



Risk Assessment of Endophytes

Joint Meeting of Working Groups

1 and 4

3-7 November 2014

İzmir, Turkey

COST Action FA 1103

Endophytes in Biotechnology and Agriculture



Chair of Action

- ✓ Carolin Schneider

Scientific Committee

Working group 1: Ecology of endophytes

- ✓ Leo van Overbeek
- ✓ Andrea Campisano

Working group 2: Identification of new competent endophytes

- ✓ Angela Sessitsch
- ✓ Birgit Mitter

Working group 4: New industrial products in life sciences

- ✓ Anna Maria Pirttilä
- ✓ Frank Surup

Local Organizer Committee

Ege University

- ✓ Hatice ÖZAKTAN
- ✓ Lalehan YOLAGELDI
- ✓ Mustafa AKBABA
- ✓ Sercan PAZARLAR

COST Action FA1103: Endophytes in Biotechnology and Agriculture

WG1-4 Meeting on “RISK ASSESSMENT OF ENDOPHYTES”

03-07 November 2014

University of Ege, Faculty of Agriculture,
Department of Plant Protection, Bornova - İzmir/TURKEY

PROGRAM OF THE MEETING

Monday, 3th November

- 13:30-14:00 Registration
- 14:00-14:30 Opening address, **Dr. Carolin Schneider**
Welcome address and presentation of the hosting university by local organizers
-

1st session (WG2): Mutualistic and pathogenic plant-endophyte interactions: what makes the difference?

Chair person: **Birgit Mitter**

- 14:30-15:00 Determination of enzymatic activity of endophytic *Fusarium* spp. and their interaction with Fusarium crown rot disease, **Tunalı Berna**
- 15:00-15:30 Risks evaluation of endophytic bacteria's anarchic proliferation within *in vitro* tissue cultures, **Fki Lotfi**
- 16:00-16:30 **Coffee break**
- 16:30-17:00 Is endophytes expression a constraint in olive tree micropropagation? **Maalej Muhammed**
- 17:00-17:30 Ecological functions of fungal endophytes of grasses, **Zabalgogazcoa Iñigo**
- 18:00-20:00 **Dinner (Rectorate Restaurant in Bornova)**

Tuesday, 4th November

1st session (WG2): Mutualistic and pathogenic plant-endophyte interactions: what makes the difference?

Chair person: Birgit Mitter

- 09:30-10:00 Fraxinus Dieback In Europe: Understanding Disease Phenomenon And Elaborating Guidelines For Sustainable Management, **Vasaitis Rimvys**
- 10:00-10:30 Plant-associated microbes as biocontrol agents against common ragweed, **Widhalm Siegrid**
- 10:30-11:00 **Coffee break**

2st session (WG1): Human pathogens in plants

Chair person: Andrea Campisano

Keynote lecture

- 11:00-12:00 Plants as reservoir for human pathogenic bacteria, **Schmid Michael**
- 12:00-12:30 The human-associated bacterium *Propionibacterium acnes* as a grapevine endophyte, **Campisano Andrea**
- 12:30-13:00 Genotypic differences in three novel *Pantoea ananatis* strains having different impacts on the host plant, **Sheibani-Tezerji Raheleh**
- 13:00-14:00 **Lunch (Faculty's restaurant)**

Keynote lecture

- 14:00-14:45 **Jackson Robert**
- 14:45-15:15 *Mycobacterium* sp. as endophytes – rare or unnoticed? **Pirttilä Anna Maria**
- 15:15-15:45 Importance of endophyte *Aspergillus terreus* in biotechnology and public health, **Mohamed Mahmoud Fadhela**
- 15:45-16:15 **Coffee break**
- 16:15-17:00 Strategies used by *Salmonella* to colonize plants, **Schikora Adam**

- 17:00-17:30 Genotyping and phylogeny of *Phaeoacremonium* genus associated with necrotic wood and infections in human, **Berraf-Tebbal Akila**
- 17:30-18:00 A whole-genome comparison of virulence traits in endophytic genomes of Enterobacteria, **López-Fernández Sebastian**
- 20:30 **Social Dinner (Endülüs, Turkish Tavern in Alsancak)**

Wednesday, 5th November

3st session (WG4): Production of mycotoxins, phytotoxins and other undesired secondary metabolites by biocontrol agents

Chair person: Frank Surup

- 09:30-10:00 Taxonomic diversity of *Epichloë* endophytes affects the alkaloid profile of *Lolium perenne* in natural pastures, **Soto-Barajas M. Carlos**
- 10:00-10:30 New Natural Products From Phytopathogenic Fungi, **Surup Frank**
- 10:30-11:00 Fungal crafted phenalenones and their phylogenetic diversity, **Tejesvi Mysore V.**
- 11:00-11:30 **Coffee break**
- 11:30-12:00 Risk assessment of an innovative biological control strategy using endophytic entomopathogenic fungi, **Alkhayat Dalia Muftah**
- 12:00-12:30 Obstacles in developing a biocontrol agent with endophytes, **Schulz Barbara**
- 13:00-14:30 **Lunch (Faculty's restaurant)**
Turkish coffee will offer in the faculty café
- 14:30-15:00 Mycotoxins and other exometabolites from *Fusarium* and related genera, **Thrane Ulf**
- 15:00-15:30 Novel biologically active compounds isolated from the symbionts of plants and insects, **Kolařík Miroslav**
- 15:30-16:00 Using the RPB2 single-copy gene as an alternative molecular marker compared with ITS and investigated on the *in vitro*-assembled mock community, **Zelenka Tomas**

Thursday, 6th November

09:00-11:00 Management committee meeting

Field Trip to Organic Farm in Kuşadası and guided tour in Ephesus Ancient City

Meeting point: Agricultural Faculty at 10.45 a.m.

13:00-14:00 Lunch (Değirmen restaurant in organic farm)

14:00-15:00 Visit to organic farm

15:30-17:00 Guided tour in Ephesus Ancient City

Friday, 7th November

10:00-13:00 Guided tour in Izmir

SESSION 1
WORKING GROUP 2

Mutualistic and pathogenic plant-endophyte interactions: what makes the difference?

Determination of enzymatic activity of endophytic *Fusarium spp.* And their interactions with *Fusarium* crown rot disease

Berna Tunalı¹, Bayram Kansu¹, Filiz Gürel², Fatih Ölmez³, Elif Çepni²

¹Ondokuz Mayıs University, Agriculture Faculty, Plant Protection Dept., 55139, Atakum, SAMSUN

²İstanbul University, Faculty of Science, Molecular Biol. and Genetic, 34134, Vezneciler, İSTANBUL

³Field Crop Central Research Institute, Biothecnology Dept.,06172, Yenimahalle ANKARA

Endophytes are of potential antagonist group against many pathogenic fungi in plants. The most common one of these pathogenic fungi is *Fusarium* genus, but includes endophytes, saprophytes and other features on its host or soil.

The aim of this study was evaluate to biological effect and enzymatic activity of seventy-eight endophytic *Fusarium spp.* (EFS) from some species of *Gramineae* family, *in vivo* and *in vitro*. While antibiosis effect, dual test and spore production of endophytes against pathogenic *F. graminearum* and *F. culmorum* have conducted for biological parameters, protease, cellulose and chitinase enzymes were used for enzymatic activity.

As a result of this work, twenty-four and forty-eight isolates of EFS have founded enzymatic zone for protease and cellulose enzymes, respectively. Until now, twenty-two isolates of EFS were tested and only seven isolates were founded active for chitinase. The colony diameter of isolates that had activity on media was changed between (max. – min.)0.36 - 5.7cm, 0.1 – 0.25cm and 0.33 – 4.6cm after cellulose, protease and chitinase analyses results respectively.

For the antibiosis, the first factor on biological effects, two isolates of *F. oxysporum* have founded the most effectiveness against pathogenic *F. graminearum* and *F. culmorum*. Secondly, for the dual test, while three isolates belong to *F. poae*, *F. equiseti* and *F. culmorum* have showed the most effective against pathogenic *F. graminearum*, one isolates of *Fusarium sp.* had the most effective against pathogenic *F. culmorum*. Lastly, to the effect on spore production of endophytes, two *F. oxysporum*, one *F. proliferatum* and one *Fusarium sp.* were founded as the most effective against the pathogen spores.

