsiella* spp. (9), *Pantoea* spp. (7), *Pseudomonas aeruginosa* (5), *Pseudomonas aeruginosa* (5), *Serratia ficaria* (5), *S. liquefaciens* (3), *Providencia alcalifaciens* (2), *Escherichia* spp. (2), *Raoultella* spp. (1), *S. odorifera* (1) *and S.marcescens (1).* This indicates the prevalence of *Enterobater* spp. with its representing species *E.sakazaki* (44), *E. cloacae* (17) and *E. aerogenes* (4),). Based on biochemical reactions and API profiles, clustering analysis clearly separated representatives of Enterobacteriaceae into sub-groups according to their environmental origin, manure, soil or plant rhizosphere; an indication of the environment effect (Fig. 1). The rhizosphere effect of 9–16 fold increases was demonstrated. The two strains of *E.coli* 1 isolated from waters of both examined areas, and 51 representative isolates were selected for further characterization and identification. This included 16S rDNA gene sequencing (accession numbers KX036525 to KX036557). All tested strains proved to be negative for the pathogenic marker genes (stx1, stx2, eae and ehly), analyzed using TaqMan multiplex qPCR and serotyping.

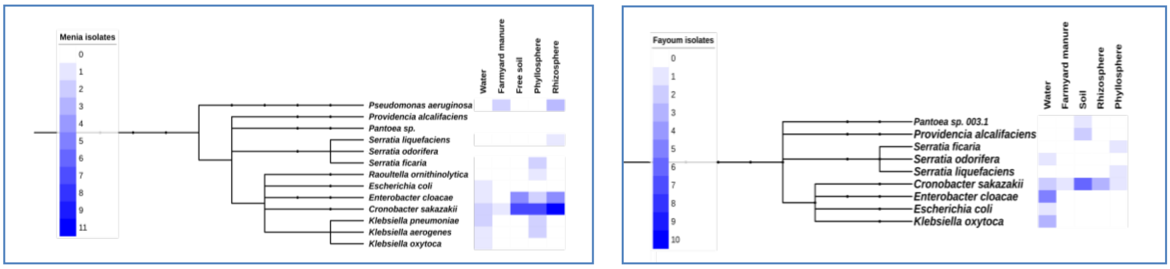

Fig.1: Environmental samples of Menia and Fayoum fields: Clustering analysis clearly separated representatives of Enterobacteriaceae into sub-groups according to their environmental origin, namely water, soil, manure, plant rhizosphere as well as phyllosphere.

Keywords: Fenugreek plants (*Trigonella foenum graecum L.*), Pathogenic *E.coli*, *E.coli* 0157:H7 and 0104:H4 strains, Enterobacteriacea

[a] **The presented research work was funded by the German-Egyptian Research Fund (GERF-STDF 5032)**

COMMUNITY STRUCTURE AND PLANT GROWTH-PROMOTING POTENTIAL OF CULTIVABLE BACTERIA ISOLATED FROM CAMEROON SOIL

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Exploiting native plant growth-promoting rhizobacteria (PGPR) in Cameroonian agro-ecosystems provides a means to improve plant–microbe interactions that may enhance ecosystem sustainability and agricultural productivity in an environmentally eco-friendly way. Consequently, we aimed to investigate the community structure and functional PGPR diversity of maize grown in Cameroon in order to select the best bacterial candidates for agriculture under specific environmental conditions found in Cameroon. Native bacteria isolated from Cameroon maize rhizosphere soil were identified by partial 16S rRNA gene sequencing and screened for traits particularly relevant for Cameroon low-fertility soil conditions, such as their abilities to tolerate high concentrations of salt, to solubilize phosphate on agar plates supplied with tricalcium phosphate, hydroxyapatite and rock phosphates (RP) of different origins (Malian, Cameroonian, Algerian, Mexican and Moroccan RP) as well as their potential to fix atmospheric nitrogen and to produce siderophores.

A total of 143 bacterial isolates were identified and assigned to 20 genera. Based on their *in vitro* characterization, 88.1% were salt tolerant at 2% NaCl, but only 16.8% could tolerate 8% NaCl, 50.4% solubilized phosphate regardless the type of phosphate source, 10.5% possessed the *nifH* gene and 19.6% produced siderophores. Among the different inorganic phosphate sources tested, tricalcium phosphate with 46.2% was the phosphate source most easily solubilized whereas Moroccan RP with only 2.8% was the most recalcitrant. Six isolates affiliated to the most abundant genera identified, *Bacillus* and *Arthrobacter*, carrying multiple or single PGP traits were selected and their effect on growth and phosphorous (P) uptake of tomato plants was investigated in greenhouse under different combinations of P and salt stresses. In general, two bacterial strains composing of all tested PGP traits showed best plant growth-promoting results under all growth conditions.

Our results indicate the potential of using native bacterial strains to enhance plant growth under specific stress conditions in Cameroon. Finally, their performance under field conditions should be assessed before being recommended for commercial applications.

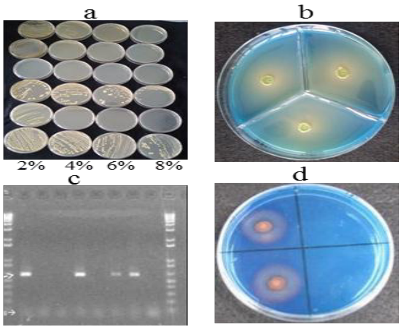

Fig.1. *In vitro* characterization of bacterial strains: (a) salinity tolerance at different NaCl concentrations, (b) phosphate solubizing activity on plate, (c) nitrogen fixing ability by *nifH* gene detection and (d) siderophores production activity on plate.

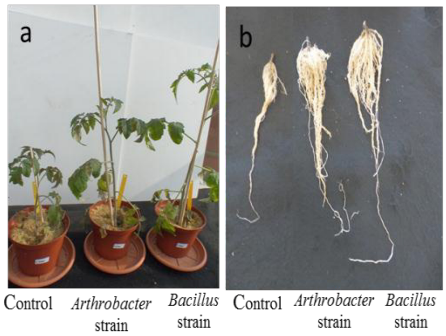

Fig.2. Effects of *Arthrobacter* and *Bacillus* strains compared to the negative control on shoot (a) and root (b) of tomato plants grown in greenhouse.

PLANT INDUCED CHANGES IN SOIL MICROBIAL COMMUNITIES

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Plants and microbes that inhabit the soil live in close association (Berendsen et al., 2012). In addition to being an anchor and a nutrient uptake system for the plant, root serves also as a signaling platform between the plant and its surroundings (Chaparro et al., 2012). The rhizosphere, a part of the soil where microbial communities are influenced by plants, is one of the world's most densely populated areas containing up to 10^9 bacterial cells per gram. The composition of a particular microbial community can be governed by the host plant genome, most likely through the flow of organic compounds from root to the rhizosphere (rhizodeposition) (Sergaki et al., 2018). Such compounds, known as root exudates act as substrates and signals for microbes initiating a complex relationship between the plants and the microbiome (Chaparro et al., 2012). Therefore, soil microbial communities are shaped differently depending on the plant species (Sergaki et al., 2018). Elevated levels in species richness and diversity produce high functional redundancy within the soil microbiome, allowing it to quickly recover during stress (Chaparro et al., 2012).

Here, we explore the changes in the total microbial community in soil after the introduction of a plant host as well as the effect of air dried sewage sludge compost addition on the soil microbiome. Additionally, we explored *Salmonella enterica* persistence in soil and its impact on the soil microbiome. Sampling of the soil with or without the amendment occurred at three time points, i.e. 30 days prior to planting, on planting day and 35 days thereafter. Chinese cabbage (*Brassica rapa* L.) was chosen as the model plant. To gain insight in the soil microbiome change depending on the presence of the plant and soil amendment addition 16S rRNA gene fragment was amplified, sequenced and analyzed. The persistence analysis focused on the introduction of *Salmonella enterica* together with the soil amendment or *via* irrigation water.

There was no difference between the microbiome of soil samples without sewage sludge compost analyzed 30 days prior to planting and on planting day. On the other hand, the addition of sewage sludge compost, as shown by the samples analyzed at planting day, changed the soil microbiome. Furthermore, the same effect can be seen after 35 days. The main shift in the microbiome of both soil with or without sewage sludge compost occurred between planting day time point to 35 days of vegetation. Therefore, the change in soil microbiome composition was induced by the presence of the Chinese cabbage plant. The effect of the addition of sewage sludge compost is less pronounced at day 35 because samples without sewage sludge compost show higher variation in soil microbiome composition compared to samples with sewage sludge compost. Our results suggest that the addition of sewage sludge compost creates a more stable microbial community, less susceptible to the plant induced change. The presence of *Salmonella enetrica* has no impact on the relative structure of the microbiome. Although the persistence of *Salmonella enetrica* is influenced primarily by the incubation period the addition of sewage sludge changes the survival dynamics compared to control soils. The main difference between the two scenarios is in the soil and sewage sludge incubation period prior to the introduction of the plant host. In the scenario where *Salmonella enetrica* was introduced together with the sewage sludge it had an equal opportunity as other competitive microbes. In this scenario we see an enhanced *Salmonella enetrica* survival rate compared to control samples. In the scenario where the sewage sludge compost, and consequently a broader diversity of microbes, was added to the soil before the introduction of *Salmonella enetrica* we see a lower survival rate compared to the control sample.

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IMPACT OF BIOCHAR, BIOFERTILIZER (*Bacillus pumilus* TUAT-1) AND THEIR COMBINATION ON RHIZOSPHERE AND ROOT MICROBIAL COMMUNITY AND PLANT GROWTH IN RICE

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Biochar application has achieved a great deal of positive effects, in terms of soil fertility and crop yield, and also changes the soil microbial community. It has not yet been addressed if co-application of biochar with plant growth-promoting bacteria inoculant biofertilizer synergy effects on crop growth and microbial community in rhizosphere and root of host plant. We therefore performed in pot experiments here to evaluate the effects of biochar (BC) and *Bacillus pumilus* strain TUAT-1 biofertilizer (Bio) and their combination (BB) on growth, and microbial community of rhizosphere and rice root at 2 and 5 week after transplanting (WAT). At both growth stages, rice growth was improved with either of BC or Bio. At 2WAT, TUAT-1 was readily detected in rhizosphere soil with more abundance in BB than those of Bio alone, whereas 5 WAT it could not clearly detected using quantitative real-time PCR.

The metagenomic analysis showed that the relative abundances of the bacteria belonging to the phylum *Proteobacteria*, *Elusimicrobia* and *Actinobacteria* were higher in rhizosphere soil of BC, whereas the frequency of phylum *Bacteroidetes* were lower than those of control (CT) at 2WAT. These differences become smaller at 5WAT. Microbial community structure of root also changed due to biochar and biofertilizer. The phylum abundance of *Acidobacteria* and *Firmicutes* in root was higher in order of Bio>BB>BC>CT. The microbial diversity both rhizosphere and root increased due to Bio, BB and BC when compared with CT, and was higher in 5WAT than those of 2WAT. Overall our results demonstrate that either of biochar or biofertilizer could improve plant growth, affect rhizosphere and root microbial community structure depending on time of treatment duration.

This research was supported by grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (the special scheme project on regional developing strategy).

BIOLOGICAL CONTROL OF *Ralstonia solanacearum*: INSIGHTS INTO THE COMPLEX INTERACTION BETWEEN ANTAGONISTS, PATHOGEN AND THE HOST PLANT RHIZOSPHERE MICROBIOME

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Abstract

Ralstonia solanacearum (*Rs*, biovar2, race3) is an epidemic soil-borne phytopathogen. In this study, three greenhouse experiments with tomato plants were performed to evaluate the rhizocompetence of *in vitro* antagonists and their ability to control *Rs*. The population densities of *Rs* and antagonists were estimated by selective plating and in total community DNA by means of real-time PCR and *fliC* gene PCR-Southern blot hybridization. *Bacillus vallismortis* (B63) and *Pseudomonas brassicacearum* (AL2YTEN-142)-inoculated plants showed a pronounced delay or no disease symptoms and significantly decreased in *Rs* population compared to the non-inoculated plants. However, despite the absence of wilting symptoms *Rs* was detected in low numbers in the stem of tomato plants inoculated with AL2YTEN-142. Amplicon sequencing of 16S rRNA gene fragments amplified from total community DNA revealed pronounced treatment-dependent shifts in bacterial communities in the tomato rhizosphere. Most important, the strong reduction of *Rs* in the presence of the antagonists was confirmed and dynamic taxa in response to *Rs* or the inoculants were identified. Confocal laser scanning microscopy uncovered colonization patterns of the AL2YTEN-142. Gfp-positive cells were detected in lateral roots, root hairs and epidermal cells and within xylem vessels. Both inoculants hold great promise to control *Rs* under field conditions.

Key words: *Ralstonia solanacearum,* biocontrol, latent infection, *fliC,* amplicon sequencing

PLANT SPECIES DEPENDENT ENRICHMENT AND DIVERSITY OF INCP-1 PLASMIDS IN THE RHIZOSPHERE

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Horizontal gene transfer mediated by plasmids plays a circular role in the bacteria adaptation to the environmental stresses. Broad host range IncP-1 plasmids are assumed to be important vectors contributing to the dissemination of the antibiotic resistance and catabolic genes and their abundance is thought to be correlated with anthropogenic pollution. Recently, unexpected enrichment of IncP-1 plasmids carrying populations was observed in the rhizosphere of lettuce grown in non-polluted Diluvial sand soil compared to bulk soil. Here we aimed at elucidating the reasons behind the enriched relative abundance of IncP-1 plasmids in the rhizosphere of lettuce, as well as their potential enrichment in other plant species. IncP-1 plasmids relative abundance was found to be increased in the rhizosphere of lettuce and tomato plants and less so in potato plants, based on *korB* quantification by qPCR of total community DNA. Successful isolation of IncP-1 plasmids was achieved exploiting their ability to mobilize the IncQ plasmid pSM1890. Restriction patterns of plasmid DNA revealed a high diversity as 11, 10 and one different restriction patterns were observed for lettuce, tomato and potato, respectively. The vast majority belong to the IncP-1β subgroup. IS*1071* was detected in almost all IncP-1β plasmids except one. The results showed that the plant species influence the relative abundance and diversity of IncP-1 plasmids in the rhizosphere. Present study provides insights into the natural role of IncP-1 plasmids in bacterial adaptation in the rhizosphere.

Key words: Total community DNA, tomato rhizosphere, IncP-1 plasmids, PCR, hybridization

CRISPR LOCI AS POTENTIAL TOOL FOR *Azospirillum* TRACKING IN SOIL

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Studies on plant growth-promoting bacteria (PGPB) have significantly increased during last decade, but the most of reports (>80%) do not consider the colonization of inoculated PGPB, using tracking or monitoring approaches (Figure 1). The PGPB colonization have been assessed by reporter genes-, immune- and nucleic acid-based methodologies (Figure 2). Nucleic acid-based methodologies are based on different molecular markers allowing partial discrimination of the inoculated PGPB from indigenous bacteria. However, these methods are poor sensitive and/or expensive.

In a large percentage of bacteria and archaea, clustered repetitive interspaced short palindromic repeats (or CRISPR) has particular repetitive sites which has been found to be specific on certain PGPB strains (such as *Azospirillum* spp.). Therefore, the focus of this project was to develop an inexpensive and easy-to-use PGPB tracking method using CRISPR loci as molecular marker.

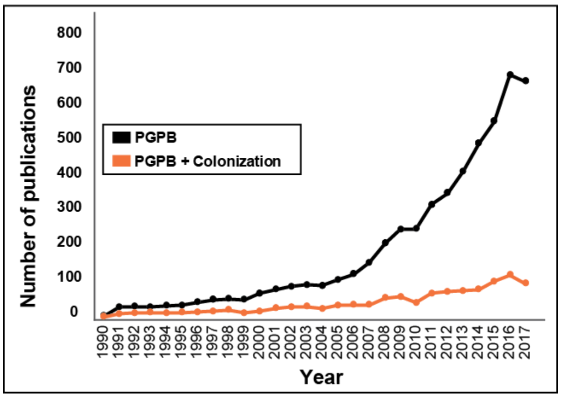


Figure 1. Studies on PGPB and PGPB + colonization as focus. Data obtained from Thompson Reuters Web of Knowledge (http://www.webofknowledge.com).