Tunalı *et. al.* (2011) reported that some of the EFS isolates were analyzed for three important mycotoxins (zearelenone, nivalenol and derivates of deoxynivalenol) of *Fusarium* and measured very diverse amounts of these mycotoxins.

The EFS isolates, had reported by Tunalı and Kansu, (2010) and Kansu *et. al.* (2013) that no pathogenic effect on wheat, are continuing to test for pathogen-endophyte interaction into the different reaction types (resistance, tolerant and susceptible) of wheat cultivars. If all of these findings can combine for each EFS isolate, we could

have been risk assessment as a potential biological control agent for both hosts and pathogens.

Tunali, B., Kansu, B., 2010. Pathogenicity study on endophytic *Fusarium* spp. from cultivated wheat (*Triticum aestivum* L.) plants. *11th European Fusarium Seminar, 20-23 September, Warsaw-Poland*, p.335.

Tunali, B., Kansu., B., Aksu, P., Büyük, O., 2011. The determination of mycotoxin producing ability of some endophytic and pathogenic *Fusarium* isolates from wheat samples in Turkey. *2nd Mediterranean Workshop on Mycotoxins and Toxigenic Fungi, 12-14 October, Istanbul-Turkey*, p.264.

Kansu, B., Tunali, B., Ölmez, F., 2013. The effects of endophytic *Fusarium* treatment on wheat. *Endophytes for Plant Protection, the state of the art, 27-29 May, Berlin-Germany*, p.188.

*This study was supported by TUBITAK-TOVAG No. 111O835

Risk evaluation of endophytic bacteria's anarchic proliferation within *in vitro* tissue cultures

L. Fki^{1*}, A. Nasri, O. Chkir¹ M. Maalej¹, H.M. Shumacher² and N. Drira¹

¹ Laboratory of Plant Biotechnology, Faculty of Sciences of Sfax- Route Sokra BP 1171, 3000 Sfax, Tunisia, University of Sfax, Tunisia,

² Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures GmbH, Inhoffenstr. 7b, 38124 Braunschweig, Germany

Endophytic bacteria expression during *in vitro* propagation of plants is a major constraint which obstructs large scale propagation schemes. This study has been designed to analyze the consequences of the anarchic proliferation of endophytic bacteria during *date palm* micropropagation and to develop a strategy to minimize culture losses. Endophytic bacteria expression was generally detected when cultures were under stress conditions such as temperature fluctuation and delayed subculture. Embryogenic culture and bud clusters proliferation were seriously affected by the anarchic proliferation of endophytic bacteria. They slowly turned yellow and brown. Although some part of the tissue continued to form new shoot buds and somatic embryos, within 2 weeks all the tissues necrosed. It became impossible to continue cultures any longer. Only juvenile leaves could be used to establish relatively clean *in vitro* tissue cultures. Immaturity of vascular tissue in these explants may explain the scarcity of contaminated cultures. Defining physico-chemical conditions hampering bacterial growth, without affecting plant cell proliferation can be a promising way to overcome anarchic proliferation of endophytic bacteria. To sum up, we can conclude that endophytes have negative consequences on *in vitro* tissue cultures when they become out of plant cell control.

Fki L., Bouaziz N., Chkir O., Benjemaa-Masmoudi R., Swennen R., Rival A., Drira N. & Panis B. (2013). Cold hardening and sucrose treatment improve cryopreservation of date palm meristems. *Biologia Plantarum* 57 (2): 375-379.

Fki L., Bouaziz N., Kriaa W., Benjemaa-Masmoudi R., Gargouri-Bouazid R., Rival A. & Drira N. (2011). Multiple bud cultures of 'Barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. *Journal of Plant Physiology* 168: 1694 – 1700.

Fki L., Bouaziz N., Sahnoun N., Swennen R., Drira N. & Panis B. (2011). Palm cryobanking. *CryoLetters* 32(6): 451-462.

Fki L., Masmoudi R., Kriaa W., Mahjoub A., Sghaier B., Mzid R., Mliki A., Rival A. & Drira N. (2011). Date palm micropropagation via somatic embryogenesis. Jain S.M., Al-Khayri J.M., and Johnson D.V. (eds.), *Date Palm Biotechnology*, Springer, Netherlands. pp. 47-68.

Is endophytes expression a constraint in olive tree micropropagation?

M. Maalej¹, L. FKI¹, A. Chaari², N. Drira¹

¹ Laboratory of Plant Biotechnology, Faculty of Sciences of Sfax- Route Sokra BP 1171, 3000 Sfax, Tunisia, University of Sfax, Tunisia,

² Olive tree institute- Route Elmatar, 3000 Sfax Tunisia

The effect of endophytes expression in in-vitro tissue cultures has long been an issue of debate. This study has been designed to assess negative effects of endophytes proliferation during olive tree micropropagation. The presence of endophytic bacteria wasn't a serious constraint in olive tree micropropagation. These bacteria started their proliferation and became an issue only after many months from the day of initiation. In vitro cultures established from explants taken from plants that are under severe atmospheric pollution were found cleaner than from control ones. Indeed, percentage of contaminated cultures that are initiated from such explants was significantly lower (2 %) than those initiated from control explants (10 %). Endophytic bacteria expression has negative effects on olive tree in vitro shoots. Indeed, besides growth inhibition, leaves start to be yellow because of total chlorophyll rates decrease and then fall down. Up to our preliminary investigations, we can conclude that endophytes proliferation is well controlled in olive tree. Furthermore, endophytes population in olive tree is sensitive to atmospheric pollution and their visual proliferation, although scarce, has negative consequences on olive tree in vitro tissue cultures.

Ecological functions of fungal endophytes of grasses

Beatriz R. Vázquez de Aldana and Iñigo Zabalgozcoa

Institute of Natural Resources and Agrobiolgy of Salamanca (IRNASA-CSIC). Spain

i.zabalgo@irnasa.csic.es

The capability for asymptomatic endophytism is common for a large number of fungal taxa, as deduced from the results of surveys of endophytes in different plant species. However, the asymptomatic nature that defines endophytic occupation of tissue is a barrier for the understanding of the possible functions that such fungi might perform in their host, in other plant species, or in an ecosystem. Knowledge of the life cycle of endophytic species and of their functional roles could serve to ascertain the potential value of some endophytes for agricultural or industrial applications, but also to determine the safety derived from a commercial, upscale use of a given endophyte. For instance, plant pathogens might act as endophytes in alternate hosts, which in turn could serve as disease inoculum reservoirs, or latent saprobes might massively sporulate, producing allergenic spores (1).

Surveys conducted in four grass species adapted to distinct habitats (*Dactylis glomerata*, *Holcus lanatus*, *Elymus farctus*, *Ammophila arenaria*), produced a list of more than 200 different fungal taxa that were isolated as endophytes from asymptomatic samples of leaves, roots, and other plant parts (2). In the present work, we have searched the scientific literature for descriptions of the biological roles that might have been attributed to these fungi. As a result, it was found that these species in addition to their capability as endophytes in grasses, perform a variety of other functions, being saprobes, soil or dung inhabitants, pathogens of their host or other plant species, animal pathogens affecting invertebrates and mammals, including humans, or producers of antimicrobial compounds.

The results indicate that many endophytic species have life cycles independent of the host where they were isolated as endophytes. The knowledge of the complete life cycle of such fungi is important to assess the risk linked to industrial applications. Also, the widespread capability of asymptomatic occupation of plants observed among fungi indicates the “permeability” of plant defenses against invading fungi, an issue that confronts some current models of plant defense against pathogens.

1. Vázquez de Aldana BR, Bills G, Zabalgozcoa I. 2013. Are endophytes an important link between airborne spores and allergen exposure? *Fungal Diversity* 60:33-42.
2. Sánchez Márquez S, Bills GF, Herrero N, Zabalgozcoa I. 2012. Non systemic fungal endophytes of grasses. *Fungal Ecology* 5: 289-297.

***Fraxinus* dieback in Europe: understanding disease phenomenon and elaborating guidelines for sustainable management**

Rimvys Vasaitis

Swedish University of Agricultural Sciences

Currently, severe dieback of *Fraxinus* spp. is observed in most European countries. This is an emerging disease, which results in massive tree mortality, threatening the existence of *Fraxinus* over the continent. It is caused by *Hymenoscyphus fraxineus*, alien and invasive fungus, origin of which remains is Far East Asia. Initially, many European countries started national research programs on *Fraxinus* dieback, focusing on numerous aspects of the biology and ecology of the disease, but the activities were scattered. Aim of the FRAXBACK is, through sharing and synthesis of available knowledge, generate comprehensive understanding of *Fraxinus* dieback phenomenon, and to elaborate state of the art practical guidelines for sustainable management of *Fraxinus* in Europe. The Action is implemented through innovative interdisciplinary approach, and includes forest pathologists, tree breeders and silviculturists. Its deliverables: i) guidelines for sustainable management of *Fraxinus* in Europe; ii) European database for dieback-resistant *Fraxinus* genotypes /families/populations and established/planned progeny trials; iii) illustrated digests/leaflets/brochures on *Fraxinus* dieback; iv) disease distribution maps; v) website; vi) book. FRAXBACK is comprised of four Working Groups: WG1 Pathogen; WG2 Host; WG3 Silviculture; WG4 Dissemination and knowledge gaps. Its duration is 4 years (2012 – 2016), including two 2 - 3 MC/WG meetings and up to 17 STSMs per year, and several international conferences. Options for collaboration with the COST Action FA1103 “Endophytes in Biotechnology and Agriculture” will be discussed.

Plant-associated microbes as biocontrol agents against common ragweed

Widhalm, S.; Sessitsch, A. & Trognitz, F.

Ragweed is rapidly spreading in Europe and naturalized in many areas mainly through contaminated sunflower- and bird seeds. The pollen which is highly allergenic and can cause allergenic rhinitis and seasonal asthma. Unfortunately, effective agents to control this weed of the *Asteraceae* family are limited.

Novel approaches for more environmental friendly and effective agents to combat this weed are demanded. Application of plant-associated bacteria as bioherbicides could be a successful strategy to win the battle against the invasive common ragweed (*Ambrosia artemisiifolia* L.). Such bacteria could either be rhizobacteria or endophytes, which are in close relationship with ragweed plants. Because of their selectiveness of association with the host plant, such bacteria can be applied on agriculture land, without harming the crops. Another aspect of using natural occurring bacteria is the low risk of non-target effects which can be a great issue when introduction exotic biocontrol agents into a new environment.

The exploitation of naturally occurring phytotoxic microbes as biocontrol agents would enable an effective weed management in areas where the application of synthetic pesticides is restricted.

Microbial herbicides can be produced and applied at high concentration, but they are not expected to persist and survive in the environment beyond the season of application.

In frame of this research project we are looking for rhizobacteria and endophytes from common ragweed, which can reduce germination, growth and pollen production.

Common ragweed plants were collected from three federal states in Austria. Plant-associated bacteria were isolated from the rhizosphere, stem and roots. The endophytic bacteria from stem and root were isolated through surface sterilization. Furthermore, a stock collection for all cultivable bacteria was generated. Taxonomic classification of bacterial isolates from the federal state 'Burgenland' was facilitated through 16S rRNA PCR followed by sequencing and sequence comparison with the NCBI BLAST tool.

In the screening for bioherbicidal effects we include the production of hydrogen cyanide, indole acetic acid and antimetabolite toxins like tabtoxin and coronatine. Positive tested isolates will be screened on ragweed seeds and seedlings as well as non-target plants for herbicidal activity. Simultaneously an effective inoculating technique will be established. Additionally, the major allergen production of treated-compared to non-treated plants will be evaluated.

Up to this point, the abundance and diversity was established for the sample site in Burgenland. Bacteria belonging to the genus of *Bacillus*, *Pseudomonas*,

Microbacterium and *Arthrobacter* were found in all sample sites as well as in the root, stem and rhizosphere. Additionally, a PCR based screening of tabtoxin and coronatine production in *Pseudomonas* isolates was carried out. The antimetabolite production in *Pseudomonas* and *Bacillus* isolates was tested.

12 % of the *Pseudomonas* and 38.5 % of the *Bacillus* isolates were tested positive for antimetabolite production. Nevertheless tabtoxin or coronatine producing *Pseudomonas* isolates could not be identified. However, HNC producing bacterial isolates could be detected. Furthermore, selected candidates are screened for potential phytotoxic effects in planta enabled through flood-inoculation technique. To avoid enhanced production of allergenic pollen, the allergen will be monitored during the bioherbicide application.

SESSION 2
WORKING GROUP 1

Human pathogens in plants

The human-associated bacterium *Propionibacterium acnes* as a grapevine endophyte

Andrea Campisano¹, Lino Ometto¹, Stephane Compant², Alessandro Bergna³, Daniela Bulgari³, Livio Antonielli², Omar Rota-Stabelli¹

1. Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010, S. Michele all'Adige (TN), Italy

2. AIT Austrian Institute of Technology GmbH, Department of Health & Environment, Bioresources Unit, Konrad-Lorenz-Strasse 24, A-3430, Tulln, Austria

3. Dipartimento di Scienze Agrarie ed Ambientali-Produzione, Territorio, Agroenergie, Università degli Studi di Milano, Italy

Animals and plants have established a long-lasting cohabitation with a variety of microbes, including pathogens, commensals and beneficials. Studies investigating such associations documented numerous cases of bacterial host switches (usually from domestic animals to humans).

The exchange of microbial symbionts between humans and plants is much less investigated. We reported a surprising example of horizontal interkingdom transfer of the human opportunistic pathogen (*P. acnes*) to grapevine (*Vitis vinifera* L.). *P. acnes* was interestingly most common inside the plant's pith tissue. Phylogenetic and population analyses place that the establishment of the grapevine-associated *P. acnes* likely during the Neolithic, when grapevine was domesticated. The endophytic subspecies of *P. acnes* was named *P. Zappae*.

An analysis of Propionibacteria in the grapevine endosphere showed that *P. Zappae* is far from being the only species present in this plant as an endophyte.

Campisano, A., Ometto, L., Compant, S., Pancher, M., Antonielli, L., Yousaf, S., Varotto, C., Anfora, G., Pertot, I., Sessitsch, A., and Rota-Stabelli, O. (2014). Interkingdom transfer of the acne causing agent, *Propionibacterium acnes*, from human to grapevine. *Mol. Bio. Evol.* 2014 May;31(5):1059-65.

Yousaf S., Bulgari D., Bergna A., Pancher M., Quaglino F., Casati P., Campisano A. (2014). Pyrosequencing detects human-and animal pathogenic taxa in the grapevine endosphere. *Front Microbiol.* 2014 Jul 8;5:327.

Genotypic differences in three novel *Pantoea ananatis* strains having different impacts on the host plants

Rahelah Sheibani-Tezerji^{1,2}, Muhammad Naveed¹, Marc-André Jehl², Angela Sessitsch¹, Thomas Rattei² and Birgit Mitter¹

¹Health & Environment Department, Bioresources Unit, AIT Austrian Institute of Technology GmbH, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria

²University of Vienna, Department of Microbiology and Ecosystem Science, Division of Computational System Biology, Althanstrasse 14, 1090 Vienna, Austria, rahela.sheibani@univie.ac.at

Pantoea ananatis is a host-associated bacterium that is able to colonize a wide range of ecological niches and showing a vast range of variation in phenotypic traits from endophytic to pathogenic (Coutinho and Venter 2009). Bacterial endophytes show a range of properties playing an important role in plant production including plant growth promotion, improvement of nutrient uptake, increasing stress tolerance, antagonizing plant pathogens and induction of systemic resistance (Sessitsch et al., 2004; Mitter et al., 2013; Naveed et al., 2014). The genetic and physiological characteristics determining the difference between pathogenic and beneficial plant-microbe interaction are less understood. In this study, we investigate three novel closely related *P. ananatis* strains (named S6, S7 and S8) and their effect on the maize host plant. Each of these strains shows a different phenotypic behaviour, ranging from pathogenic (S7), commensal (S8) to a beneficial, growth promoting effect on maize (S6). We have studied their genomes in order to identify genetic attributes that could explain the phenotypic differences between closely related endophytic, commensal and pathogenic bacteria regarding their interaction with the host plant. Our comparative analysis indicates genomic differences mainly in cell surface components, motility related proteins, type VI secretion system, transposase/integrase/phage related genes and eukaryotic-like domain containing effector proteins.