Through several genomic prospection procedures, primer design and end-point PCR standardization, we obtained strain specific primers sets for PGPB *Azospirillum* sp. B510 based on CRISPR repetitive sites on its genome. Specificity was confirmed with some collection (B506, B6) and native (J178 and J142) *Azospirillum* sp. strains. Then, we inoculated sterile and non-sterile substrates (2:1:1 peat:soil:perlite) with *Azospirillum* sp. B510 and its presence was observed by end-point PCR (Figure 3). Currently, experiments on rhizosphere and root endosphere of wheat are being conducted in order to assess the methodology effectiveness and sensitivity *in planta*. An inexpensive and easy-to-use tracking method for *Azospirillum* spp. could be highly value for agriculture in South America countries (such as Brazil and Argentine) where *Azospirillum* strains are extensively used as biofertilizers and monitored by certification laboratories.

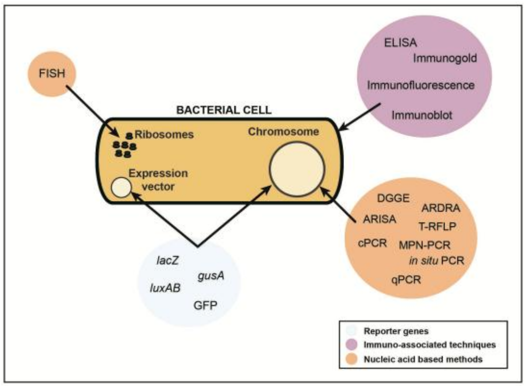


Figure 2. Methodologies commonly applied for PGPB tracking.

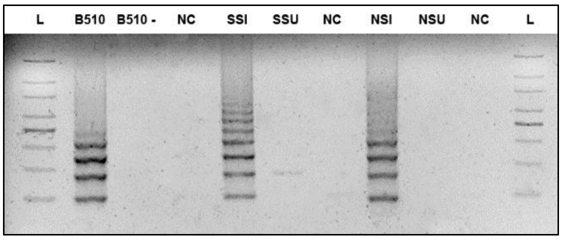
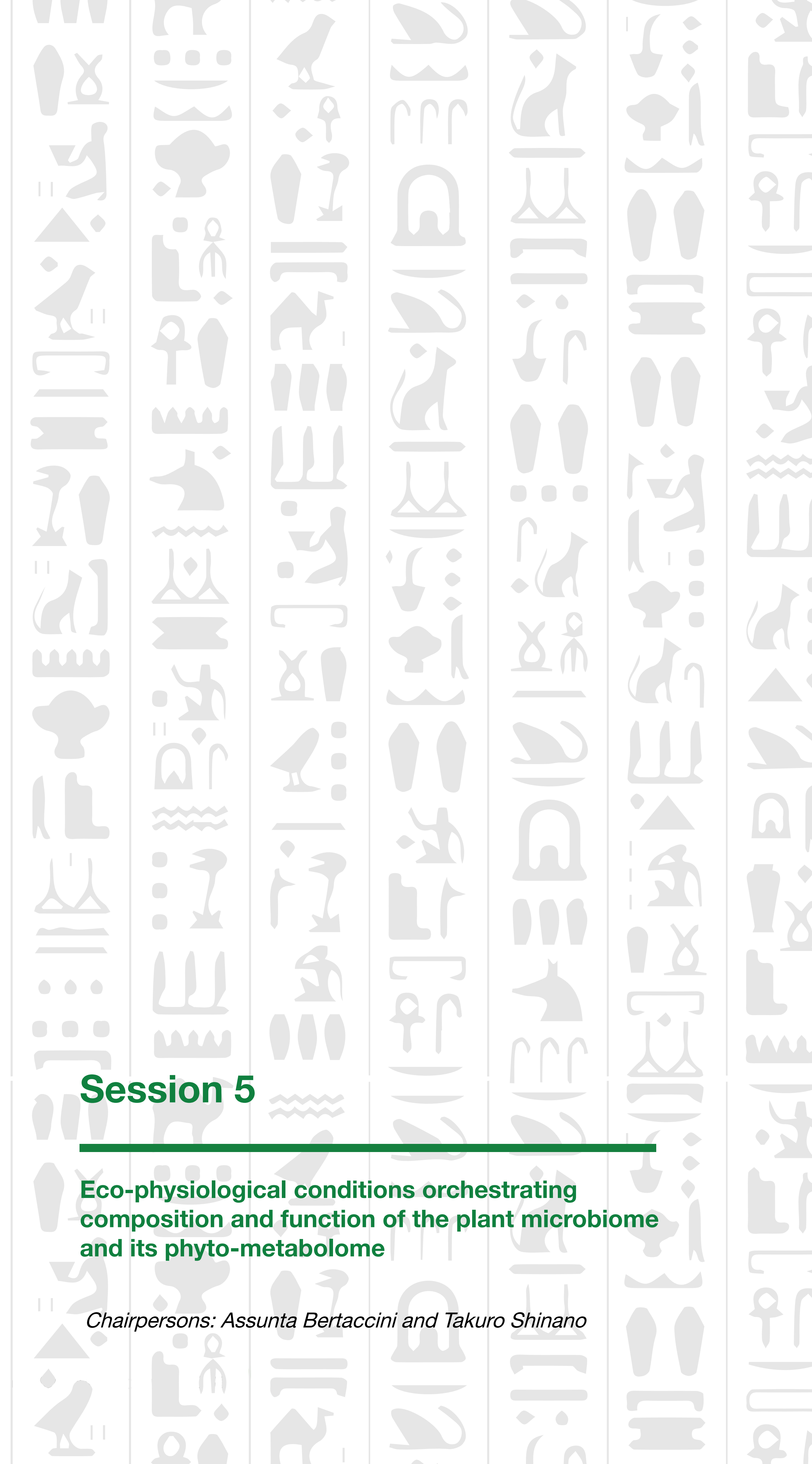


Figure 3. Banding pattern on agarose electrophoresis of by CRISPR-PCR method. **B510** (*Azospirillum* sp. B510, DNA positive control); **SSI** (sterile substrate inoculated); **NSI** (non-sterile substrate inoculated); **SSU** (sterile substrate uninoculated; **NSU** (non-sterile substrate uninoculated; **NC** (negative control). **L** = 100 bp Ladder.

Acknowledgements: FONDECYT (no. 1160302, 11160112, 1181050); CONICYT National Doctorate scholarship no. 21150794; and INACH code RT_02_16.



Session 5

Eco-physiological conditions orchestrating composition and function of the plant microbiome and its phyto-metabolome

Chairpersons: Assunta Bertaccini and Takuro Shinano

THE PLANT MICROBIOME AS A RESOURCE FOR OXIDATIVE BIOCATALYSTS

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Acetic acid bacteria (AAB) are aerobic, acidotolerant, fastidious bacteria often found associated with plant surfaces, fruits, nectar, plant saps, fruit juices etc. (see Ehrenreich & Liebl 2017). These bacteria have a rather unique metabolism in which substrate oxidation and carbon assimilation are often largely uncoupled processes. As an important mode of energy generation, membrane-bound dehydrogenases (mDHs) of AAB can oxidize various substrates such as sugars, polyols and alcohols, which occur abundantly in plant exudates or injured plant tissues. mDHs are oriented towards the outside of the cytoplasmic membrane and incompletely oxidize substrates without prior uptake into the cytoplasm. The mDH-supported metabolic lifestyle of AAB is interesting for biotechnology because mDHs can perform stereo- and regio-specific oxidation reactions which are often difficult to achieve with classical organic chemistry. Until recently, the engineering of AAB strains towards improved performance was hampered by the lack of the necessary genetic tools.

Efficient genetic tools for AAB, including e.g. conjugative gene transfer and selection/counterselection strategies were developed in our laboratory. These tools now allow site-specific modification of the genomes of AAB, such as the construction of *Gluconobacter oxydans* strains with deletions and insertions of mDH genes (Kostner et al. 2013, 2015). This targeted approach allows the analysis of the effects of such modifications on the physiology and whole-cell biocatalytic function of AAB. Also, the substrate specificities, physiological roles and application potential of selected mDHs can be studied in depth, and strains with specific oxidation capabilities can be tailored (Mientus et al. 2017). Finally, these tools and strains constructed with their aid are useful for the characterization of new mDHs from the cultured and uncultured biodiversity in nature (Peters et al. 2017). The microbiota on plant surfaces, in plant exsudates or other plant-derived fluids and their (meta)genomes can serve as a largely untapped resource for novel and biotechnologically interesting mDHs.

Ehrenreich & Liebl (2017) In König et al. (Eds) Biology of Microorganisms on Grapes, in Must and in Wine, Springer; Kostner et al. (2015) Appl Microbiol Biotechnol 99: 375; Kostner et al. (2013) Appl Microbiol Biotechnol 97: 8341; Mientus et al. (2017) Appl Microbiol Biotechnol 101: 3189; Peters et al. (2017) Appl Microbiol Biotechnol 101: 7901.

BACTERIAL QUORUM SENSING MOLECULES AS POSSIBLE WAY TO PROTECT CROP PLANTS AGAINST PLANT AND HUMAN PATHOGENS

Abhishek Shrestha, Ahmed Gomaa, Shimaa Adss, Gwendolin Wehner, Christoph Böttcher, Holger Heuer, Frank Ordon and Adam Schikora*

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During the cultivation of crop plants, priming for enhanced resistance using biocontrol agents is an efficient disease management strategy. It results in robust resistance and higher yield. The beneficial effects of the bacterial QS molecules, e.g. *N*-acyl homoserine lactones (AHL), on resistance and plant growth have been shown in different plants. Presence of AHL influences the transcriptional of various defense and growth-related genes and modifies the physiology of primed plants. Here, we present the effects of the AHL oxo-C14-HSL and AHL-producing bacteria on the priming capacity of barley plants. Barley is one of the most important crop worldwide and an enhanced resistance against pathogens, such as the powdery mildew causing fungus *Blumeria graminis*, is of high importance to agriculture. We demonstrate here that barley, primed with the beneficial bacterium *Ensifer meliloti*, expresses enhanced resistance against *B. graminis*. We show also that the capacity to induce priming varies among different barley cultivars. This suggests that appropriate genetic equipment is required in order to induce AHL-priming, at the same time it bears the potential to use this genetic feature for new breeding approaches. We further show that priming for enhanced resistance in barley involves stronger activation of the barley ortholog of the MPK6 kinase, regulation of defense-related *PR1* and *PR17b* genes and remodeling of the chemical composition of the cell wall. Noticeable was the stronger accumulation of lignin upon priming after chitin challenge. Interestingly, the global metabolomic changes in barley during priming are rather subtle and specific. The use of biologicals or beneficial bacteria represents therefore a good strategy for sustainable plant protection measures and opens new opportunities for breeding approaches.

PLANT SPECIALIZED METABOLITES IN THE RHIZOSPHERE: DYNAMICS AND FUNCTIONS IN BIOLOGICAL COMMUNICATION

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Plants synthesize a wide variety (200,000-1,000,000) of low molecular weight compounds, which function in plant defenses against both biotic and abiotic stresses. These compounds have been described as “specialized” rather than “secondary” metabolites. Specialized metabolite is synthesized by a few plant families or a few species, suggesting adaptive advantages for these plants to acquire the ability to synthesize specialized metabolites. Part of specialized metabolites functions in the rhizosphere, a small region around the roots, defined as the area affected by plant roots. Around 10-40 % of photosynthates including both primary and specialized metabolites are shown to be secreted into the rhizosphere. Despite of the accumulation evidence that plant specialized metabolites are important in the biolocigal communication between plants and microbes in the rhizosphere, there still remain largely unknown at the molecular level. The aim of our research is to elucidate the dynamics and function of plant specialized metabolites in the rhizosphere in order to gain the clear picture of the rhizosphere. We used isoflavone as a model to analyze the rhizosphere dynamics of plant specialized metabolites. Isoflavones are a class of flavonoid found predominantly in legume plants. Isoflavones act as a signal molecule to induce the expression of rhizobial nod genes. It has also recently been shown that isoflavones modulate the microbial communities. We analyzed the secretion of isoflavones into the rhizosphere in both hydroponic culture and field. Isoflavone secretion is higher during the vegetative stages than during the reproductive stages. The dynamics of isoflavones were simulated using the analysis of isoflavone decomposition and distribution and then validated using the rhizobox study. In addition, changes of bacterial communities were analyzed using the artificial rhizosphere condition.

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APPROACH TO INVESTIGATE THE ROOT AND MICROORGANISM INTERACTION BY ‘RHIZOMICS’

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Rhizosphere is designated as a soil area where the plant root interacts directly by using secreting compounds. We have been tried to apply proteome analysis in the rhizosphere by using aseptic rice cultivation in hydroculture. About 1 to 2 µg protein was collected from the solution and subjected to the proteome analysis by using nano LC-MS/MS. More than 100 different protesin were identified and about half of the proteins were categorized as PR protein. Furthermore, several proteins were the first report to be identified as a protein. Based on these findings, it is considered that rhizosphere is able to be recognized as like one plant organ 1, 2). On the other hand, it should be mentioned that actual rhizosphere is existed in soil and the interaction between microorganism can not be ignored. Before initiating a study on rhizosphere soil, we have applied positron-emitting tracer imaging system (PETIS) and imaging experiment with $^{11}CO_2$. It is because to define the exact location of rhizosphere in the soil. After applying $^{11}CO_2$ to the leaf of plant then ^{11}C move to the root within in one hour. After that root compartment is removed from the soil compartment then the radioactivity of soil was analyzed (Fig. 1). By using PETIS, it is able to define the area where the shoot secretion is active. Based on the real time mapping of rhizosphere, the soil was collected and metagenome analysis was carried out. The soil obtained as active rhizosphere shows a clear difference in microbial composition based on 16S rRNA amplicon whole genome sequencing. We are now trying to apply this method to the radionuclide contaminated field to make clear the mechanism why some plant species actively uptake radioactive cesium from soil.

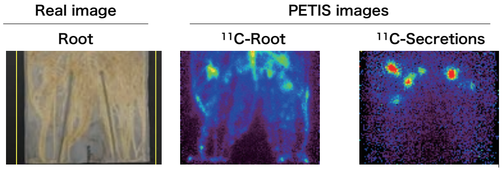

Fig. 1 Image of the root and ^{11}C distribution to root and secretions.

Reference
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WHOLE-CELL MASS SPECTROMETRIC ANALYSIS FOR RAPID CLASSIFICATION OF CULTURABLE MICROBES

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With an increased understanding of the diversity of plant-bacterial associations, future biotechnological applications for increased crop production, conserved biodiversity and sustained agro-ecosystems are foreseeable. A prerequisite for this are high throughput methods for characterisation and classification of culturable microorganisms isolated from plants grown under diverse environmental conditions. Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) is a novel tool for fast and high resolving identification of microorganisms. The method is based on the measurement of the molecular weight of most abundant proteins in a crude extract and using the mass spectra information as a fingerprint for a particular organism. This talk will highlight the applicability of this tool for studying microbial diversity and phylogeny.

THE PLANT GROWTH-PROMOTING BACTERIA *KOSAKONIA RADICINCITANS* IMPROVES FRUIT YIELD AND QUALITY OF *SOLANUM LYCOPERSICUM*

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Production and the quality of tomato fruits have a strong economic relevance. Microorganisms such as the plant growth-promoting bacteria *Kosakonia radicincitans* (DSM 16656) were demonstrated to improve shoot and root growth of young tomato plants, but data on yield increase and fruit quality by *K. radicincitans* are lacking. Therefore, we investigated how *K. radicincitans* affects tomato fruits. After inoculating tomato seeds with *K. radicincitans* or a sodium chloride buffer control solution, we monitored stalk length, first flowering and the amount of ripened fruits produced by inoculated and non-inoculated plants over a period of 21 weeks. Inoculation of tomato seeds with *K. radicincitans* accelerated flowering and ripening of tomato fruits. We also analyzed sugars, acidity, amino acids, volatile organic compounds, and carotenoid contents in the fruits. We found that the plant growth-promoting bacteria (PGPB) affect the amino acid, sugar and volatile composition of ripened fruits, contributing to a more pleasant-tasting fruit without forfeiting selected quality indicators.