Coutinho, T., and Venter, S. (2009) *Pantoea ananatis*: an unconventional plant pathogen. *Mol. Plant Pathol.* 10:325 - 335.

Mitter, B., Petric, A., Shin, M.W., Chain, P.S., Hauberg-Lotte, L., Reinhold-Hurek, B., Nowak, J., and Sessitsch, A. (2013) Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Frontiers in plant science* 4:120.

Naveed, M., Mitter, B., Reichenauer, T. G., Wiczorek, K., and Sessitsch, A. (2014) "Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17." *Environmental and Experimental Botany* 97(0): 30-39.

Sessitsch, A., B. Reiter, and G. Berg. (2004) Endophytic bacterial communities of field-grown potato and their plant-growth-promoting and antagonistic abilities. *Can. J. Microbiol.* 50:239-249.

***Mycobacterium* sp. As endophytes- rare or unnoticed?**

Pirttilä Anna Maria¹, Quambusch Mona², Koskimäki Janne¹, Tejesvi Mysore¹, Haller Kristin², Bartsch Melanie², Winkelmann Traud²

¹Department of Biology, University of Oulu, Oulu, Finland am.pirttila@oulu.fi, mvtejesvi@gmail.com, janne.koskimaki@oulu.fi

²Woody Plant and Propagation Physiology Section, Institute for Horticultural Production Systems, Leibniz Universität Hannover, Herrenhaeuser Strasse 2, 30419 Hannover, Germany. quambusch@baum.uni-hannover.de, bartsch@baum.uni-hannover.de, traud.winkelmann@zier.uni-hannover.de, kristinhaller@web.de

Environmental mycobacteria are found as saprophytes, commensals or symbionts of animals, humans and protozoans. Some strains in the genus *Mycobacterium* are known to consist serious human pathogens, causing persistent infections. The *Mycobacterium* genus is notorious for being hard to culture, as some strains need more than two weeks to complete cell division. Strains producing a visible colony within a week are called fast-growing strains. Some species of *Mycobacterium* are obligate parasites never found as free-living organisms, causing latent infections. Besides contamination from other humans, the majority of sources of human *Mycobacterium* pathogens are water and food. The mycobacterial cell wall is unique and differs from that of other bacteria, making the bacteria persistent towards most anti-infective treatments, such as antimicrobials and hypochlorite. The *Mycobacterium* species are classified mainly based on the diseases caused, tuberculosis, Hansen's disease or leprosy, and nontuberculous mycobacteria (NTM).

Mycobacterium strains are occasionally isolated from plants as endophytes. So far, members of the genus have been discovered from Scots pine (*Pinus sylvestris* L.), wild cherry (*Prunus avium* L.), rock plant (*Pogonatherum paniceum*(Lam.) Hack.), arrowhead vine (*Syngonium podophyllum* Schott.), and peace lily (*Spathiphyllum* × Schott). The bacteria were isolated from tissue-cultured plants at various steps of the micropropagation process. Most of the *Mycobacterium* strains were unidentified; the strain colonizing arrowhead vine and peace lily was classified as *M. scrofulaceum* and the strain from Scots pine is a close relative of *M. insubricum*, and the strain from wild cherry is closely related to *M. colombiense*. The rock plant hosted several *Mycobacterium* strains, closely related to *M. chubuense*, *M. poriferae*, *M. obuense*, *M. diernhoferi*, *M. intracellulare*, *M. llatzarense* and *M. cookii*. The majority of these strains were detected only by PCR using *Mycobacterium*-specific primers, and only one close relative of *M. cookii* was isolated from the tissue. In addition to the micropropagated plants, mycobacteria have been found in roots of rice and wheat, and in *Sphagnum* mosses. The low isolation frequency of mycobacteria from plants as endophytes may be due to the fact that culturing was mainly used as the detection method in the past, and most universal primers used routinely for amplification and sequencing of the bacterial 16S rDNA may exclude *Mycobacterium* spp. due to sequence differences. Species of *Mycobacterium* have not been reported as plant pathogens and their role as endophytes remains ambiguous, whether harmful

(parasitic) or beneficial. Due to the potential pathogenicity to humans, these results should stimulate further studies on the diversity and function of mycobacteria as endophytes.

Koskimäki, J.J., Nylund, S., Suorsa, M., Pirttilä, A.M. (2010) Mycobacterial endophytes are enriched during micropropagation of *Pogonatherum paniceum*. *Env.Microbiol.Rep.* 2: 619–624.

Quambusch M, Pirttilä AM, Tejesvi MV, Winkelmann T, Bartsch M (2014) Endophytic bacteria in plant tissue culture: differences between easy- and difficult-to-propagate *Prunus avium* genotypes. *Tree Physiol.* 34: 524–533.

Importance of endophyte *Aspergillus terreus* in biotechnology and public health

¹⁾Fadila Mohamed Mahmoud, ²⁾Anissa RAZALI, ³⁾Zoulikha Krimi, ⁴⁾Said Boudffer

¹Department of Cellular and Molecular biology, Faculty of Natural and Life Sciences, Blida 1 University, BP 270, Blida, Algeria. Email: m-fadhela@netcourrier.com

^{2, 3}Department of Biotechnology, Faculty of Natural and Life Sciences, Blida 1 University, BP 270, Blida, Algeria. Email: krimizlk@yahoo.fr

⁴Phytopathology Laboratory of National Agronomy Institut of Adrar, Algeria. Email: saidboudeffeur@yahoo.fr

The endophytic fungi are known by their colonizations of internal tissues of plants, in particular leaves, stems and roots without causing disease on their hosts. They are found ubiquitously in various angiosperms and gymnosperms including aquatic and desert plants. Date palm is one of the hosts that endophytic fungi are colonized and exteriorized her potential to get adaptation to stress conditions where the date palm is cultivated. Among the endophytic fungi isolated from this plant *Aspergillus terreus* was the most common one with very abundance in date palms infected or not with bayoud (caused by *Fusarium oxysporum* f.sp. *albedinis*) and those cultivated in Sebra of Tamentit with very high concentration of salt from South of Algeria of Adrar province. *Aspergillus terreus*, this sea fungus cause opportunistic infection in people with deficient immune systems, and cause in immunocompetent individuals allergic respiratory diseases. *A. terreus* has been recognised as a cause of aspergilloma, onychomycosis, aural cavity disease, subcutaneous abscesses, and keratitis, then is very dangerous for public health. All these infections caused by this endophyte return her isolation difficult and represent one of assessments of works with endophytes. In biotechnology, *Aspergillus terreus* is very important fungi, because it can produce some secondary metabolites like indol-3-acetic acid (IAA), Hydrogen Cyanid (HCN) and solubilize phosphorus. It affects also the inhibition of different phytopathogenic fungi, *Fusarium oxysporum* f.sp. *albedinis*, *Fusarium oxysporum* f.sp. *lycopersici* and *Gaeumannomyces graminis* var. *tritici* in our experiments realized *in vitro*.

References: unpublished data.