Keywords: Plant growth-promoting bacteria, *Kosakonia radicincitans*, fruit metabolites, *Solanum lycopersicum*

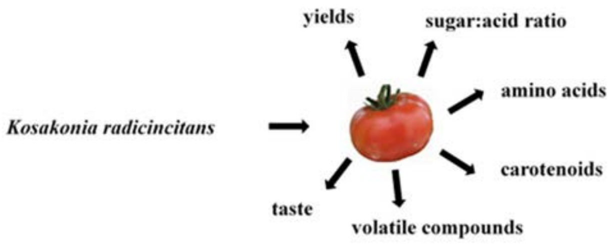

Fig1. Graphical abstract

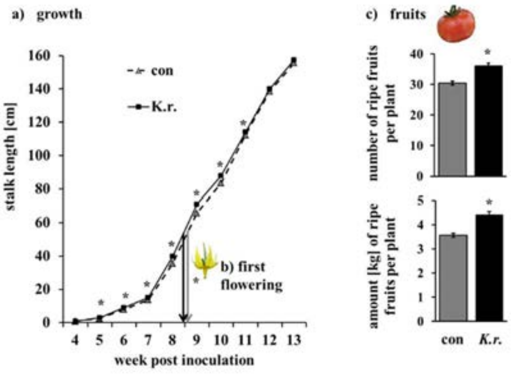

Fig2. a) Stalk length (growth) and b) date of first flowering of tomato plants treated with either 0.05 M NaCl solution (con, grey symbols, dotted line/ grey arrow) or 10^8 cfu ml^{-1} *K. radicincitans* cells (K.r., black symbols, solid line/ black arrow). c) Number and amount (fruits) of harvested red fruits per plant within an 18-weeks cultivation period from plants treated with either 0.05 M NaCl solution (con, grey bars) or 10^8 cfu ml^{-1} *K. radicincitans* cells (*K.r.*, black bars) (n=30).

Reference: Berger, B.; Baldermann S.; Ruppel, S. (2017). The plant growth-promoting bacteria *Kosakonia radicincitans* improves fruit yield and quality of *Solanum lycopersicum*. *Journal of the Science of Food and Agriculture* 97(14), 4865-4871.

OSMOADAPTATION AND HYDROXYECTOINE ACCUMULATION ENHANCE ENDOPHYTISM OF ENCAPSULATED *Kosakonia radicincitans* IN RADISH PLANTS

[1]Mauricio Cruz Barrera*, [2]Desiree Jakobs-Schoenwandt, [3]Matthias Becker, [2]Anant Patel, [1]Juan Serrato, [4]Martha Gómez and [3]Silke Ruppel

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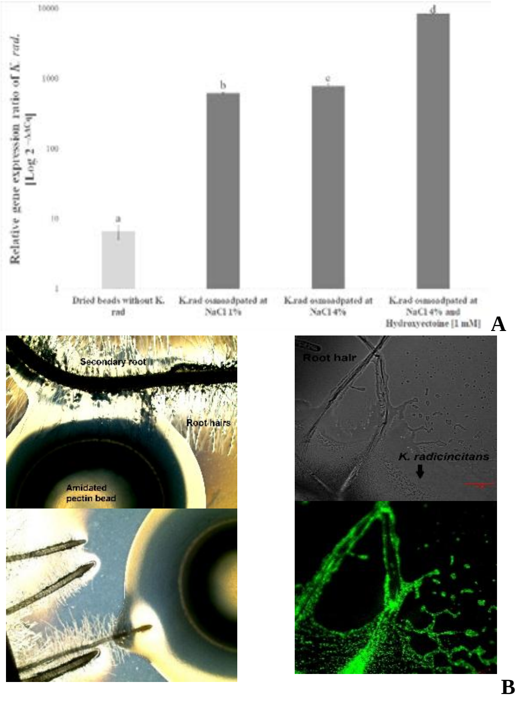
Recent studies on endophytic *Kosakonia radicincitans* DSM 16656^T demonstrated a wide plant growth-promoting activity [1,2]. The application of this Gram-negative bacterium as a biostimulator could be improved by developing a formulation that protects the cells during drying and storage and supports plant colonization [3]. Here we set out to elucidate the influence of compatible solutes accumulation of *K. radicincitans* triggered by osmotic stress on its capacity to colonize radish plants. We found that physiological modifications by osmotic stress treatments and accumulation of compatible solutes during cultivation, improve the capability of *K. radicincitans* formulated in dry amidated pectin beads to colonize radish roots and to promote radish growth under greenhouse conditions. Thus, pre-conditioning of cells in defined media (DM) with 4% NaCl and by adding compatible solutes such as hydroxyectoine, induced a 10-fold higher *K. radicincitans* colonization of radish plant tissue, in comparison to 4% NaCl pre-conditioned cells (Fig.1A). Additionally, with the osmoadaptation and with the presence of this osmolyte, either dry matter of tuber and leaves increased by 29.03% and 13.10%, respectively, in comparison to beads with bacterium grown in DM 1% NaCl. Results suggest that the immobilization in amidated pectin beads allowed the successful colonization of plant tissues by the endophyte through mainly root hairs and secondary roots (Fig. 1B). In general, these results indicated that plant colonization was enhanced by synergistic effects of pre-conditioned cells by osmoadaptation and formulation performance. The best of our knowledge, the current study is the first to deal with the pre-conditioning of cells and the amidated pectin encapsulation of a bacterial endophyte as a formulation alternative.

Fig. 1. A: Accumulation of *K. radicincitans* DNA in inoculated radish plants with dried amidated pectin beads. **B:** Interaction of encapsulated bacterium in amidated pectin beads with radish seedlings, microscopy approach (Zeiss LSM 510 META laser scanning confocal microscope, excitation/emission 488 nm).



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PHYTOCHEMICAL AND MICROBIOLOGICAL STUDY OF LEAVES AND FLOWERS OF *Ipomoea carnea* Jacq. (FAMILY Convolvulaceae) GROWN IN EGYPT

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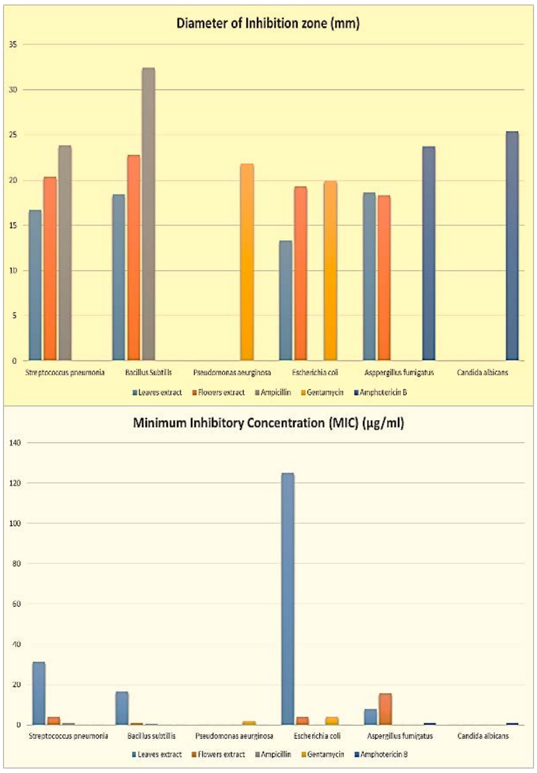
Abstract

Background: *Ipomoea carnea* was recorded along canals, drains, road sides, railways, waste lands and fluid edges in the Nile Delta **(Shaltout *et al.*, 2006).** The present study aimed at finding antimicrobial potential of alcoholic leaves and flowers extracts of *Ipomoea carnea* Jacq.

Methods: Antimicrobial activity was evaluated against four bacterial strains and two fungal strains by determining zone of inhibition and minimum inhibitory concentration (MIC) **(Lorian, 2005)**. Diameters of the zone of inhibition were compared with standard antibiotics. Preliminary phytochemical screening was done according to standard protocol. All experiments were conducted in triplicate and values were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed for statistical justification. Phytochemical assay of rutin and *β*-sitosterol was performed for leaves and flowers alcoholic extracts of *I. carnea* Jacq.



Results: Maximum zones of inhibition were found in case of alcoholic flowers extract in comparison with alcoholic leaves extract in the following order *Escherichia coli* > *Bacillus subtillis* > *Streptococcus pneumonia* respectively but alcoholic leaves extract showed slightly higher antifungal activity than flowers ethanol extract against *Aspergillus fumigatus* while both leaves and flowers alcoholic extracts showed no activity against *Pseudomonas aeurginosa* and *Candida albicans*. The concentration of rutin in leaves and flowers ethanol extracts of *I. carnea* by HPLC were (9.174 and 2.733 mg/g dry wt.) respectively. The concentration of *β*-sitosterol in leaves and flowers chloroform extracts of *I. carnea* were (0.463 and 17.085 mg/g dry wt.) respectively.

Conclusion: The antimicrobial study revealed that flowers extract showed powerful antimicrobial activity mainly against *Escherichia coli* than leaves extract which may be attributed to the presence of higher concentration of *β*- sitosterol in flowers extract **(Sen *et al.*, 2012)** and these results could be recommended for further clinical studies to evaluate the antimicrobial activity of leaves and flowers ethanol extracts of *I. carnea.*

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Session 6

Water quality and availability, and environmental stresses shape the plant microbiome and challenge future agriculture productivity

Chairpersons: Wolfgang Liebel and Kauser Malik

CHALLENGES AND POSSIBILITIES FOR WASTER RE-USE IN IRRIGATION OPTIMIZATION OF TECHNOLOGIES OF WASTE WATER TREATMENT AS A POTENTIAL RESOURCE FOR FUTURE AGRICULTURE IN SEMI- /ARID DESERTS

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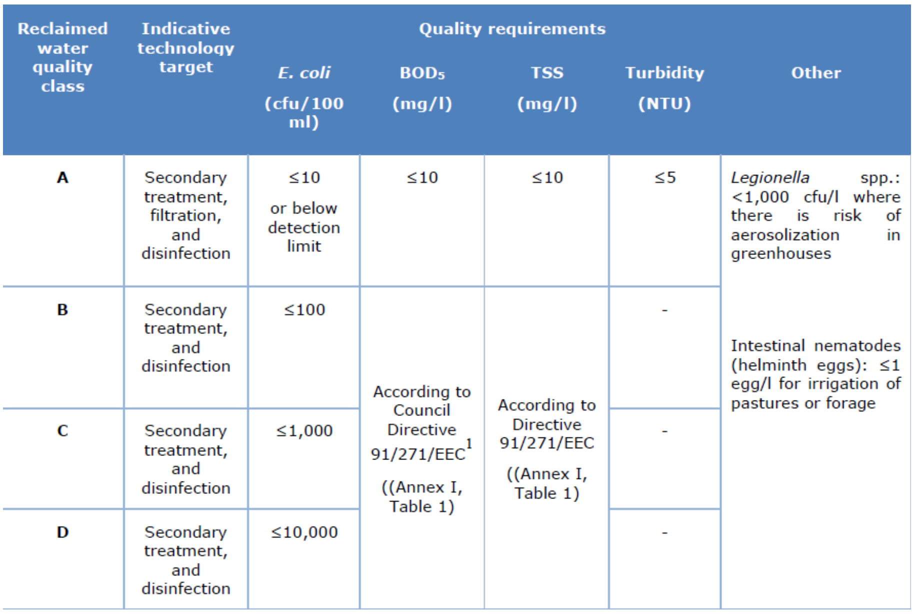
Optimized wastewater treatment for the purpose of reuse is gaining more importance worldwide due to the increasing lack of water. Not only climate change but also growing populations stress our water resources. Water scarcity means either the physical shortage of water or the lack of infrastructure to supply water (economical scarcity). Today, around 43 countries suffer from water scarcity (Global Water Institute, 2013). As an effect of climate change, the frequency and intensity of droughts and their environmental and economic damages have drastically increased over the past thirty years. The number of areas and people affected by droughts went up by almost 20% and the total costs of droughts amounted to EUR 100 billion (EC, 2012). Wastewater as a constant produced resource of water can close the lack of missing water. The WHO has been stating in several publications that different water uses can apply different quality of water, so that the high quality of water can be saved for drinking water purposes. For arid and semi-arid regions, the utilization of wastewater for agriculture or landscaping is often implemented. This not only decreases the use of commercial fertilizer and irrigation but also increases agricultural production and closes the water and nutrient loop. Nevertheless, the direct use of un-disinfected wastewater can cause public health problems (WHO, 2006). Therefore, standards for the water quality are given in the table below (e.g. EU):



Reclaimed water quality class	Indicative technology target	Quality requirements				
		E. coli (cfu/100 ml)	BOD_5 (mg/l)	TSS (mg/l)	Turbidity (NTU)	Other
A	Secondary treatment, filtration, and disinfection	≤10 or below detection limit	≤10	≤10	≤5	*Legionella* spp.: <1,000 cfu/l where there is risk of aerosolization in greenhouses
B	Secondary treatment, and disinfection	≤100	According to Council Directive 91/271/EEC[1] ((Annex I, Table 1)	According to Directive 91/271/EEC ((Annex I, Table 1)	-	Intestinal nematodes (helminth eggs): ≤1 egg/l for irrigation of pastures or forage
C	Secondary treatment, and disinfection	≤1,000			-	
D	Secondary treatment, and disinfection	≤10,000			-	

Table 1: Reclaimed water quality requirements for agricultural irrigation Annex I 2018

It is obvious that the different qualities can only be produced with adjusted and optimized wastewater treatment technologies. It is often reported that plants irrigated with wastewater have better growth and higher production than those treated with drinking water. Nevertheless, the accumulation of heavy metals and pathogenic organisms in the plant-soil-microbiome system require a careful handling. Often reused water is used for forest or park irrigation, because there are less health problems than using for edible plants. Future investigations using various water qualities and crop types need to be intensified to trace the impact on the interacted plant microbiome.

HALOPHYTE MICOBIOME: REVEALING ITS DIVERSITY, FUNCTIONS AND APPLICATIONS

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Salicornia europaea (*herbacea*) L. (Amaranthaceae) is one of the most salt-accumulating halophytes known, that has generated significant interest as a multi-purpose plant. Halophytes have developed several primary adaptive mechanisms that are triggered in response to salinity. However, only a limited number of genes have been evaluated to characterize the molecular mechanisms underlying salt tolerance. The secondary mechanisms that might mitigate salt stress in plants under unfavorable conditions may consist of its associated microbiome - endophytes. The close interaction of endophytes within their host can directly affect plant properties, particularly under stress conditions. These research aspects are looked into by our group members that have chosen *S. europaea* as a model species for exploring the salt tolerance mechanisms and its microbiome due to its high salt tolerance and its great agronomic value.

Three specific research questions will be addressed during this talk: (1) How environmental factors drive the gene expression in *S. europaea*?; (2) Does *S. europaea* have specific and unique endophytes protecting its host under unfavorable saline conditions?; Are the Salicornia bacterial and fungal endophytes compatible in other non-host plants?

Some of these outcomes will be presented, along with the most interesting experiments on selected halotolerant endophytes isolated from *S. europaea*.

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Acknowledgments

The author would like to thank Dr. Sonia Szymańska and Ms. Bliss Furtado for conducting all experiments. This study was financially supported by a grant from the National Science Centre (Poland) (DEC-2012/07/B/NZ9/01801) and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 676480.

Salicornia europaea L. (Phot. Katarzyna Hrynkiewicz)

Oral VI-3

COMPARISON OF MICROBIOME OF HALOPHYTE (*Salsola stocksii*) AND NON-HALOPHYTE (*Triticum aestivum*) USING CULTURE-INDEPENDENT APPROACHES

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Halophyte microbiome contributes significantly to plant performance and can provide information regarding complex ecological processes involved in osmoregulation of these plants (Dodd and Perez-Alfocea 2012; Turner et al. 2013). The objective of this study is to investigate the microbiomes associated with belowground (rhizosphere), internal (endosphere) and aboveground (phyllosphere) tissues of halophyte (*Salsola stocksii*) and non-halophyte (*Triticum aestivum*) through metagenomics approach. Metagenomic analysis from rhizosphere, endosphere and phyllosphere of *Salsola* showed that approximately 29% bacteria were uncultured and unclassified. *Proteobacteria* and *Actinobacteria* were the most abundant phyla in *Salsola* and wheat. However, *Firmicutes, Acidobacteria, Bacteriodetes, Planctomycetes, Cyanobacteria, Thermotogae, Verrucomicrobia, Choroflexi* and *Euryarchaeota* were predominant groups from halophyte whereas *Cyanobacteria, Acidobacteria, Bacteriodetes, Planctomycetes* and *Verrucomicrobia* were predominant phyla of wheat samples. Sequences from *Euryarchaeota, WPS-1, Ignavibacteriae, Chlamydiae,* and *Nanohaloarchaeota* were identified only from the rhizosphere of halophytes.