Strategies used by *Salmonella* to colonize plants

Adam Schikora

IPAZ, Justus Liebig University Giessen, Germany

Salmonella are one of the most prominent causes of food poisoning. The high number of infections with nontyphoidal *Salmonella* strains is constant and a not negligible threat to human population. In the last two decades salmonellosis outbreaks were increasingly associated with infected fruits and vegetables, indicating that contaminated vegetables are a concern for human health. Successful infection demands either avoidance or suppression of the host immune system. These can be achieved by different strategies. One is an injection of bacterial effector proteins into host cells, other the modification of bacterial structures usually recognized by immune system (so called pathogen-associated molecular patterns). In this talk I will present the function of *Salmonella* effector protein in plant cell, supporting the new concept of trans-kingdom competence of these bacteria. We screened a range of *Salmonella* Typhimurium effector proteins for interference with plant immunity. Among these, the phosphothreonine lyase SpvC attenuated the induction of immunity-related responses when present in plant cells. Moreover, the requirement of *Salmonella* SpvC was shown by the decreased proliferation of the $\Delta spvC$ mutant in *Arabidopsis* plants. Another strategy consists of modification of bacterial flagellin, the protein normally recognized by the plant FLS2 receptor. Usually, such detection is followed by an immune reaction. We demonstrate that certain *Salmonella* strains can avoid this reaction by a modification of the flg22 epitope. These results suggest that some *Salmonella* bacteria developed diverse strategies exploited during proliferation in different hosts. The fact that *Salmonella* and other *Enterobacteriaceae* use plants as hosts strongly suggests that plants represent a much larger reservoir for animal pathogens than so far estimated.

Genotyping and phylogeny of *Phaeoacremonium* genus associated with necrotic wood and infections in human

A. Berraf-Tebbal¹, Z. Bouznad², and A.J.L. Phillips³

¹Département des Biotechnologies, Faculté des Sciences de la Nature et de la Vie, Université Blida 1. 09000 Blida, Algeria

²Département de Botanique, Ecole Nationale Supérieure d'Agronomie (ENSA), El-Harrach, Algeria

³Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal

E-mail: berraf.a@hotmail.fr

The genus *Phaeoacremonium* is an ecologically important taxon which includes species associated with two declining diseases on grapevine (*Vitis vinifera*) namely Petri disease and esca. In recent years, *Phaeoacremonium* spp. have been isolated from human patients with phaeohyphomycosis infections. Some of these species were reported from human and have also been isolated from grapevines showing esca symptoms.

The identification of species in *Phaeoacremonium* genus can be a difficult task if based solely on morphological and cultural characters. In this respect, the application of molecular methods, particularly PCR-based techniques, may provide an important contribution. MSP-PCR (microsatellite primed-PCR) fingerprinting has proven useful in the molecular typing of fungal strains. The high discriminatory potential of this method is particularly useful when dealing with closely related or cryptic species. In the present study, the application of PCR fingerprinting was performed using the microsatellite primer M13 for the purpose of species identification and strain typing of 49 *Phaeoacremonium*-like isolates collected from grapevines with typical symptoms of dieback.

The isolates were clustered on the basis of their profiles in a consensus dendrogram built with GELCOMP version 4.1, using Pearson's correlation coefficient and UPGMA. Seven meaningful groups were formed from the reproducibility level (70%). Representative isolates from each group were selected for sequencing of the β -tubulin and actin genes. The TUB and ACT sequences of the 17 isolates selected from the MSP-PCR profiles were combined and aligned with sequences of 50 isolates retrieved from GenBank. The isolates obtained in this study clustered with four previously published species, namely, *Pm. aleophilum*, *Pm. venezulense*, *Pm. parasiticum*, *Pm. hispanicum*.

Pm. aleophilum is recognized as the most common species on grapevines worldwide and thus was expected to be the common one in this study. The next most frequent species were *Pm. parasiticum* and *Pm. venezuelense*. *Pm. parasiticum* is the most

common species causing human infection, and was first reported in 1974 as *Phialophora parasitica*. *Pm. venezuelense* has rarely been encountered on grapevines and is represented worldwide by only five strains, of which three were from human infections; the fourth was from a grapevine and the fifth strain from an unknown host. Also of interest was the single isolate of *Pm. hispanicum*, which has the ability to grow at 37°C, and which suggests that it has the potential to survive at human body temperature. This finding is quite interesting in relation to the ecology of *Pm. parasiticum* and *Pm. venezuelense*, as these thermotolerant species are associated with *Phaeoophomycosis* in humans but have also been isolated from grapevines and other woody hosts. Infections in humans appear to have become more common over the last two decades. This finding further supports the hypothesis that human pathogenic *Phaeoacremonium* species may have originated from woody host plants. To confirm this hypothesis, further studies are needed.

Berraf-Tebbal, A., Marco A. Guerreiro and Alan J. L. Phillips. 2014. Phylogeny of *Neofusicoccum* species associated with grapevine trunk disease in Algeria with description of *Neofusicoccum algeriense* sp. nov. *Phytopathologia Mediterranea*. DOI 10.14601/Phytopathol_Mediterr 14385.

Berraf-Tebbal, A., Bouznad, Z. and Phillips, A.J.L. 2014. Molecular and morphological characterization of *Phaeomoniella* isolats associated with eutypa dieback and esca of grapevines in North Algeria. *Agrobilgia*. N°6, 53-59.

B.T. Linaldeddu, A. Deidda, B. Scanu, A. Franceschini, S. Serra, A. Berraf-Tebbal, M. Zouaoui Boutiti, M. L. Ben Jamaa, A. J. L. Phillips. 2014. Species diversity of *Botryosphaeriaceae* associated with grapevine and other woody hosts in Italy, Algeria and Tunisia, with descriptions of *Lasiodiplodia mediterranea* and *Lasiodiplodia exigua* sp. nov. *Fungal Diversity*. DOI 10.1007/s13225-014-0301-x.

A whole-genome comparison of virulence traits in endophytic genomes of enterobacteria

López-Fernández, Sebastián^{1,2}; Sonogo Paolo¹; Pancher, Michael¹; Engelen, Kristof¹; Campisano, Andrea¹

1. Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010, S. Michele all'Adige (TN), Italy.

2. Technische Universität Braunschweig, Institut fuer mikrobiologie, Spielmannstrasse 7, 38106. Braunschweig, Germany.

Endophytes are roughly defined as microorganisms living inside plants without causing them harm. Plant pathogens and endophytes co-exist and often interact with the plant host and within its microbial community. The outcome of this interaction may lead to healthy plants or to plant disease, through the inducible production of molecules known as virulence factors.

It is now clear that some of the endophytes of grapevine have a human origin. For example, species known as human pathogens were transmitted to plants during the domestication of consumable species (Campisano et al., 2014). This is in agreement with other findings showing that the endosphere of grapevine is filled with species belonging to groups categorized as pathogenic (Yousaf et al., 2014). These studies suggest that there is a connection between endophytes and pathogens that might refer to the origin of endophytes as a group. However the very keys of the endophytic and pathogenic lifestyles rest in the genomes of bacteria.

Genomics is an outstanding tool for analysing the ecology, biotechnological properties and life history of microorganisms. Bacterial comparative genomics has highlighted how the genomes of endosymbionts and free living organisms differ, in that the former have drastically being reduced and re-arranged. Nonetheless, the genomic differences between bacterial endophytes and plant pathogens are not quite clear.

In this study we compare the genomes of 7 endophytic strains belonging to the genera *Pantoea*, *Enterobacter* and *Erwinia* with a panel of reference strains known to be pathogenic or endophytic. A whole-genome alignment revealed that the genomes of endophytes seem to be smaller than the ones from pathogens and analysis of orthologous-gene content showed that the core genomes contain 2648, 2301 and 2913 gene families in *Enterobacter*, *Erwinia* and *Pantoea* respectively. We show that pathogens and endophytes share genes coding virulence factors like cell wall degrading enzymes, protein secretion systems, siderophores and pathogenicity islands, but also genes that might be determinant for the association with plants and that to the best of our knowledge were never before reported, like the NUDIX complex and several two component systems.

Finally we quantify the number of virulence factors in endophytic and pathogenic strains in the core and the accessory genomes and show that endophytes and pathogens have very little differences in content of virulence factors, however the distribution and gene context varies between the two groups.