Dominant halophilic bacteria and archaea identified in this study included *Agrococcus, Halomonas, Armatimonadetes Nocardioides, Solirubrobacter, Halalkalicoccus, Haloferula* and *Halobacterium.* Diversity and differences of microbial flora of *Salsola* and wheat suggested that functional interactions between plants and microorganisms contribute to salt stress tolerance and having a complete inventory of the halophilic and halotolerant bacteria from the rhizosphere of halophyte will help in the discovery of potential inoculants for crops growing on salt-affected land (Mukhtar et al. 2017).

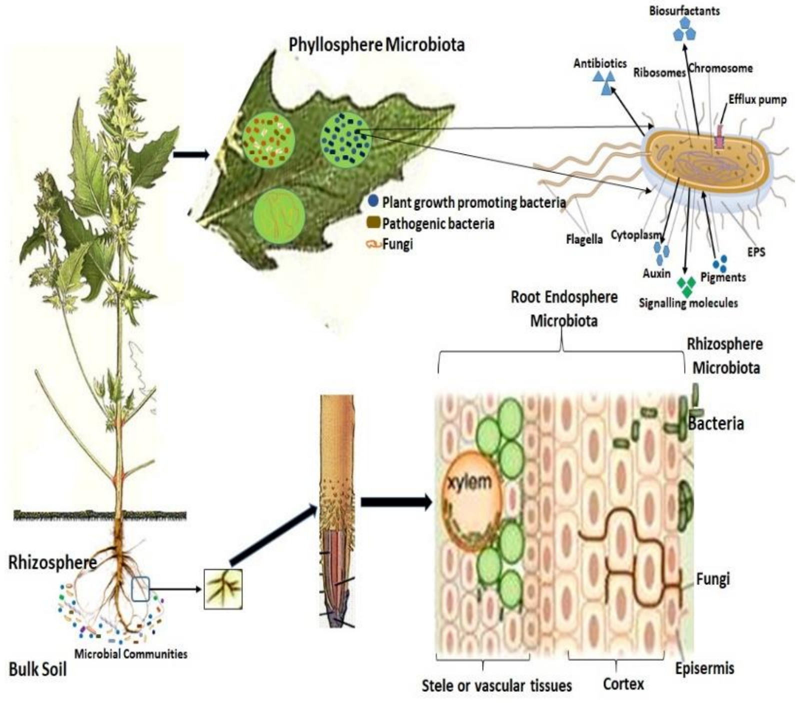

FIGURE 1. Overview of a plant microbiome.

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INFLUENCE OF BIOTIC AND ABIOTIC STRESS ON TOMATO ENDOPHYTES.

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Tomato is an economically important crop plant often grown in the greenhouse. Here we assessed the plant growth-microbiota-stress triangle in tomatoes under biotic (*Verticillium dahliae*, *Fusarium oxysporum*) and abiotic (drought, salinity) stress. We determined plant performance parameters, isolated and characterized bacteria from plant roots and determined the root-associated bacterial community composition by 16S rRNA sequencing.

Both biotic and abiotic stress retarded plant growth but to a different degree and with differential effects on plant physiological parameters. Our culturing effort yielded 683 bacteria from surface-sterilized tomato roots, and we found an enrichment of *Paenibacillus* spp. in *F. oxysporum*-infected plants, while the genus *Agrobacterium* was depleted in most abiotic and biotic stress conditions. Bacterial isolates obtained from stressed plants were enriched for drought tolerance but not for salt tolerance. Salt tolerant isolates were more likely to produce known PGP-traits *in vitro*, e.g. 1-aminocyclopropane-1-caroxylate (ACC) deaminase or siderophores, among others. Many of our isolates were able to promote plant-growth *in vivo*, with salt-tolerant isolates being more likely to promote plant-growth. 16S rRNA profiling of tomato-root-associated bacterial communities revealed that ionic stress depleted bacterial α-diversity of root endophytes while infection with *V. dahliae* modulated bacterial abundance with no statistically significant effect on bacterial α-diversity.

In summary we detected a strong effect of biotic and abiotic stress on plant performance and bacterial root-endophytes. We have obtained an extensive collection of tomato root endophytes which will serve as a basis for mechanistic investigations into the influence of microorganism on plant performance.

Table 1: Plant treatments in the greenhouse experiment.

Treatment	Abiotic stress	Biotic stress
C	Control	Control
I	Salinity	-
O	Drought	-
F	-	*F. oxysporum*
V	-	*V. dahliae*
IO	Salinity + drought	-
IF	Salinity	*F. oxysporum*
IV	Salinity	*V. dahliae*
IOF	Salinity + drought	*F. oxysporum*
IOV	Salinity + drought	*V. dahliae*
OF	Drought	*F. oxysporum*
OV	Drought	*V. dahliae*

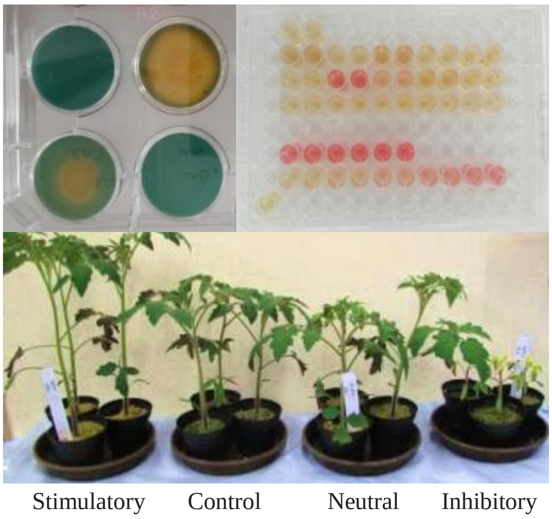

Figure 1: TOP: *In vitro* test for plant-growth promoting traits of bacterial isolates. BOTTOM: *In vivo* test for plant growth promotion of bacterial isolates.

MICROBIAL DIVERSITY IN THE RHIZOSPHERE OF MESO-, HALO- AND XEROPHYTES

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The microbial community in the rhizosphere can provide information regarding various mechanisms involved in the survival of plants under extreme conditions. Rhizobacteria play an important role in plant health and soil fertility. The main objective of the present work was to study microbial diversity and plant growth promoting potential of these bacteria, isolated from the rhizosphere, rhizoplane and root endosphere of mesophytes (wheat, cotton), halophytes (para grass, *Salsola*, *Atriplex*, *Kochia*, *Suaeda*) and xerophytes (cactus, *Zygophyllum*). From these plants more than 200 bacterial isolates were identified on the basis of 16S rRNA gene sequence. Phylogenetic analysis showed that strains of *Bacillus* spp. were dominant in the rhizosphere of Meso-, Halo- and Xerophytes while strains *of Kocuria* spp. were dominant in the rhizosphere of Halophytes and Xerophytes. In addition, *Enterobacter* and *Pseudomonas* were dominant in the rhizosphere of Mesophytes; *Halobacillus* in the rhizosphere of Halophytes and *Staphylococcus* was dominant in rhizosphere of Xerophytes. In total, 189 bacterial isolates were screened for various plant growth promoting abilities including IAA production, phosphorous solubilization, nitrogen fixation, siderophore and HCN production. Fifty three isolates were identified as IAA producer, 61 isolates solubilized phosphate, 41 were capable of fixing nitrogen, 19 isolates produced siderophore and 15 isolates showed HCN production.

From this vast bacterial collection, five strains, including three of *Bacillus* sp., one of *Enterobacter aerogenes* and one of *Aeromonas veronii*, were selected and used as inoculants for maize. Inoculum was applied in two forms; 1) as seed coat, 2) inoculum enriched-soil with rock phosphate. Plants were grown in climate room and harvested after four weeks. It was observed that all bacterial strains positively affected the plant growth as compared to un-inoculated plants. Plants inoculated with *Bacillus* sp. HL3RS14 enriched soil, showed maximum increase in dry weight of roots (51-104%) and shoots (35-114%) as compared to control (soil + rock phosphate, no inoculum). These results indicated that bacterial strains with plant beneficial traits are more promising candidates as biofertilizer when used with carrier material as compared to directly coated on seeds.

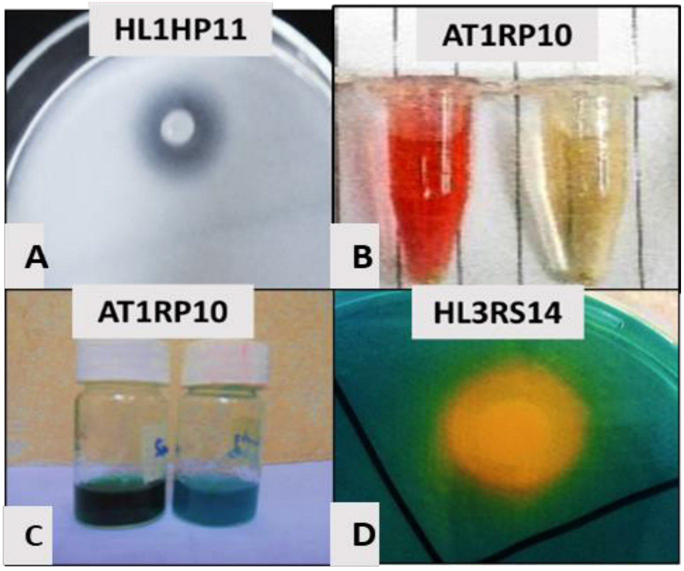

Figure 1. Plant growth promoting abilities of isolates. (A) Phosphate solubilization (B) IAA production (C) Nitrogen fixation (D) Siderophore production

BAMBARA GROUNDNUT-BACTERIA INTERACTION; SOURCE OF FOOD SECURITY

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Abstract

With the rise in world population and decrease in food supply due to global climate change, food security becomes very pertinent. Malnutrition, food scarcity and poverty have consistently affected population growth. This issue has driven scientists to seek out other plants that have been under-studied but have potential for food security. African soils now contain essential nutrients in very low quantities leading to low fertility. This makes it unable to support plant growth as efficiently as it used to due to continuous land use without a proper soil management programme.

The use of underutilized and neglected food crops has been observed to be the way out of over-use and dependence on staple foods. This review aims to determine the effect of using soil environment of underutilized leguminous crops to be able to accomplish maximum yields to improve crop yield and invariably food security. The problem of low yield from continuous farming has led to more cultivation of land and less use of mineral fertilizers due to the inability to afford such fertilizers cum its hazardous effect on soil and crop. Planting of legumes that are able to increase the nitrogen content of soils by nodulation with rhizobacteria is a non-chemical solution. The use of rhizobacteria is very important to improving crop yield and most especially rhizobacteria from legumes like bambara groundnut. Bambara groundnut and its interaction with various rhizobacteria in the soil could play a vital role in biocontrol and biofertilization. This, in turn, will help to increase crop yield by resisting pests and disease and improving plant growth and productivity.

Figure 1: **(a)** Bambara groundnut seeds in different colours and sizes; **(b)** Uprooted bambara groundnut plant showing lateral roots and nodules attached to them.

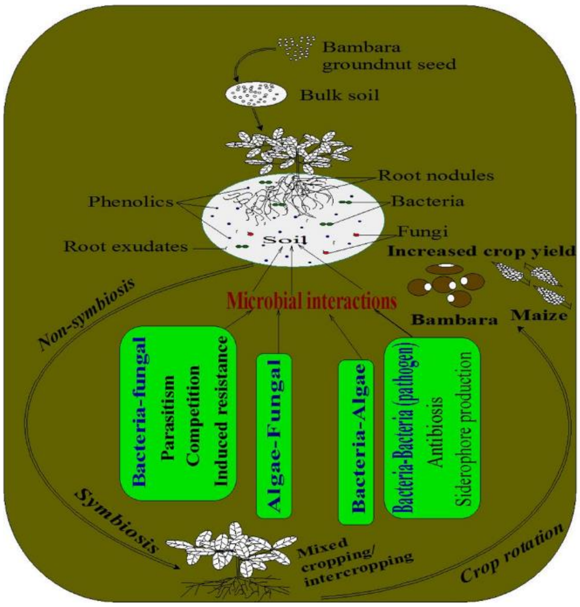

Figure 2: Associations involved in bambara groundnut-bacterial interactions for food security

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BACTERIAL ENDOPHYTES FOR IMPROVED PLANT GROWTH UNDER NITROGEN AND WATER LIMITED CONDITIONS.

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Nitrogen and Water are the key resources limiting world agricultural production. Nitrogen fertilizers, provided by the energy demanding Haber–Bosch process, have enhanced crop yields at huge environmental cost. Hence, low-input sustainable alternatives to chemical fertilizers are urgently needed. Additionally, drought due to fresh water scarcity has traditionally been manged by using drought tolerant plant genotypes, relying on lengthy and costly plant breeding programs. We propose a feasible alternative strategy by utilizing the abilities of certain novel plant endophytes harbouring beneficial traits improving plant growth under biotic and abiotic stress conditions.

This work aims to 1. Systematically compare the capacity of novel bacterial endophytes to improve plant growth under limited water and nitrogen conditions, using the model plant *Brachypodium distachyon* in the National Plant Phenomics Centre, Aberystwyth, UK. 2. Determine plant molecular components involved in beneficial plant endophyte interactions via RNAseq. 3. Identify genomic features of endophytes associated with beneficial mode of actions via whole genome sequencing of 48 endophytes.

Promising results for the novel endophytes will be compared with results reported using known plant growth promoting bacterial strains such as *Azoarcus Olearius* DQS4 and *Azospirillum brasilense* Cd.

Mitigation of Salinity Stress on Nodulation, Nitrogen Fixation and Growth of *Cicer arietinum* by Triple Microbial Inoculation

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Plant-associated microorganisms play a critical role in agriculture productivity. Symbiotic microorganisms participate with each other and allow host plants to maintain optimal nutrients for enhancing the plant growth. Therefore, the goal of the present study was to investigate the synergistic interaction of different symbiotic microbes for ameliorating the nodulation efficiency and the growth of salt-affected chickpea plants (*Cicer arietinum* L.). *Rhizobium pusense* ASU5 (MG865432) was isolated from root nodules of chickpea plants. *In vitro*, the effect of magnetite nanoparticles (Fe_3O_4-NPs) and bulk $FeCl_3$ at concentration 10, 50, 100, 150, 200, and 250 µg ml^{-1} on the growth of *Rhizobium pusense* was investigated. Magnetite nanoparticles at 150 µg ml^{-1} enhanced the growth of *Rhizobium*. The impact of seven soil salinity levels (0, 25, 50, 75, 100, 150 and 200 mM) on the germination and the subsequent growth was addressed. The salinity levels ranged from 25 to 150 mM significantly inhibited the growth while 200 mM hindered the germination of chickpea plants. The impact of triple microbial inoculation of chickpea plants grown under soil salinity levels at 0, 75 and 150 mM was studied. Inoculation of chickpea with mycorrhizal fungi, *Rhizobium pusense* activated with magnetite nanoparticles (Fe_3O_4-NPs), and endophytic *Stenotrophomonas maltophilia* grown at salinity level of 75 and 150 mM significantly improved nodulation, nitrogenase activity, leghaemoglobin content and growth compared with their control plants. The mitigation of the adverse effect of salinity stress is due to the improvement in the nutritional status of plants as determined by K and P, carbohydrates and protein content **(Table 1)**. In summary, treated *Rhizobium* with Fe_3O_4-NPs, AMF, and *Stenotrophomonas maltophilia* build a beneficial symbiotic association in chickpea plants and their interaction can act on enhancing the plant growth, nodulation, nitrogenase activity and some physiological processes under salt stress condition. This could be successful bio-fertilizer that can contribute to protecting chickpea plants against salinity by attenuating the salt-induced oxidative damage.

Table 1: Effect of different symbiotic microorganisms; *Rhizobium pusense* ASU5 stimulated with Fe_3O_4-NPs (RP-Fe_3O_4-NPs), Arbuscular mycorrhizal fungi (AM), Endophytic bacteria *Stenotrophomonas maltophilia* C6 (KY515467.1) (SM) on plant growth, nodulation, nitrogenase activity, Leghaemoglobin content, K and P plant contents and soluble carbohydrate and protein of eight-week-old chickpea plants grown under different levels of salt stress.

Parameter	Salt level	0 mM NaCl		75 mM NaCl		150 mM NaCl	
		Inoculation					
		Cont.	RP-Fe_3O_4-NPs+SM+AM	Cont.	RP-Fe_3O_4-NPs+SM+AM	Cont.	RP-Fe_3O_4-NPs+SM+AM
Root length		19.3 ± 1.5^{a3}	26.3 ± 1.1^{b5}	16.77 ± 0.25^{a2}	23.5 ± 0.8^{b4}	14.17 ± 1^{a1}	23.5 ± 0.8^{b4}
Root F.W. (g)		0.93 ± 0.06^{a12}	4.59 ± 0.5^{b5}	0.85 ± 0.06^{a12}	2.39 ± 0.3^{b34}	0.76 ± 0.03^{a1}	1.99 ± 0.2^{b3}
Root D.W. (g)		0.11 ± 0.002^{a2}	0.53 ± 0.05^{b5}	0.05 ± 0.02^{a1}	0.21 ± 0.01^{b34}	0.04 ± 0.03^{a1}	0.16 ± 0.01^{b23}
Shoot length		24.2 ± 2.3^{a4}	41.2 ± 3.3^{b5}	16.5 ± 1.8^{a12}	25.4 ± 0.6^{b4}	13.9 ± 1^{a1}	19.27 ± 1.2^{b3}
Shoot F.W. (g)		5.98 ± 0.8^{a5}	12.99 ± 1.5^{b6}	1.41 ± 0.3^{a12}	3.29 ± 0.3^{b4}	1.29 ± 0.7^{a1}	2.83 ± 0.2^{b34}
Shoot D.W. (g)		1.11 ± 0.1^{a5}	2.08 ± 0.07^{b6}	0.18 ± 0.07^{a12}	0.51 ± 0.1^{b4}	0.008 ± 0.006^{a1}	0.29 ± 0.05^{b23}
Nodule number/plant		4.67 ± 1.1^{a3}	21.33 ± 0.5^{b5}	2.67 ± 0.5^{a2}	14.33 ± 1.1^{b4}	0^{a1}	1.67 ± 0.5^{b2}
Nodule FW/ plant (g)		0.19 ± 0.007^{a34}	0.69 ± 0.06^{b6}	0.073 ± 0.008^{a2}	0.34 ± 0.004^{b5}	0^{a1}	0.079 ± 0.02^{b2}
Nodule DW/ plant (g)		0.003 ± 0.0005^{a12}	0.058 ± 0.004^{b4}	0.003 ± 0.0004^{a12}	0.026 ± 0.003^{b3}	0^{a1}	0.007 ± 0.0005^{b12}
Nitrogenase activity (µmoles C_2H_4 plant^{-1} h^{-1})		2.98 ± 0.1^{a3}	10.36 ± 0.07^{b5}	0.32 ± 0.01^{a2}	3.35 ± 0.2^{b4}	0^{a1}	0.35 ± 0.01^{b2}
Leghaemoglobin (g/l)		77.2 ± 0.06^{a4}	147.3 ± 0.8^{b6}	33.6 ± 2.0^{a2}	80.9 ± 0.8^{b45}	0^{a1}	37.5 ± 0.8^{b23}
Root K^+ (mg/gm. DW)		14.7 ± 0.4^{a5}	43.04 ± 0.6^{b6}	9.28 ± 0.05^{a2}	14.32 ± 0.3^{b45}	6.87 ± 0.7^{a1}	12.64 ± 0.1^{b3}
Shoot K^+ (mg/gm. DW)		11.82 ± 0.2^{a3}	20.44 ± 0.6^{b6}	9.55 ± 0.4^{a2}	19.34 ± 0.3^{b5}	7.28 ± 0.2^{a1}	17.76 ± 0.6^{b4}
Root P (mg/gm. DW)		2.89 ± 0.09^{a3}	6.9 ± 0.04^{b}	1.58 ± 0.04^{a2}	5.23 ± 0.2^{b5}	0.94 ± 0.06^{a1}	4.08 ± 0.08^{b4}
Shoot P (mg/gm. DW)		3.78 ± 0.6^{a3}	7.51 ± 0.2^{b5}	3.08 ± 0.3^{a2}	6.47 ± 0.06^{b4}	1.11 ± 0.04^{a1}	6.19 ± 0.1^{b4}
Soluble carbohydrate		19.09 ± 0.2^{a3}	46.32 ± 0.1^{b6}	14.15 ± 0.2^{a2}	34.29 ± 0.6^{b5}	10.46 ± 0.4^{a1}	28.57 ± 0.3^{b4}
Soluble protein		366.33 ± 0.5^{a4}	439.93 ± 3.2^{b6}	307.78 ± 1.5^{a2}	372.59 ± 3^{b5}	173.27 ± 0.5^{a1}	324.53 ± 3.8^{b3}

Means with the same superscript letter between the treatments within each salinity level and with the same superscript number among the salinity levels are not significantly different, according to SPSS test "Duncan's multiple range".

Acknowledgement: M. H. Abd-Alla is grateful for Alexander von Humboldt support.

DARK SEPTATE ENDOPHYTES (DSE$_S$) RESPONSES TO ABIOTIC STRESS AND THEIR ROLE IN ALLEVIATION OF SALT STRESS IN TOMATO PLANT

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Endophytes are a group of microorganisms that live inside the tissues of the host plant without causing diseases. In natural ecosystems, all higher plants can be hosts to one or even hundreds of endophytic organisms which may be fungi or bacteria. Dark septate endophytes (DSEs) are considered as a subgroup of endophytic fungi characterized by their morphology of melanized, septate hyphae. In the current study, we used three models of dark septate endophytes that have been previously isolated and characterized from stressed soil sites in Hungary and France.

We studied the responses of the three dark septate endophytes models; *Periconia macrospinosa* (DSE 2036), *Cadophora* sp. (DSE 1049), *Leptodontidium* sp. (Me07, melanised Wild type) and the corresponding mutant of *Leptodontidium* sp. (non-melanized albino mutant) to abiotic stresses like salt, heavy metals and heat *in vitro*. The impact of tomato plant inoculation with DSEs models on plant growth, nutrients uptake and the content of toxic Na$^+$ ions uptake under two levels of salt stress (5.5 ds/m and 10.5 ds/m) were studied.

Results of DSEs growth and morphology on different synthetic culture media subjected to high temperature, salt and heavy metals suggested that these endophytes have a high competence to tolerate abiotic stresses. Plants inoculated with DSEs aside from *Leptodontidium* sp. (Wild type) showed higher growth biomass under salt stress than non-inoculated plants. Moreover, in inoculated plants, DSEs improved the total carbon content in plant shoot which indicate the increment of the photosynthetic efficiency. In addition, the Na$^+$ ions content decreased in inoculated plants under low level of salt stress. We conclude that DSEs tolerance to abiotic stresses serve to alleviate abiotic stress on tomato plant. This suggests that DSEs could serve as biological agents in plant production systems increasing abiotic stress tolerance of different crops.

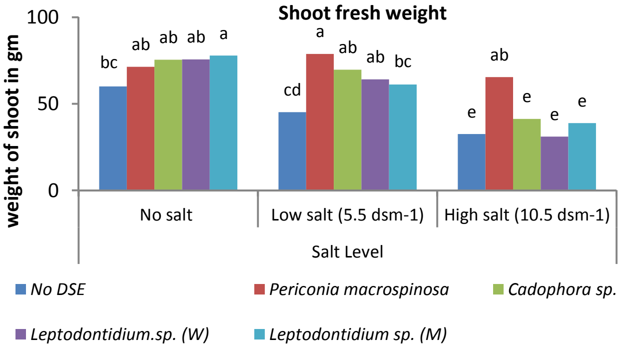


Figure shows fresh weight of tomato plants inoculated or not with DSEs and harvested after 6 weeks.

No salt

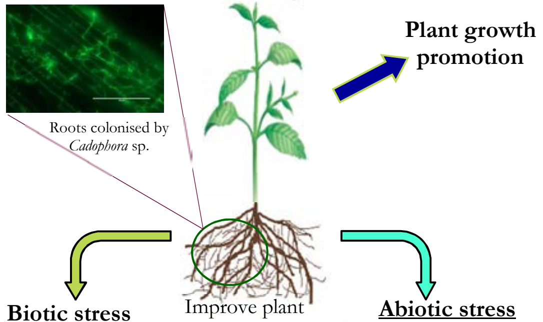
- DSEs *Cado.* *Per.* *Lep.* *Lep. (M)*

Low salt

- DSEs *Cado.* *Per.* *Lep.* *Lep. (M)*

High salt

- DSEs *Cado.* *Per.* *Lep.* *Lep. (M)*



Roots colonised by *Cadophora* sp.

Plant growth promotion

Improve plant performance against

Biotic stress
Fungi Bacteria
Insects Viruses

Abiotic stress
Salinity HMs Heat
pH Drought Radiation

PLANT GROWTH PROMOTING POTENTIAL OF BACTERIAL ROOT ENDOPHYTES OF STRESSED PLANTS

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Plants are constantly exposed to environmental stress. Changes in global climate will increase problems in agriculture with saline soils and drought and by exacerbating problems associated with soil-borne pathogens, e.g. *Fusarium oxysporum*and *Verticilliumdahliae*. Root-associated bacterial communities play an important role in enhancing the plant host's capability to cope with stress.We hypothesized a) that stress selects for root endophytes with enhanced resistance to this stress and b) that the selected endophytes confer stress resistance to their host.For this, we grew tomatoes under abiotic (salinity, drought) and biotic (*V. dahliae*) stress, isolated bacteria from their roots and determined the growth-promoting potential of the obtained isolates *in vitro* and *in vivo*.

Stunted growth was observed in plants as a response to abiotic and biotic stress. A total of 1085 bacterial endophytes were isolated from surface sterilized roots of tomatoes grown under conditions specified in Table 1. 523 unique isolates were obtained following dereplication using their protein fingerprint (MALDI Biotyper). We also observed a stress-associated enrichment of endophytes with certain plant growth promoting properties (Figure 1):For example, isolates from plants treated with osmotic stress and *V. dahliae*(OV) were enriched for IAA, siderophore and ACC deaminase production. Drought tolerant endophytes were enriched in most stress exposed treatmentswhereas the effect on salt-tolerance was much less pronounced.

Overall, our results indicate enrichment of plant growth promoting traits of bacterial endophytes isolated from stressed plants. Elucidating the effect of combined environmental stresses on the plant microbiome is required in order to fully exploit the potential of microbial communities in enhancing plant performance and designing unique agricultural solutions.

Table 1: Plants were exposed to the following stresses

Treatments	Abiotic stress	Biotic stress
C	None	None
I	Salinity	None
O	Drought	None
IO	Salinity + Drought	None
V	None	*V. dahliae*
IV	Salinity	*V. dahliae*
OV	Drought	*V. dahliae*
IOV	Salinity + Drought	*V. dahliae*

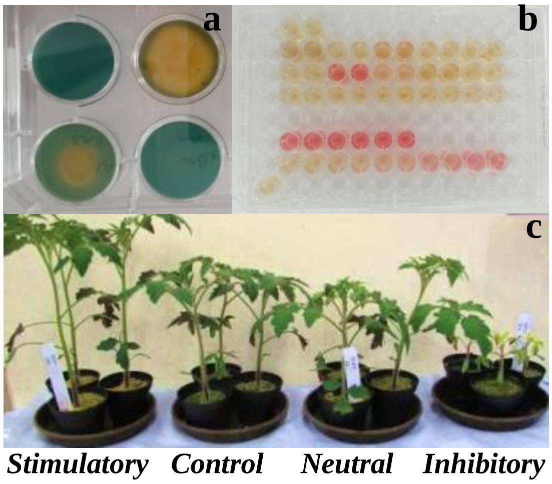

Fig 1: PGP traits of the isolates (a) Siderophore (b) IAA production (c) *invivo* assays showing the effect of stimulatory, neutral and inhibitory isolates on plant growth.

Poster VI-4

BIOPRODUCTS DESIGNED FOR AGRICULTURAL PURPOSES: THE AGROSAVIA´s STRATEGY

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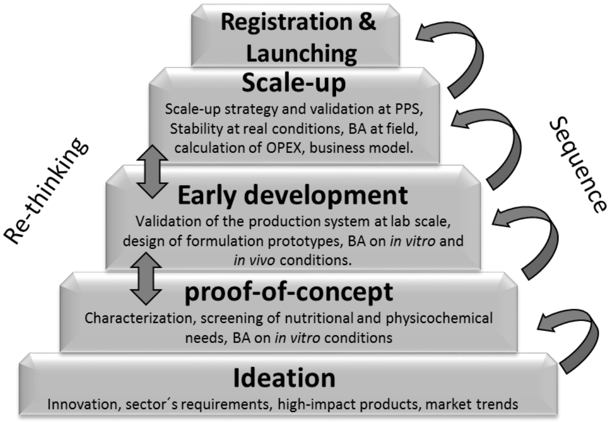
Abstract. In Colombia, the Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, is a public institution with the objective of developing research and technology (R+D+i) and transfer them to the agricultural sector. Part of these activities is carried out by the Department of Bioproducts (DoB), which oversees the bioprocess and formulation design of bioproducts, their registration and transfer through production agreements with national and international companies. At DoB, a stage-gate (SG) strategy is used for their development process and comprises five steps, (1) ideation, (2) proof-of-concept, (3) early development, (4) scale-up, and (5) registration and bioproduct launching.

Three successful examples of bioproducts designed by this SG strategy are shown next. TRICOTEC® is a granulated biopesticide based on *Trichoderma koningiopsis*, it has a minimum concentration of 1x10^{9} conidia/g, and an efficacy up to 50 % over pathogens such as *Rhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. [1]. On the other hand, a biostimulant based on plant growth promoting (PGP) endophytic *Bacillus amyloliquefaciens* is under design. A fermentation strategy was selected to reach a spore concentration of 1x10^{10} spores mL^{-1} with a spore efficiency of 90%. Its biological activity as a PGP had been demonstrated in cape gooseberry increasing in order of magnitude of 100 for fresh weight; 100 and 40 for total dry weight and leaf area, respectively; and 5 for shoot length and root length [2]. Additionally, the DoB is working on a biopesticide based on a *Rhodotorula glutinis* strain to control *Botrytis cinerea*. The production process was established obtaining up to 15 g L^{-1} of dry biomass weight and a cellular concentration of 2x10^{9} CFU mL^{-1}. On efficacy test, the reduction of the gray mold in blackberry crop and postharvest stages of rose crops is close to 60 %, with a minimum concentration of 1x10^{7} CFU mL^{-1} [3, 4].



*BA: Biological activity; PPS: Pilot Plant Scale; OPEX: Operating expenses

Figure 1. Pyramid scheme of the SG strategy developed by the Department of Bioproducts of AGROSAVIA.

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VARIATION AMONG PEANUT GENOTYPES IN TOLERANCE OF DROUGHT

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Peanut is an important edible oil crop which is greatly affected by drought. Water scarcity is the most serious environmental factor and a primary constraint for agriculture worldwide.

Two field experiments were laid out under drip irrigation system in Abo-Ghaleb, Giza, Egypt (located between 30°14'39.8"_ 30°15'45.9"N and 30°55'39.7" _ 30°56'50"E with an altitude of 18 meters) during summer 2017-2018. The objectives of the research were to study the effect of different irrigation levels on peanut yield as well as to determine the best mutant lines under drought. Forty nine peanut genotypes developed by directly irradiating the seeds of two cultivars (Giza 6 and NC). Mutagenesis was carried out using three different radiation doses of gamma rays (10-20-30 K. rad). The tested 49 genotypes (47 mutant lines and 2 parent varieties Giza 6 & NC) were evaluated under two irrigation levels as shown in (Fig., 1) namely low level by applying 50% and control by applying 100% irrigation. A split-plot design in a randomized complete block arrangement was used with two replications. The results displayed significant and highly significant differences in all studied traits in all genotypes. The mutant line no. 7 (20 K.rad Giza 6 irradiated mutant line) recorded the highest values in all studied traits except seed index and shelling percentages. Hierarchical clustering analysis categorized the tested 49 peanut genotypes intro three distinct clusters as shown in (Fig., 2). Cluster I includes mutant no. 7 only, while mutants no. 35, 42, 49 and 25 belonged to cluster II, while cluster III constituted of other genotypes.

Future research:

Mapping by sequencing will be applied for mutant identification. Proline, chlorophyll and mineral elements will be estimated.

Fig., 1 Irrigation levels of peanut genotypes under drip irrigation system.