Campisano, A., Ometto, L., Compant, S., Pancher, M., Antonielli, L., Yousaf, S., Varotto, C., Anfora, G., Pertot, I., Sessitsch, A., and Rota-Stabelli, O. (2014). Interkingdom transfer of the acne-causing agent, *Propionibacterium acnes*, from human to grapevine. *Mol Biol Evol* 31, 1059-1065. doi: 10.1093/molbev/msu075.

Yousaf, S., Bulgari, D., Bergna, A., Pancher, M., Quaglino, F., Casati, P., and Campisano, A. (2014). Pyrosequencing detects human and animal pathogenic taxa in the grapevine endosphere. *Frontiers in Microbiology* 5. doi: 10.3389/fmicb.2014.00327.

SESSION 3

WORKING GROUP 4

**Production of mycotoxins, phytotoxins and other
undesired secondary metabolites by biocontrol
agents**

Taxonomic diversity of *Epichloë* endophytes affects the alkaloid profile of *Lolium perenne* in natural pastures

MC Soto-Barajas, BR Vázquez de Aldana, A Alvarez-Pascua, J Gómez-Fuertes, I Zalbalgoeazcoa

Institute of Natural Resources and Agrobiology, IRNASA-CSIC, Salamanca, Spain,
carlos.soto@irnas.csic.es

Some species of *Epichloë* endophytes have a mutualistic relationship with grasses (1, 2). The plant provides the fungus with an habitat, nutrients, and a medium of dispersion through its seeds. The endophyte produces secondary metabolites that act as a defensive barrier for the plant, providing anti-herbivore defense (3). *Epichloë* endophytes are being used for *Lolium* cultivar improvement; however, fungal alkaloids such as lolitrems are toxic to mammals, and undesirable in forage cultivars. The aim of this research was to characterize the effect of the species diversity of *Epichloë* endophytes on the production of the alkaloids peramine and lolitrem B in natural populations of *L. perenne*.

The material examined were 378 plants of *L. perenne* sampled at eight different locations (from semiarid grasslands to coastal meadows). *Epichloë* endophytes were detected in 38% of the plants analyzed, ranging from 12% to 60% among locations. Morphological features of *Epichloë* cultures (growth rate, shape, color and sporulation) lead to their classification in four groups (M1: brain-like colony; M2: cottony aerial mycelium; M3: slow-growth with sparse aerial mycelium; NM: mixed group with other morphologies). A genotypic characterization of the cultures was conducted with ITS and β -tubulin gene nucleotide sequences. All sequenced strains clustered in four clades which coincided with the morphological groups. G1(49%)= M1(47%) + NM(2%); G2a(22%)= M2(19%) + NM(3%), with a genotype similar to *Epichloë typhina*; G2b(3%)= M2; and, G3(26%)= M3, and genotype similar to *Epichloë festucae* var. *lolii*. These results also indicated the presence of different *Epichloë* species living in sympatry in *Lolium* populations.

Peramine was detected in 70% of the infected plants (range: 2.0 – 31.5 $\mu\text{g g}^{-1}$), and lolitrem B in 46% of them (range: 0.5 – 4.1 $\mu\text{g g}^{-1}$). A 39% of infected plants contained both alkaloids and 9% none of them. Plants infected by fungi with genotype G1 had a similar content of peramine (\bar{x} = 3.7 $\mu\text{g g}^{-1}$), and a variable range of lolitrem B (0.6 – 4.1 $\mu\text{g g}^{-1}$). Grasses with G2 endophytes produced the highest concentration of peramine (up to 31.5 $\mu\text{g g}^{-1}$), the lowest of lolitrem B (\bar{x} = 0.6 $\mu\text{g g}^{-1}$), and most plants did not produce lolitrem B. In plants infected by G3 endophytes the concentration of peramine occurred in a medium-low range (2.1 – 17.2 $\mu\text{g g}^{-1}$), and the variation in lolitrem B concentration was wide (0.8 – 3.8 $\mu\text{g g}^{-1}$).

Considering the diversity of *Epichloë* endophytes found and their direct relation with alkaloid production, some of these fungal strains could be suitable to improve *L. perenne* cultivars.

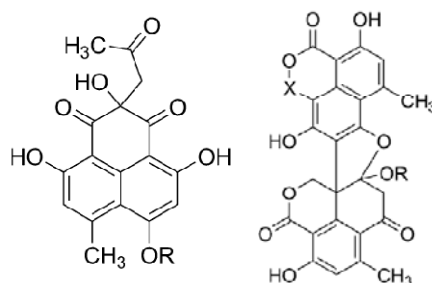
1. Vázquez de Aldana BR, García Ciudad A, García Criado B, Vicente-Tavera S, Zabalgoeazcoa I. 2013. Fungal endophyte (*Epichloë festucae*) alters the nutrient content of *Festuca rubra* regardless of water availability. *PlosOne* 8: e84539.
2. Vázquez de Aldana BR, Zabalgoeazcoa I, García Ciudad A, García Criado B. 2013. An *Epichloë* endophyte affects the competitive ability of *Festuca rubra* against other grassland species. *Plant and Soil* 362: 201-213.
3. Vázquez de Aldana BR, Zabalgoeazcoa I, Rubio de Casas R, García Ciudad A, García Criado. 2010. Relationships between the genetic distance of *Epichloë festucae* isolates and the ergovaline and peramine contents of their *Festuca rubra* hosts. *Annals of Applied Biology* 156: 51-61.

Fungal crafted phenalenones and their phylogenetic diversity

Tejesvi Mysore V¹., Elsebai Mahmoud F¹., Pirttilä Anna Maria¹,

¹Department of Biology, University of Oulu, Oulu, Finland mvtejesvi@gmail.com,
elsebai72@yahoo.com, am.pirttila@oulu.fi,

Phenalenones are a distinctive class of natural polyketides with diverse and significant biological activities including antimicrobial, anticancer and cytotoxic activities. Endophytes and other environmental fungi produce more than 72 phenalenone compounds. Phenalenones are structurally diverse and are reported as heptaketides, hexaketides, homodimers and heterodimers with vast chemical diversity. Endophytic *Penicillium* sp. isolated from *Aegiceras corniculatum* produces an immunomodulator phenalenone derivative, demethyl FR-901235. Bacillosporin C & D were isolated from the unidentified endophytic fungus SBE-14 from a mangrove plant of South China Sea. To study the phylogenetic relationship of secondary metabolism in fungi, we constructed a phylogenetic tree of 32 phenalenone-producing fungal strains using Internal Transcribed Spacer2 Region (ITS2). The phylogenetic analysis was performed by the Neighbour-Joining method using Molecular Evolutionary Genetics Analysis (MEGA5). Majority of the fungi were distributed to two main clusters, irrespective of the compounds produced. In the first cluster, there were 28 fungi including *Penicillium herquei*, *Polytolypa hystricis*, *P. aurantiovirens*, *P. atrovventum*, *Aspergillus niger*, *Aspergillus tubingensis*, *Talaromyces bacillisporus*, *Paecilomyces carneus*, *Neonectria* sp., *Sirococcus tsugae*, *Godronia cassandrae* and *G. abietina*. The other cluster constituted of *Coniothyrium cereale*, *Phaesphaeria rousseliana* and *Scytalidium* sp. The fungal genera producing unique compounds were clustered together along with different species of the same genera. Considering the several possibilities for branching in the phylogenetic tree, the secondary metabolism does not reflect the phylogeny of the fungi but the gene clusters for metabolites are horizontally obtained.



- 1) Demethyl FR-901235 (R= CH₃), 2) Bacillosporin C (X =CH₂, R= H)

New natural products from phytopathogenic fungi

Frank Surup,¹ Sandra Halecker,¹ Ajda Medjedović,² Hans-Josef Schroers,² Marc Stadler¹

¹ Helmholtz Centre for Infection Research, Dept. Microbial Drugs; Inhoffenstrasse 7, D-38124 Braunschweig, Germany; e-mail: frank.surup@helmholtz-hzi.de

² Agricultural Institute of Slovenia, 1000 Ljubljana, Slovenia.