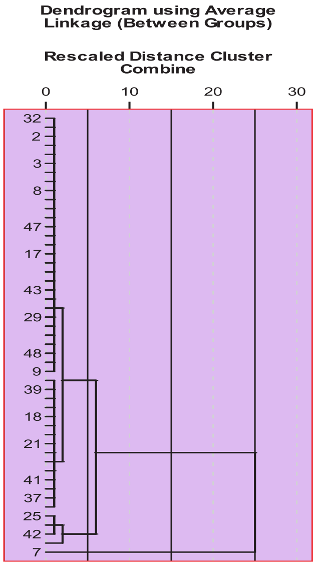
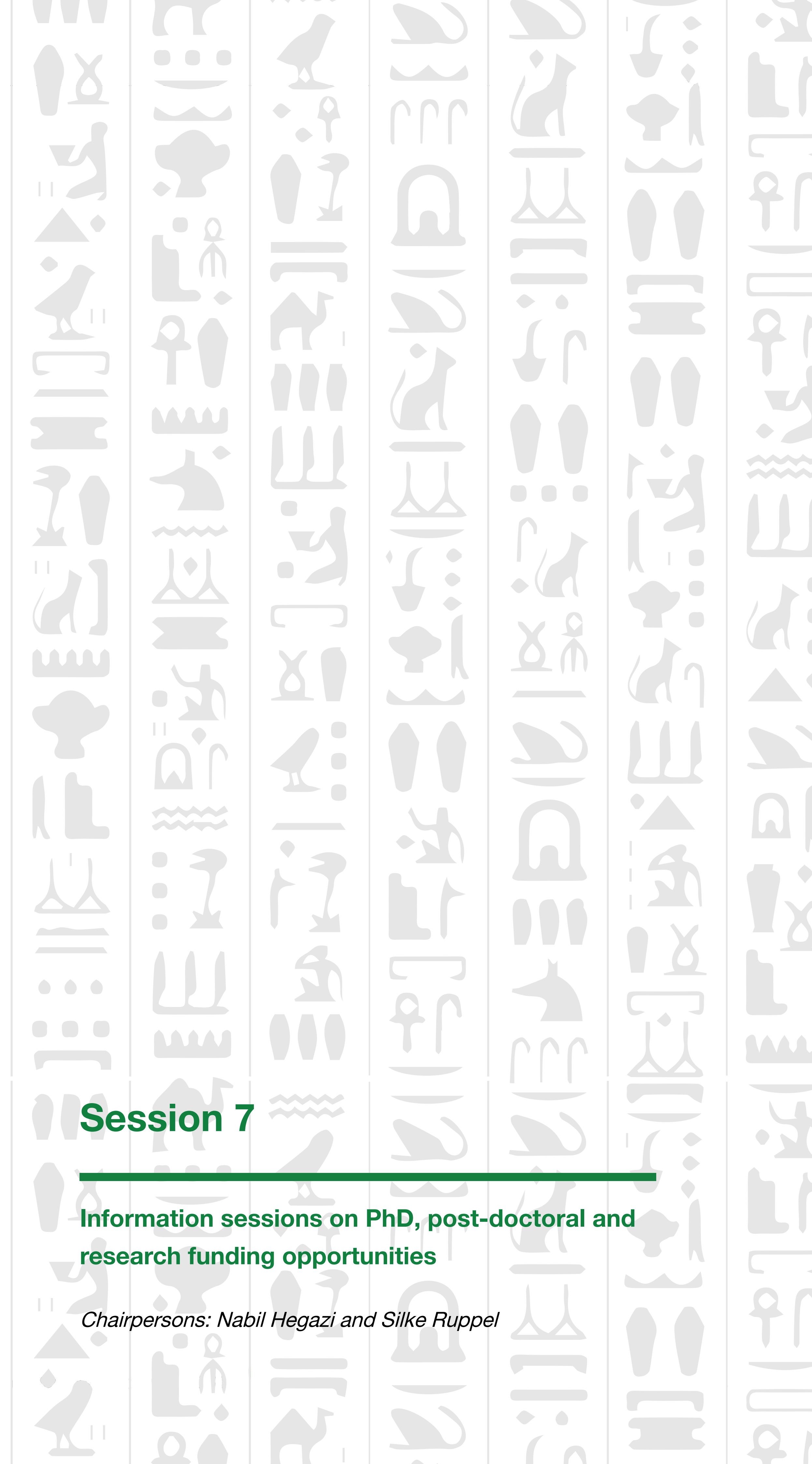


Fig., 2 Similarity levels of the estimated peanut genotypes using hierarchical cluster analysis.

Keyword: Peanut, mutagensis, cluster analysis, drought stress.

***We acknowledge the** support of Dept. of Agronomy, Faculty of Agriculture, Cairo University.

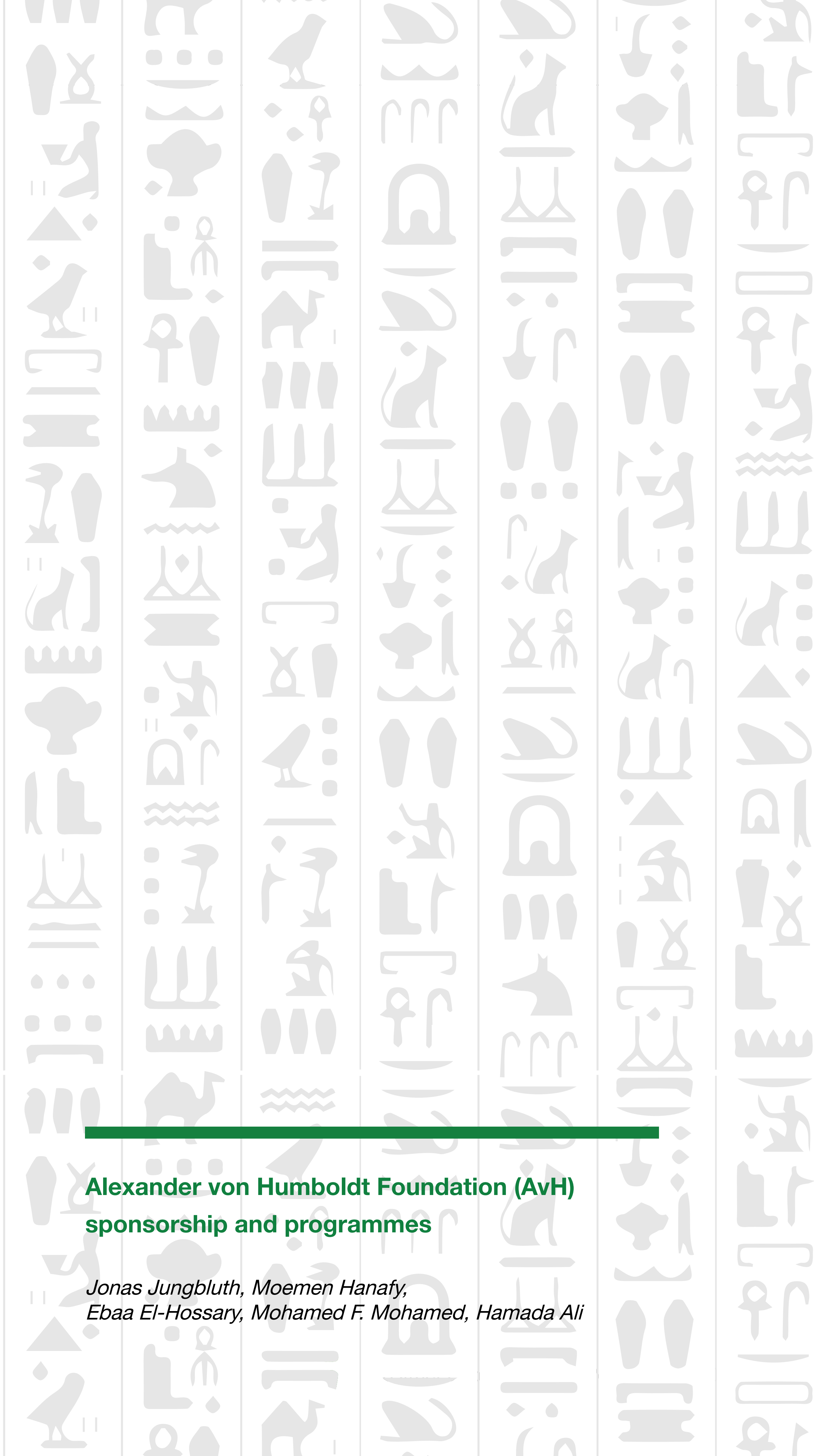
An early painting depicts an Egyptian couple harvesting crops



Session 7

Information sessions on PhD, post-doctoral and research funding opportunities

Chairpersons: Nabil Hegazi and Silke Ruppel



Alexander von Humboldt Foundation (AvH) sponsorship and programmes

Jonas Jungbluth, Moemen Hanafy,
Ebaa El-Hossary, Mohamed F. Mohamed, Hamada Ali

Alexander von Humboldt
Stiftung / Foundation

Greeting on the occasion of the Humboldt Kolleg „The plant Microbiome: Exploration of Plant Microbe Interactions for improving Agricultural Productivity”

November 18-22, 2018 Hurghada, Egypt

Dear Humboldtians,
Ladies and Gentlemen,

It is a great pleasure for me to visit Egypt again and to participate in the Humboldt-Kolleg „The plant Microbiome: Exploration of Plant Microbe Interactions for improving Agricultural Productivity”. On behalf of the Humboldt-Foundation I’d like to thank most cordially Professor N. Hegazi and his team for organizing this highly relevant Humboldt-Kolleg.

The Alexander von Humboldt-Foundation aims at supporting excellence and creating a global network of cultural and scientific dialogue at the highest level. We have sponsored more than 30,000 scientists never setting quota for countries of origin nor fields of research in the selection of future Humboldt fellows. Our only criterion was – and always will be – scientific excellence.

The Humboldt-Network in Egypt is of utmost importance for the Humboldt-Foundation in the MENA region. To date, we have granted research fellowships and research awards to 1.099 scholars and scientists from the MENA region and to 418 from Egypt. In the upcoming years we hope to increase jointly the number of applications from researchers who are highly qualified and aspire to research in Germany.

The purpose of Humboldt-Kollegs is to support the regional Humboldt-Network, to strengthen worldwide scientific cooperation, and to raise the interest of young scientists in our funding programmes and in Germany as a country of research. We are most grateful to all of you devoted Humboldt fellows who support our aims, our goals and the next generation of scientists by living up to our motto: "Once a Humboldtian, always a Humboldtian".

For this Humboldt-Kolleg, an eminent topic was chosen: “Exploration of Plant Microbe Interactions for improving Agricultural Productivity”. The question on “how microbes can feed the world” has become an exciting frontier of research. I am grateful that the Humboldt-Kolleg is contributing to this field of research and I am looking forward to the fruitful talks and discussions of the next days.

I wish you a memorable and splendid meeting with many new contacts, interesting scientific discussions, and private conversations.

Most cordial greetings,

Dr. Thomas Hesse
(Deputy Secretary General, Alexander von Humboldt-Stiftung)

POTENTIAL OF MARINE NATURAL PRODUCTS AGAINST DRUG-RESISTANT BACTERIAL INFECTIONS

Ebaa M. El-Hossary*, Miaomiao Liu, Tobias A. Oelschlaeger, Mohamed S. Donia, Ronald J. Quinn, Usama Ramadan Abdelmohsen

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Natural products have been a rich source of compounds with structural and chemical diversity for drug discovery. However, antibiotic resistance has been found in nearly every antibiotic once it is placed into clinical practice. In the last decade, pharmaceutical companies diminished their natural product discovery projects due to challenges, such as high costs, low return rates and high rediscovery rates. The largely unexplored marine environment harbors significant diversity and is a large resource to discover novel compounds with novel modes of action, which is essential for the treatment of drug-resistant bacterial infections. In this study, we report the compounds derived from marine sources, such as marine plants, bacteria, algae and others, that have shown *in-vivo* and *in-vitro* efficacy against drug-resistant bacteria. Analysis of the physicochemical properties of these marine natural products with activity against drug-resistant bacterial revealed 60% of compounds showed oral bioavailability potential. The aim of this review is to summary the diversity of these marine natural products, with a special focus on an analysis of drug bioavailability. Such biologically active compounds, with high degrees of bioavailability, have potential to be developed as effective drugs against infectious diseases in the near future.

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WILD COMMON EDIBLE MACROFUNGI DETECTION IN ASSIUT GOVERNORATE

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Macrofungi have been considered a great potential natural bioresource for the human diet and medication. They were regarded as early as the ancient Egyptian civilization era. Their role in recycling of organic wastes and relieving environmental pollution has been now well recognized (Mohamed et al., 2016). There are a number of field investigations conducted on assessment of wild edible macrofungi in Egypt. *Agaricus campestris*, *Agaricus rodmani* and *Collybia sp*. have been reported to be detected in wild natural status in Egypt. However, the previous studies neither conducted in Upper Egypt nor have reported the existence of *Agaricus arvensis* and/or *A. comtulus*. The aim of the present investigation was to identify edible wild mushrooms in different places of Assiut governorate.

The collected fruiting bodies samples were photographed, noted and reserved for laboratory assessment. The identification then was done according to Moser (1983). Herein we report detection of two wild *Agaricus* species in field expedition in Assiut governorate. Key macroscopic and microscopic morphological characteristic were identified and illustrated (Fig.1A, B and C and Fig. 1 D, E and F).

It is worth to mention that *Agaricus arvensis* and *A. comtulus* are not only edible but already have been used as human food and for medication (Mao, 1998). ***A. arvensis* can be domesticated and its anticipated that *A. arvensis* intercrop with higher crop species would increase growers income from soil area unit as additional crop besides its possible nutritional impact enhancing the productivity of the higher crop species (Mohamed et al., 2014).**

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Mohamed et al. 2014. Production of oyster mushroom (*Pleurotus spp.*) intercropped with field grown faba bean (*Vicia faba* L.) Asian J. Crop Sci. 6(1):27-37.

Mohamed et al. 2016. Fruiting bodies yield of oyster mushroom (*Pleurotus columbinus*) as affected by different portions of compost in the substrate Int J Recycl Org Waste Agricult 5:281–288.

Moser, M. 1983. Key to *Agarics* and *Boleti* (*Polyporales, Boletales, Agaricales, Russulales*), pp535.

Fig. (1): Photograph showing fruiting bodies (Pileus, Lamellae and Stipe) and spores of *Agaricus arvensis* (Fig.1A, B and C) and *A. comtulus* (Fig.1D, E and F) mushrooms.

Climate, land-use and invasive plants change plant community functional composition: an analytical study.

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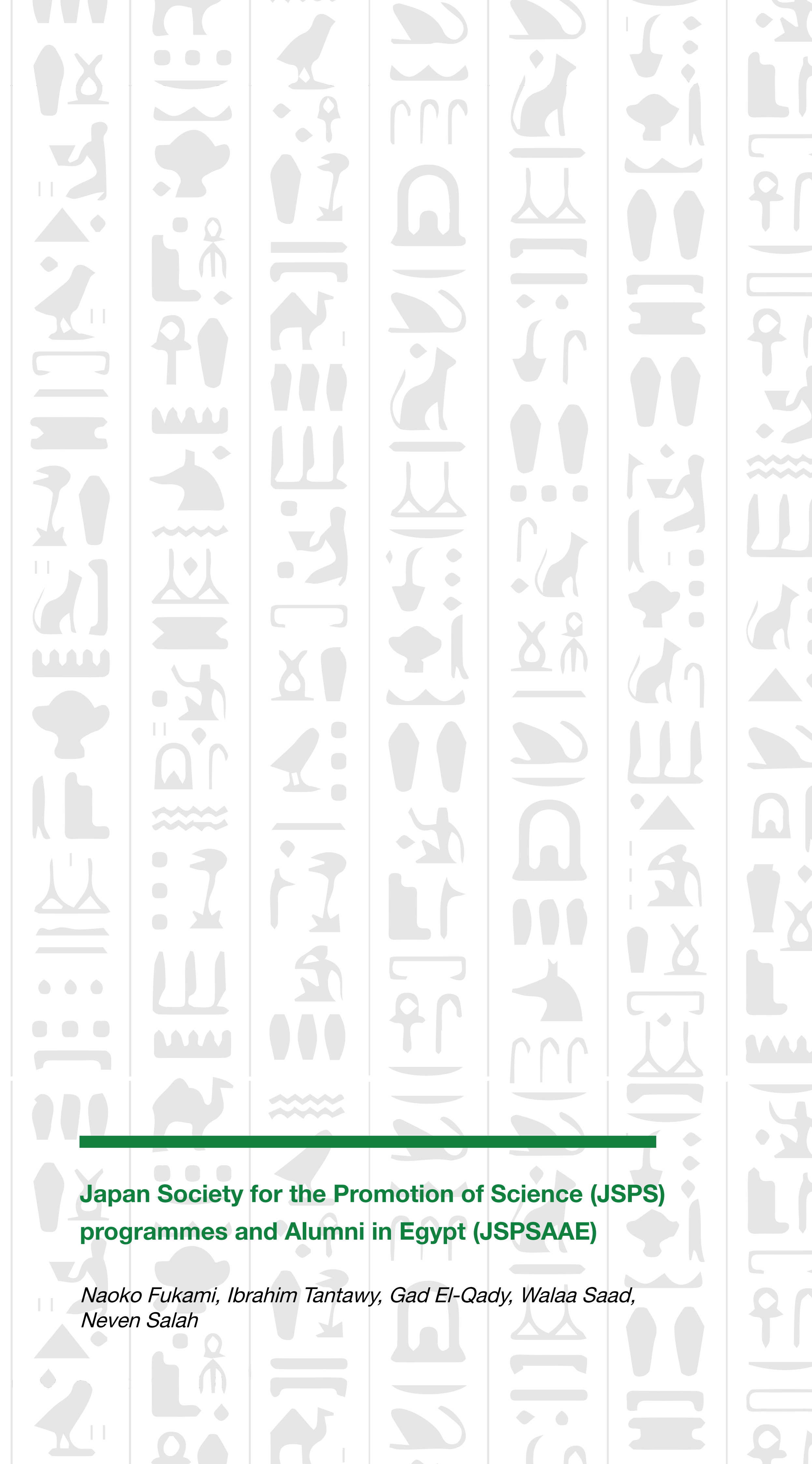
Abstract:

Understating the mechanisms of changing biodiversity is one of the most urgent issues under the situation of rapid biodiversity loss worldwide. Changes in plant species diversity can affect terrestrial ecosystem functions throughout the modifications in community functional composition. It is suggested that land-use change is the most important driver of global biodiversity change for terrestrial ecosystems. Therefore, land-use change may impact ecosystem function both directly (e.g. modifications in soil fertility and disturbance regime) and indirectly (e.g. modifications in plant functional composition and diversity). Various invasive plants are also known to decrease local plant species diversity. Changes in functional composition of plant communities due to invasive species therefore have the potential to increase ecosystem productivity and rate of nutrient cycling. Furthermore, invasive species can have higher trait phenotypic plasticity, measured as the ability to express different phenotypes in different environments. Climate change may also promote biological invasion; however, such interaction is still not clear. Therefore, understanding the interplay between land-use change, climate change and biological invasion is critical for predicting the consequences functional composition changes on ecosystem functions and services.

Here, I reviewed more than twenty different methods that can be used to link climate, land-use change and plant functional traits to ecosystem services. Moreover, I extracted data from the reviewed studies, that represented different geographic regions across the globe. Using these data, I modelled these different effects using structural equation models and leaner mixed effect models.

Keywords: climate change, ecosystem services, functional composition, invasive species, land-use change.



Japan Society for the Promotion of Science (JSPS) programmes and Alumni in Egypt (JSPSAAE)

Naoko Fukami, Ibrahim Tantawy, Gad El-Qady, Walaa Saad, Neven Salah

JSPS Cairo Research Station, Message from Director

We, JSPS (the Japan Society for the Promotion of Science) Cairo Research Station and JSPSAAE (the JSPS Alumni Association in Egypt) are very pleased that Plant Microbiome Symposium be held at Hurghada, and we, alumni members and staffs could join. Moreover, we are able to invite three up-and-coming professors from Japan and we could support six ambitious students.

Established in 1932 for the purpose of promoting science, JSPS is Japan's core research-funding agency. Its main functions are: 1) to foster young researchers, 2) to promote international scientific cooperation, 3) to award Grants-in-Aid for Scientific Research, 4) to support scientific cooperation between the academic community and industry, and 5) to collect and distribute information on scientific research activities. As part of its mission to build strong networks for advancing international joint research, JSPS has established ten overseas offices around the world. These offices serve as Japan's "Science Embassies" in their host countries and regions. As such, they promote and facilitate scientific exchange, disseminate information on scientific activities and developments in Japan, support Japanese researchers laboring abroad, and coordinate with JSPS alumni associations, among various other functions.

Among our overseas offices, the JSPS Cairo Research Station is one with the longest history. The present center was established back in 1984. It serves as JSPS's core hub in the North Africa and Middle East regions. Beginning with Egypt, the Cairo office serves countries in these regions by sustaining and advancing exchange between them and Japan. The office employs a highly diverse menu of activities in carrying out this mission.

Bringing researchers from Egypt, Germany, Japan and other countries together under one roof to engage in a discussion on plant microbiome, I believe this symposium will be the good chance to establish new research collaboration internationally including young researchers. I wish you all a very fruitful experience in today's symposium.

Naoko Fukami
The Director

JSPS Alumni in Egypt's activities.

Gad El-Qady

Prof. of Applied Geophysics

Editor and board member of JSPSAAE

In order to retain contact with Egyptian JSPS researchers and their host scientists in Japan, an Egyptian JSPS Alumni Association has been established with direct support and guidance from JSPS Office in Cairo. In the alumni association, previous as well as current JSPS fellows can become a member. We are enthusiastically welcoming Egyptian scientists who have awarded JSPS fellowships to join as a member of this association.

The objective of the Egyptian JSPS Alumni Association is to promote scientific exchange, research and cultural collaboration between Egypt and Japan, to encourage collaborative work between the JSPS fellows and host professors, and provide necessary information for JSPS fellows in Egypt for having a fruitful stay during his visit to Japan. We are inviting all former Egyptian JSPS fellows (including current fellows), who are interested to join in our alumni association activities as members. Let us act as a catalyst for a more fruitful Egyptian-Japanese scientific and cultural relationships.

On the other hand, JSPSAAE host an annual meeting as well as many regular scientific workshops and seminars organized by its members at different Egyptian universities and research institutions. These events considered as a windows for the alumni members to exchange their knowledge with their colleagues, students and young fellow. Some other events such as science café, student's forum are being organized. It is our hope that JSPSAAE grow in activities and cover all the scientific domains as well as academic and research institutions in Egypt.

THE PLANT MICROBIOME “ECOSYSTEM SERVICES” FOR HUMAN HEALTH

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Abstract:

The United Nations has set sustainable management of our planet's natural resources putting 33 goals .For reaching Goal 3; that includes reduction of communicable and non-communicable diseases which can be facilitated by practices enhancing diverse microbiota and implementation of knowledge of the microbiome. [1, 2]

Plants are a basic and essential part of our daily diet. Our food comprises all parts of plants that include their microbial habitats which can be colonized by up to 10^4–10^{10} microorganisms per gram of plant. [3]

This number of microbiota is expected to enter the body with our food. The “Ecosystem services” is a term, was described the critical functions which played by microorganisms in human health.

Humans gain health benefits from internalizing plant microbiota either through food or through breathing via air indoor and outdoor microbiomes. [4]

This review reveled that the edible plant microbiome and its diversity can influence human’s health through many different ways: (a) through increasing more diversity than our gut microbiome (b) through stimulating the human immune system, and finally (c) through the roles of indoor plant microbiome.

Conclusion: Human health has been influenced by Plant-associated microorganisms by direct and indirect ways. Plant microbiome could act as counterparts against pathogens within the microbial ecosystems. The Plant microbiome stabilizes the ecosystem, enhance biodiversity and avoid outbreaks of pathogens.

Keywords: plant microbiome- Ecosystem services – human health.

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EVALUATION OF ANTIMICROBIAL ACTIVITY OF *STREPROMYCES ALBUS* FSN2 AGAINST WOUND INFECTION IN EGYPT

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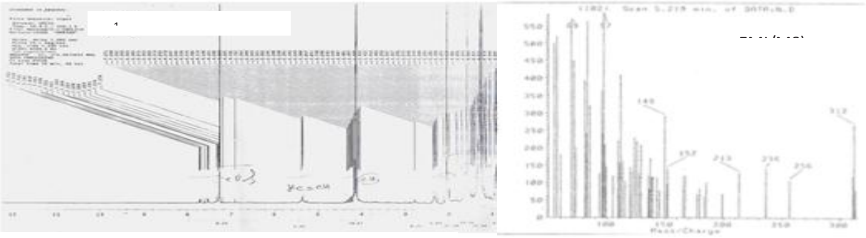
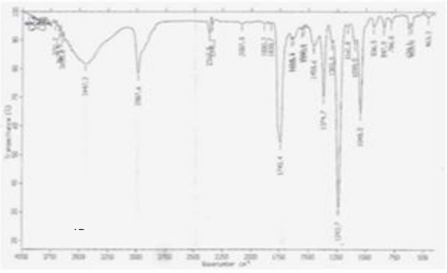
The aviability of antibiotics couldn't be the solution of infecious disesases widespread worldwide with the predection of increasing mortility. The emergence of antibiotic-resistant bacteria increased the indigence for searching of a new natural antimicrobial agent's sources. Filamentous soil bacteria, *Streptomyces* sp. considered as a useful natural source of bioactive secondary metabolites. We evaluated the effect of bioactive metabolites of *Streptomyces* isolates against MDR bacteria from wound infection.

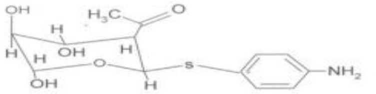
Agricultural soil samples were collected from a different area in Egypt & characterized depend on cultural, morphological & biochemical characters. Additionally, *Streptomyces* isolates were screened by disk diffusion method against fifty cases of MDR resistant pathogens obtained from the wound infection unit. Further, the growth conditions were optimized to improve the production yield of the antimicrobial agent. The extraction and purification of the antibacterial agent in addition to a physiochemical characteristic of the crude compound were done.

A total of 125 isolated actinomycetes have 45 isolates belonging to *Streptomyces* species & ten isolates showed highest antimicrobial activity against tested bacteria. Genetically, one isolate, identified as *Streptomyces albu*s FSN2 based on sequence analysis of 16S rRNA gene Figure 1, showed a broad-spectrum activity. Crude ethyl acetate extract of FSN2 was purified to homogeneity using starch at pH 7 for 5 days with a molecular mass of m/z 312. Nuclear magnetic resonance, structural elucidation showed that the compound (SN12) was aminoglycoside derivatives. Figure 2

Our finding indicates that *Streptomyces* is a useful store of a highly active compound that is recommended for infection recovery. The SN12 compound should be applied to pharmaceutical & industrial application.







P- Aminophenyl-1-thio-2-acetamido -2- deoxyglucopyranoside

Structure elucidation of SN12

References:

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Development of Novel Antimicrobial Agents Based on Plant-Derived Natural Products

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Nature continues to supply us with a wealth of chemically diverse compounds that in eons of evolution have been optimized to modulate biochemical pathways. Nowadays, both industrial and academic groups are rapidly exploring living organisms to further increase the available pool of natural products. Hence, the availability of these compounds and their uniqueness in terms of unprecedented structures will provide new leads for the pharmaceutical industry for the development of novel drugs. As part of a larger project for developing more potent and safer lead compounds based on natural product alkaloids, we have developed robust and efficient synthetic method for the natural product alkaloid, neocryptolepine (Figure 1) isolated from the shrub *Cryptolepis sanguinolenta* used in Central and West Africa in traditional medicine for the treatment of infectious diseases. A lead optimization program was started to modify the lead compound, and a series of novel neocryptolepines with different substituent at position 11 were synthesized and evaluated for their in vitro antimicrobial potency against Gram positive and Gram-negative bacteria. Most of the tested compounds exhibit significant antibacterial effect against all tested organisms at the minimum inhibitory concentrations (MICs), 1-10 μg/mL. More details about the synthetic routes of these scaffolds, their antimicrobial activities will be presented.

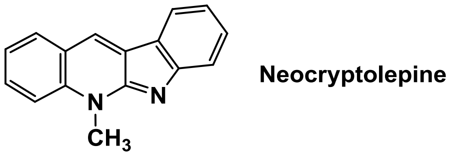

Figure 1. Natural product alkaloid, neocryptolepine from *Cryptolepis sanguinolenta*

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DAAD funding mechanisms, PhD and Postdocs study in Germany. Pursuing your PhD and Posdoc at the Technical University of Munich (TUM)

Heba Afifi

The Egyptian-German Day 2018-4: Current progress in student activities of Cairo University within the frame of cooperation with DAAD, AvH, BMBF/STDF, IGZ *(oral & poster presentations)*

Nabil Hegazi

HUMAN MIROBIOME ENGENEERING

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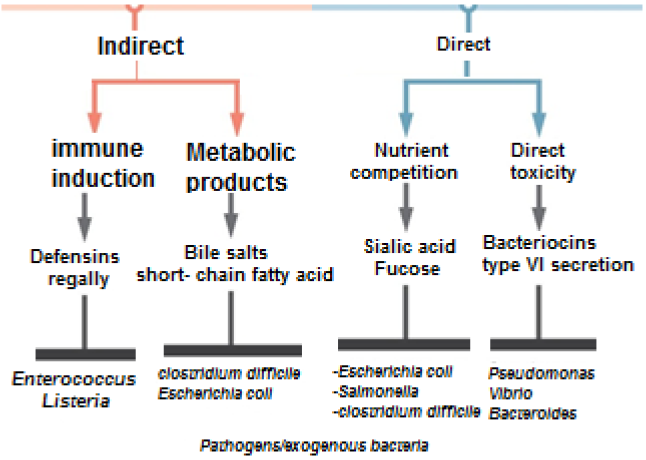
Background

- Microbiomes are composed of diverse microbial communities
- Because of their ubiquity, this microbial Consortium has a great implications on health and disease of it is host (i.e Human, plant, animal or soil)
- Reversing imbalance of microbiomes -via modifying microbiota structures- can lead to improveing human metabolism, immunity and neuro-endocrine regulation.
- Despite progress in improvement of recombinant therapeutic microbial consortia, or targeted bacteriocin, hospitalization techniques still face enormous challenges, unknowns and long-term side effects.

Theory and application

- Colonizing microbial communities resulted in interacting among themselves and host immune system by forming interlinked strong nutrients networks (as colonization resistance).
- Given the massive number and diversity of these Consortia, mechanisms of interactions between human (as host) immune system and microbiome are proposed. (Fig.1)



(Fig. 1) Intestinal microbiota oppose to the settlement and over growth of exogenous bacteria

Examples and future prospective

- Future prospective denotes a huge expectation on optimizing a DNA memory device
- This device would enable us to populate a genetically modified niche capable of releasing specific molecules (e.g. antibiotics) in response to specific stimuli (e.g.infection).(Table1)

Engineered microbe	Induced change by genetic engineering	Result
Lactobacillus casei	**Expressing protein C of pneumococci**	**Reduction of nasopharyngeal colonization of pneumococci in mice**
Lactococcus lactis	**Expression of surface-associated flagellin of *Bacillus cereus* CH strain**	**Competitive inhibition of adhesion of intestinal pathogens like *Salmonella enterica***
	Express recombinant hemagglutinin of H5N1 influenza virus (vaccine)	**Antibody response and immunity against H5N1 influenza virus**
Lactobacillus jenseni	**Colonized vaginal mucosa and secretion of cyanovirin-N (HIV-1 entry inhibitor) locally in monkeys**	**Protection from simian HIV infection**
E.coli	**Production of AI-2 and CAI-1 (autoinducers) in high concentration**	**Quorum sensing mediated repression of cholera toxin production by *V. cholerae*.**

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4606237/table/T2/?report=objectonly

(Table 1) Successful outcomes of human microbiome engineering

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WATER EFFECT ON SOIL MICROBIOME COMMUNITIES SURROUNDING PLANT ROOTS

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Water plays a basic role in the living organisms' life; representing 70% of the living organisms` bodies. This role extends to the plant microbiome, which is important to plant by various symbiosis processes.
Example 1: In recent studies, it was noticed the water effect on indigenous microbial communities in the rhizosphere of Wheat. One of the microbial communities is Phz+ rhizobacteria and one of its members is *Pseudomonas synxantha 2-79*, that produces type of phenazine called PCA that plays a role in suppressing pathogens. After three years of Wheat cultivation in, it was noteworthy that there was a decline in the number of plants colonized by Phz^+ Rhizobacteria under irrigation (23%), while 60% of the Phz+ producing PCA Rhizobacteria in non-irrigated still present (Fig.1).