Historically, fungal secondary metabolites have served as model compounds for numerous important drugs and pesticides. Because novel lead compounds are continuously needed, our work is dedicated on the discovery of novel candidate anti-infectives from natural sources. Aside from the world-wide largest and most diverse collection of gliding bacteria, we are focusing on fungi as another highly promising source for unique bioactive secondary metabolites. One of our recent projects aims for the identification and isolation of secondary metabolites from phytopathogenic fungi, which tend to be underexplored so far. From cultures of ash dieback causing ascomycete *Hymenoscyphus fraxineus* (syn. *H. pseudoalbidus*) we isolated hymenoseitin, a new 3-decalinoyltetramic acid with a strong activity against Gram-positive bacteria including MRSA. Its absolute configuration was assigned by CD spectroscopy and Hetloc data.¹

In another project, the antagonistic activity of sooty blotch and flyspeck (SBFS) fungi against apple rot causing pathogen *Colletotrichum fioriniae* was investigated. We isolated trichothecolone acetate and its novel derivative 7-hydroxytrichothecene acetate from cultures of *Microcyclospora tardicrescens* sp. 1936, a SBFS fungus causing great economic damage on apple, pear and other fruits throughout humid climates.² Furthermore, we found that *Microcyclospora malicola* sp. 1930, another SBFS fungus, is producing the bioactive obionin A besides novel derivatives of this family of pigments.

Our results demonstrate that phytopathogenic fungi are an excellent source for new bioactive secondary metabolites.

1. S. Halecker, F. Surup, E. Kuhnert, K. I. Mohr, N. L. Brock, J. S. Dickschat, C. Junker, B. Schulz, M. Stadler, *Phytochemistry* 2014, 100, 86-91.

2. F. Surup, A. Medjedović, M. Szczygielski, H.-J. Schroers, M. Stadler, *J. Agric. Food Chem.* 2014, 62, 3525-30.

Obstacles in developing a biocontrol agent with endophytes

Barbara Schulz

Institut für Mikrobiologie, Technische Universität Braunschweig, Germany

Four major obstacles must be overcome when developing a biocontrol agent with endophytes: 1) obtaining sufficient long-lasting colonization to attain the desired effect, 2) avoiding secondary metabolites that are toxic to humans or other animals, 3) formulation, 4) meeting European regulations before such a potential product can be registered. This talk will address the first two aspects.

It is in the nature of endophytic fungi to engage in multipartite symbioses with their plant hosts and with other microbial inhabitants, necessitating multiple balances of antagonisms. In a non-stressed host plant, the result is a certain level of endophytic colonization specific for each host plant and the cohabitating microorganisms. This can be a disadvantage, because whereas after application of the agent the initial colonization density is high, it often decreases as the original equilibrium is restored. Additionally, the equilibria with host and cohabitants are often quite labile and can be easily disturbed, both by biotic and abiotic factors. For example, if the host is stressed, the endophyte can become pathogenic. Also, many endophytes that interact as mutualists in one host will be pathogens in another. Examples dealing with the hosts *Hordium vulgare* (barley), *Arabidopsis*, *Fraxinus excelsior* (European ash) and *Larix decidua* and their endophytes will illustrate these difficulties.

According to our current hypothesis, in order to survive and grow *in planta*, secondary metabolites are involved in maintaining equilibria with the co-habitants and with the host plant. This may well be the reason that endophytic fungi have a particularly active secondary metabolism: approximately 60% of those that we have investigated produce antifungal, 30% antibacterial and 40% algicidal - herbicidal metabolites. And, it is exactly this attribute that is often indispensable for the desired biocontrol effect, but it may on the other hand make registration difficult. The advantages and disadvantages of these metabolites will be portrayed with the hosts *Fraxinus excelsior* and *Cirsium arvense* and their respective endophytes.

Junker, C., Draeger, S., Schulz, B. (2012). A fine line – endophytes or pathogens in *Arabidopsis thaliana*. *Fungal Ecology*, 5, 657-662.

Schulz, B., Haas, S., Andrée, N., Schobert, M. (in review) Endophytes are involved in multiple balanced antagonisms.

Schulz, B., Junker, C., Citron, C., Dickschat, J. (in prep.) Metabolic interactions between endophytes and the pathogen, *Hymenoscyphus fraxineus*, of *Fraxinus excelsior*

Risk assessment of an innovative biological control strategy using endophytic entomopathogenic fungi

Dalia Muftah Alkhatat¹, Katharina Döll², Petr Karlovsky² and Stefan Vidal¹

¹Agricultural Entomology, Georg-August University, Göttingen, Germany, email: d.muftahalkhatat@stud.uni-goettingen.de, svidal@gwdg.de

²Molecular Phytopathology and Mycotoxin Research, Georg-August University, Göttingen, Germany, email: kdoell@gwdg.de, pkarlov@gwdg.de

Since two decades it is known that several entomopathogenic fungal species, such as *Beauveria bassiana* and *Metarhizium anisopliae* s.l., are able to colonize plant tissues endophytically. This has been demonstrated for several crops such as corn, cotton, sorghum, tomato or opium poppy, respectively. The insecticidal effect of successfully colonized crops against different pests and diseases was also shown. Although these experiments showed the potential of the endophytic entomopathogenic fungi (EEFP) to be used in an innovative biological control strategy, several questions remain to be answered. One important issue refers to the possibility of the production of mycotoxins inside plant tissues as well as changes in the physiology of hosting plants, negatively influencing the fitness of the plants. We investigated the endophytic growth of two entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae* s.l.) in tomato and cotton plants. Successfully colonized plants were assessed for their insecticidal effect against *Helicoverpa armigera* and greenhouse whiteflies. Successful control of whiteflies on newly emerged leaves of tomato plants and the results of fungal biomass quantification in these leaves by real-time PCR suggests two different modes of insecticidal action: Either an effect of fungal mycotoxin production inside plants tissues or of a physiological change in colonized plants due an up- or down-regulation of phytohormones activating the defense system of plants. Based on these hypotheses two experiments were conducted:

In the first experiment: tomato plants were inoculated using different inoculation methods with two different strains of *Beauveria bassiana*, and checked for an insecticidal effect against greenhouse whiteflies (*Trialeurodes vaporariorum*). Samples collected simulatenously were analysed for mycotoxin content in plant-tissues using LC-MS. One strain of *B. bassiana* did produce Beauvericin inside plant tissues, while no detection of Beauvericin was appeared in inoculated plants using another strain. However, this strain produced Tenellin and additional secondary metabolites on different liquid media in in-vitro tests. At the same time, samples of both strains were used for metabolite profiling. The results demonstrated that inoculation of plants with *B. bassiana* down-regulated several glucosides as well as α -tomatidine.

In another experiment tomato seeds were inoculated with *Mtarhizium anisopliae* s.l. and grown under greenhouse conditions until BBCH 15 when a later infection with *Phytophthora infestans* was established. Plants were left to develop the symptoms

until harvesting time, and samples were collected for fungal biomass quantification and Destruxin or phytohormone levels investigation using LC-MS. *M. anisopliae s.l.* did not produce Destruxins inside the plant-tissues, while some phytohormones were either up-or down- regulated in relation to the fungal inoculation.

We conclude that EEPF have a considerable potential as innovative biological control agents, but risks of mycotoxin production as well as significant changes in the physiology of plants influenced by the fungal strain, the crop plant and the inoculation method should always be taken into consideration for each newly designed application system.

Ownley, B.H., Griffin, M.R., Klingeman, W.E., Gwinn, K.D., Moulton, J.K. and Pereira, R.M. (2008). *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of Invertebrates Pathology* 3: 267-270.

Tefera, T., Vidal, S. (2009). Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. *BloControl* 54: 663-669.

Vidal, S. (2011). Entomopathogenic fungi as endophytes: a new plant protection strategy? *IOBC/WPRS Bulletin* 66: 91-97.

Wagner, B.L. and Lewis, L.C. (2000). Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. *Applied Environmental Microbiology* 66: 3468-3473.