Example 2: In an experiment for irrigation pine trees *(Pinus sylvestris)* for a decade in Scots pine forest in Switzerland, it was remarkable that there was no increase in the microbial biomass at different depths. There were no shifts in fungal-to-bacterial biomass ratio was observed which was surprising as fungi are proposedly more resistant to water limitation than bacteria, which leads to lower fungal-to-bacterial biomass ratio under irrigation (Fig.2)

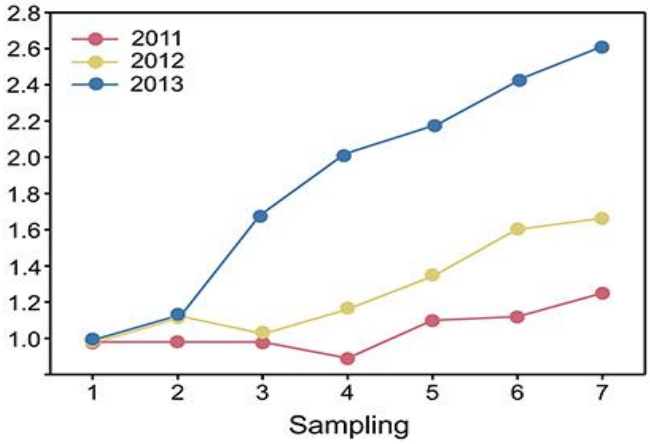

Fig. 1 Ratio of plant colonization frequencies (dryland/irrigation) by Phz+ bacteria, (vertical) log CFU per rhizosphere or gram of root fresh weight, (horizontal) sampling after each season.

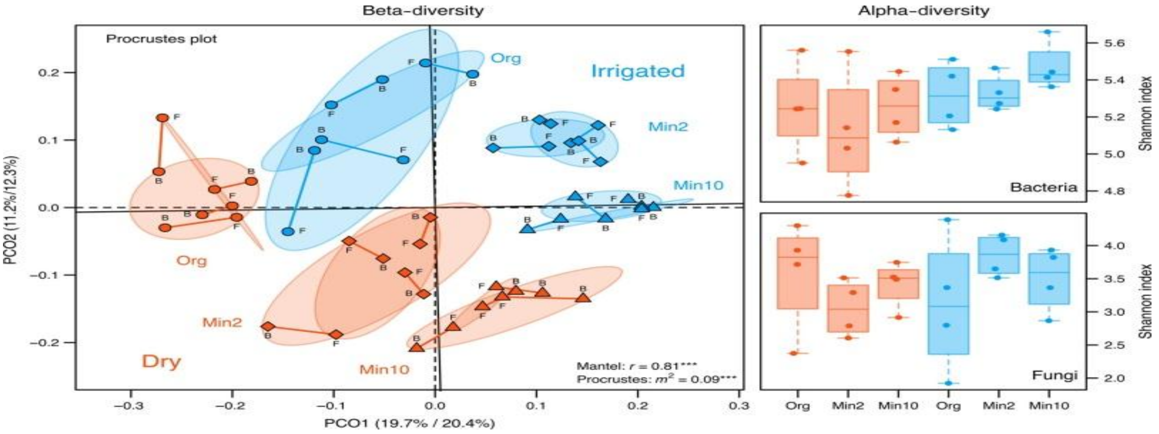

Fig. 2 The graph shows the 3 depths at which samples of soil surrounding pine trees roots containing the microbiome from bacteria and fungus were taken.

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THE ERA OF OMICS TECHNOLOGIES

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Omics is a new approach enables researchers use aspects of central dogma, which are genomics, transcriptomics, proteomics and metabolomics in scientific achievements in which they characterize, quantifies pools of biological molecules, and understand the structure, function and dynamics of biological systems.

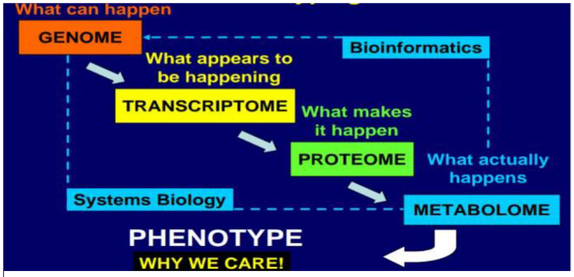

Figure 1 Central Dogma processes in biological system

Hans Karl and Tom Roderick planted the first seed of genome in 1920 and 1986 respectively. Genomics studies organism's entire DNA sequence to Identify coding and non-coding sequences and their structural and functional properties.

Transcriptomics appeared in 1991, when an attempt made to capture a partial human transcriptome and reported 609 mRNA sequences from the human brain. Transcriptomics studies all the transcribed sequences and their modifications such as 5’ G Capping, 3’ poly-A tailing, intron splicing and RNA silencing. Transcriptomics have various approaches in different aspects e.g. gene function annotation, diagnostics, disease profiling, targeted therapy and noncoding RNA

While proteomics has been developed by Marc Wilkins in 1994 to isolate, predict and display protein structures and analyze their properties. Nowadays it has a high impact in medical and environmental researches particularly plant-microbe interactions

Roger Williams developed metabolomics in 1940 while observing schizophrenics. The metabolomics studies all metabolites in an organism produced from proteins involved in central dogma. Its applications are scarce but it is applied in plant nutrition and quality, nutrigenomics, oncobiology.

Ultimately, omics technologies can build the foundation for a new era of information-guided microbial cultivation technology and reduce the inherent trial-and-error search space and how information that can be extracted from multi-omics data can be applied for the cultivation of hitherto uncultured microorganisms. The numbers and diversity of microbes in ecosystems within and around us is unmatched, yet most of these microorganisms remain recalcitrant to in vitro cultivation. Various high-throughput molecular techniques, collectively termed multi-omics, provide insights into the genomic structure and metabolic potential as well as activity of complex microbial communities.

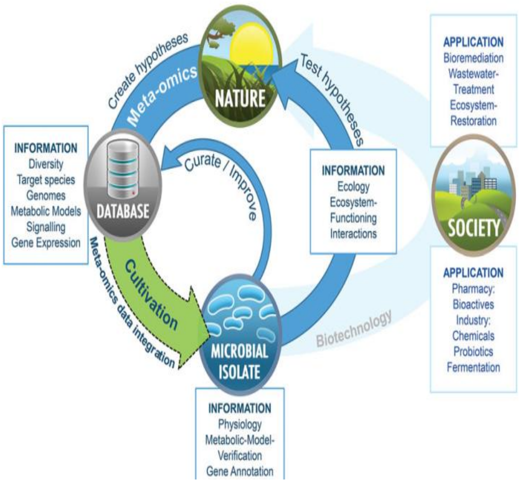

Figure 2 A model depicting the positive feedback loop between multi-omics data generation and isolation of yet uncultured microorganisms

Reference: -

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THE NECRO-MICROBIOME AND MICROBIAL CLOCK

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Post-mortem interval (PMI) is the time that has elapsed since a person has died, it is a very important tool in criminal investigations. Different methods are being used to estimate the PMI and since each method has its own limitations, researchers tend to develop new methods in order to increase the accuracy and precision of estimating the PMI. "The Necro-microbiome" is one of the emerging methods, it refers to microorganisms associated with decaying corpses (Fig. 1).

Bacteria have a great influence on the decomposition process (Fig. 2). It is a dynamic mini-ecosystem in which bacterial communities change over time (Fig. 3). Anthropological research facilities are where people choose to donate their bodies after death to be a part of forensic experiments, in which corpses are placed on soil left to decompose. Researchers take samples from different parts of the body every day performing culture-independent methods (e.g. Pyrosequencing) to determine what microbes should be there at a particular time point. If the post-mortem succession of microbial communities is repeatable and predictable (Fig. 3), researchers will be able to use acquired data to construct a Microbial Clock that begins from the moment of death till the end of decomposition. Therefore, unknown samples can be matched with different time points on the clock and by picking up their corresponding ones, we will be able to pinpoint the PMI.

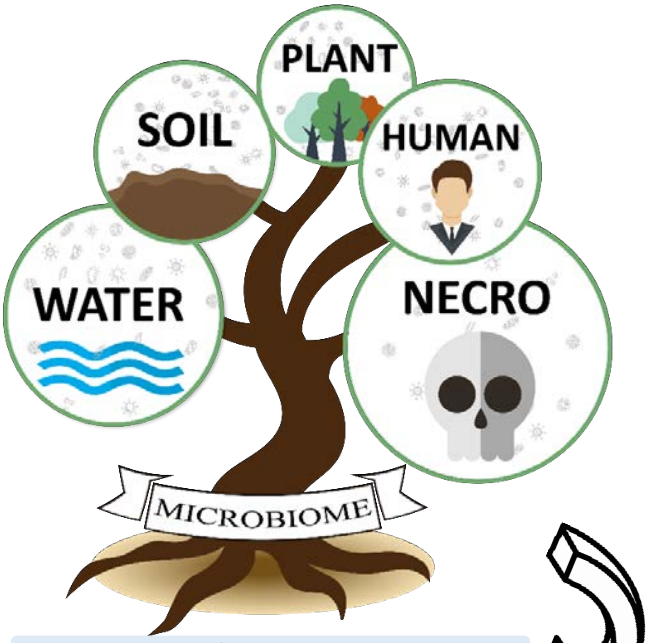

Fig. 1 An illustration of some environmental microbiomes.

FRESH
Shortly following death, circulation fails, and blood drains to the lower parts of the body, creating a blue discolouration. Muscular tissues become gradually more rigid. Cellular enzymes begin breaking down surrounding body tissue.

BLOAT
Gases begin to accumulate within the body, causing a bloated appearance, and force fluids to escape from wounds and body orifices. Ultimately, the pressure of gases and fluids can rupture the skin.

ACTIVE DECAY
Maggots and aerobic microorganisms enter the body where they feed on body tissue, leaving and returning as they go through stages of their lifecycle. Fluids accumulate around the body, accompanied by strong odours.

ADVANCED DECAY
Maggots leave the body in a state of advanced decay, with a corresponding increase in soil carbon and nutrients such as phosphorus, calcium, magnesium and—in particular—nitrogen.

DRY REMAINS
All soft tissue is gone, leaving only dry skin, cartilage and bone—bleached white where it has been exposed to the elements. There may be increased plant growth as a result of additional nutrients in the soil.

Fig. 2 An illustration of the decomposition process.

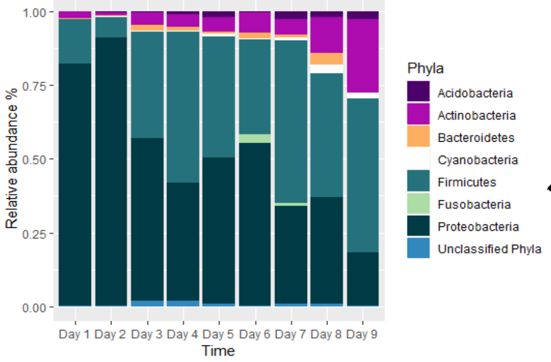

Fig. 3 Stacked bar-chart illustrates the relative abundances of the predominant bacterial phyla present in the decomposing body over time.

References:
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THE WONDER OF ARCHAEA

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Carl Woese discovered the "Archaea" as a third domain of life. They were discovered when using "Sanger RNA sequencing technique" on a class of methane-generating organism "Methanogens " the result confirmed that archaea is completely different from bacteria.

An important feature of cultivated Archaea were generally found in extreme environments includes extremes of **Temperature**, **Salinity**, **pH** or **Oxygen** availability at which life could be sustained (Table.1), Where Some are found in colonies recycle elements such as carbon, nitrogen and sulfur through their various exterme habitats (Fig.2).

From the time Archaea were discovered until recently Archaea supposed to live in their extreme environment, Now Archaea supposed to be part of human microbiome.

A team of researcher examined 13 volunteers and they were surprised to find archaea "Thaumarchaeal" on the skin which play a role in nitrogen turnover and skin health, They tested the hypothesis that the abundance of archaea on human skin is influenced by human age and skin physiology on 51 different skin samples taken from human subjects of various age (Fig.2).

This new research confirmed that bacteria are not the only mircoorganisms on our body and that archaea is playing also roles on the human microbiome.

Phylum	Environment	Place	Function	Example
Euryarchaeota	Extreme Halophiles	Dead sea	Control osmotic balance	*Halobacterium salinarum*
		Waste water	Cycle Carbon	*Methanobacterium bryantii*
Crenarchaeota	Thermoacidophilies	Sulfur spring	Sulphur Cycle	*Sulfolobus solfataricus*
	Hyperthermophilies	pool Magma	Cycle Nitrogen	
Nanoarchaeota	Thermophiles	Yellowstone National Park	Parasitism	*Nanoarchaeum equitan*
Korarchaeota	Hydrothermal	hot Little creek/Hot spring	Variety	*Korarchaeum cryptofilum*

Table.1 Classification of Archaea.

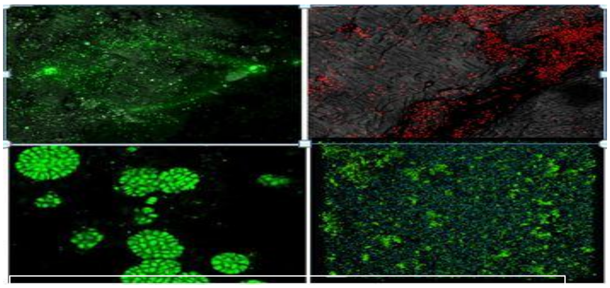

Fig.1 colony of Archaea

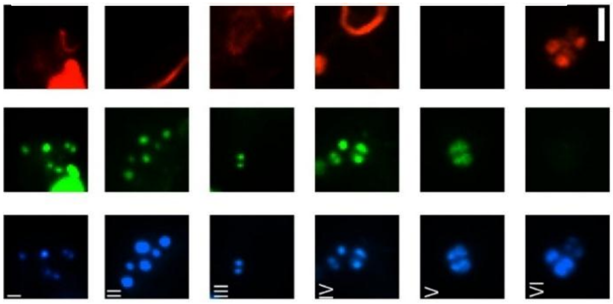

Fig. 2 Fluorescence images of archaeal cells in skin wipe samples.

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ARE ALL BACTERIA CULTURABLE?

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We are grossly ignorant of bacterial life on earth. Environmental microbiologist estimates that less than 1% of bacteria can be cultured in the laboratory. “Unculturable or Uncultivable” indicates that current laboratory culturing techniques are unable to grow a given microbiome in the laboratory; it doesn’t mean can never be cultured but, rather, signifies that we lack critical information on their biology (Fig.1)[1].

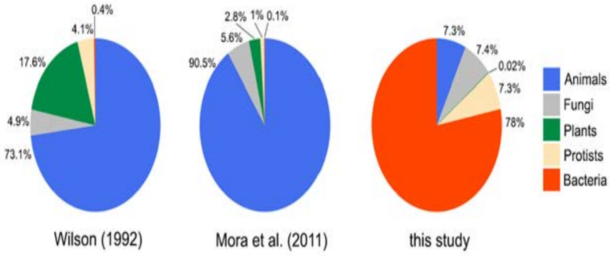

Fig.1 proportion of newly discovered bacteria along the years.

Q.1: How did we know that there are unculturable bacteria? By using several methods;
1. laboratory media: by studying the media in the laboratory and in the petri dish. We found that the number of microorganisms in the lab is more than which we can grow in the petri dish that means there are microorganisms we did not study.
2. phylogenetic identity: we have a question here; could they be non-living organisms? By checking those cells we ensure that they are living microorganisms.
3. molecular tools proved that these organisms have a bacterial genome.[2]

Why are they not growing in the lab? Because of failing to replicate essential aspects of their environment (Fig.2A) and bacterium dependence (Fig.2B) that there are types of bacteria cannot live without their dependence on other organisms.[3]

Fig. 2A

Fig. 2B

Fig.2A Bringing the environment into the lab.
Fig.2B Culturing the unculturable.

Conclusion; our knowledge about bacteria is very small because there are a huge amount of bacteria we are not able to cultivate in the laboratory, but if we study them we may be able to culture them.

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The Plant Microbiome
Hurghada, Egypt - 2018

Unterstützt von / Supported by

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