Mycotoxins and other exometabolites from *Fusarium* and related genera

Ulf Thrane

DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark. Email: ut@bio.dtu.dk

Essentially, each fungal species is able to produce a specific profile of biological active metabolites, the exo-metabolome, which is of high importance for the interaction with the surroundings. Species of *Fusarium* and related genera are no exceptions, in contrary, especially *Fusarium* is very well known for its ability to produce biologically active metabolites including mycotoxins, such as the trichothecenes, zearalenones, fumonisins, moniliformin, and beauvericins and other cyclic peptides. The metabolite production is highly influenced by the growth conditions and this information is of high value to feed and food safety, as mycotoxins are unwanted in agricultural crops. The available information on mycotoxins is overwhelming. A search for scientific peer-reviewed papers using the keywords “*Fusarium* AND mycotoxin” retrieve > 350 hits published in 2013! This equals one scientific *Fusarium*mycotoxin paper every single day. In addition, these search engines do not cover all journals and books, congress proceedings and technical reports! Unfortunately the quality of the information is varying and in this context both the identification of mycotoxin and the producing organisms are the weak points.

The rapid developments in molecular biology and gene sequence facilities have had major impact on the taxonomy of all organisms, including fungi. Most phylogenetic studies are based on nucleotide sequences of household genes, which convey little – if anything – about function of the organism. In contrast, phenotypic studies such as metabolite and enzyme profiling are much more informative concerning the ecology of the fungus. Today more and more genes coding for biosynthetic pathways of non-household products are sequenced that add to the increasing information on the genetics behind metabolite productions. Such data has also been used for phylogenetic studies and to some degree incorporated into fungal systematics. However, a whole-hearted integration of fungal phenetics and genomics, as well as critical evaluation of the exceptional amount of metabolite data being generated is crucial to ensure safe fungal products. Updated information on the species specific profiles of mycotoxins and exometabolites will be presented and discussed with a focus on future exploitation of the biotechnological use of *Fusarium* and related genera as bio-control agents, bio-fertilizers and microbial cell factories.

Thrane U, Andersen B, Frisvad JC, Smedsgaard J. 2007. The exo-metabolome in filamentous fungi. In: Metabolomics. A powerful tool in systems biology, (Nielsen J, Jewett MC, eds). Topics in Current Genetics 18: 235-252.

Frisvad JC, Yilmaz N, Thrane U, Rasmussen KB, Houbraken J, Samson RA. 2013. *Talaromyces atroseus*, a new species efficiently producing industrially relevant red pigments. PLoS ONE 8(12): 84-102.

Röhrich CR, Iversen A, Jaklitsch WM, Voglmayr H, Vilcinskas A, Nielsen KF, Thrane U, von Döhren H, Brückner H, Degenkolb T. 2013. Screening the Biosphere: The fungicolous fungus *Trichoderma phellincola*, a prolific source of hypophellins, new 17-, 18-, 19-, and 20-residue peptaibiotics. *Chemistry & Biodiversity* 10: 787-812.

Sørensen JL, Akk E, Thrane U, Giese H, Sondergaard TE. 2013. Production of fusarielins by *Fusarium*. *International Journal of Food Microbiology* 160(3): 206-211.

Leong SL, Lantz H, Pettersson OV, Frisvad JC, Thrane U, Heipieper HJ, Dijksterhuis J, Grabherr M, Pettersson M, Tellgren-Roth C, Schnurer J. 2014. Genome and physiology of the ascomycete filamentous fungus *Xeromyces bisporus*, the most xerophilic organism isolated to date. *Environmental Microbiology* (*in press*). doi:10.1111/1462-2920.12596.

Novel biologically active compounds isolated from the symbionts of plants and insects

Kolařík M.¹, Stodůlková E.², Černý J.³, Kubátová A.⁴, Novák P.⁵, Císařová I.⁶,
Flieger M.⁷

¹Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic,
mkolarik@biomed.cas.cz

²Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic,
evastod@biomed.cas.cz

³Department of Cell Biology, Faculty of Science, Charles University, Viničná 7, 128 01, Prague, Czech Republic, jan.cerny@natur.cuni.cz

⁴Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 01, Prague, Czech Republic, kubatova@natur.cuni.cz

⁵Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic,
pnovak@biomed.cas.cz

⁶Department of Inorganic Chemistry, Faculty of Science, Charles University, 128 40 Prague, Czech Republic, cisarova@natur.cuni.cz

⁷Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic,
flieger@biomed.cas.cz

Naphthoquinones (NQs) are synthesized by herbs, bacteria and fungi (mostly *Fusarium*, *Aspergillus* and *Penicillium*). The fungal NQs have a broad range of biological activities, including antimicrobial, anti-inflammatory, antiviral and cytostatic. We have studied secondary metabolites from *Quambalaria cyanescens* (Basidiomycota, Exobasidiomycetes) a fungus known as endophyte, plant saprobe, opportunistic human pathogen and bark beetle associate. Next studied lineage of fungi includes *Biatrispora* spp. (Ascomycota: Pleosporales) known as endophytes of land plants but living also in marine and estuarine environment. Seven new together with ten known NQs were isolated. Their structures were determined by single-crystal X-ray diffraction experiments, NMR and mass spectrometry. They have antibiotic effect and are active against various carcinoma cells lineages. Identified NQs exhibited low cytotoxicity to healthy human cells and have potential in anticancer therapy, similar to plant produced drugs such as shikonin and lapachol.

Selected references from own group

Stodůlková E., Man P., Kuzma M., Černý J., Císařová I Kubátová., Chudíčková Kolařík M., Chudíčková M., Flieger M. (2014) A highly diverse spectrum of naphthoquinone derivatives produced by the endophytic fungus *Biatrispora* sp. CCF 4378. *Folia microbiologica* (in press).

Kostovcik M, Bateman CC, Kolarik M, Stelinski LL, Jordal BH, Hulcr J, 2014. The ambrosia symbiosis is specific in some species and promiscuous in others: evidence from community pyrosequencing. *ISME Journal*. 1 August 2014 (doi:10.1038/ismej.2014.115)

Veselská, T. and Kolařík (2015): M. Application of flow cytometry for exploring the evolution of *Geosmithia* fungi living in association with bark beetles: the role of conidial DNA content. *Fungal Ecology* 13, p: 83-92

Pažoutová, S., Follert, S., Bitzer, J., Keck, M., Surup, F., Šrůtka, P., Holuša, J. and Stadler, M. A new endophytic insect-associated *Daldinia* species, recognised from a comparison of secondary metabolite profiles and molecular phylogeny. *Fungal Diversity* 60 (2013), pp. 107-123.

Pažoutová, S., Pešicová K., Chudíčková M., Šrůtka P., Kolařík M. (2015) Delimitation of cryptic species inside *Claviceps purpurea*. *Fungal Biology* in Press.

Using the *RPB2* single-copy gene as an alternative molecular marker compared with its and investigated on the *in vitro*-assembled mock community

Zelenka T.¹, Větrovský T.², Kolařík M.³, Žifčáková L.⁴, Baldrian P.⁵

¹Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic, zelenkt@gmail.com

²Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic, vetrovsky@biomed.cas.cz

³Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic, mkolarik@biomed.cas.cz

⁴Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic, zifcakoval@gmail.com

⁵Institute of Microbiology of the ASCR, Vídeňská 1083, 142 20, Prague, Czech Republic, baldrian@biomed.cas.cz

The mostly used fungal barcode marker is the internal transcribed spacer region of rDNA (ITS). It is well suited for taxonomic identification but the information on the relative abundance of taxa is skewed due to the multi-copy nature of rDNA and the existence of ITS paralagues. Possible alternatives are single-copy protein coding genes, such as the gene encoding the second largest subunit of RNA polymerase II (*RPB2*). To compare usability of these markers the *in vitro*-assembled mock community of 130 fungal species was created. This mixture was analysed by 454 pyrosequencing, chosen mainly because of sufficient length of reads. From the species taken to the study, 68 were presented in both datasets, whereas 29 (*RPB2*) and 33 (ITS) were found in one of the datasets only. To show the best processing approach considering known inputs, three various clustering algorithms were used. In all cases the real numbers of species were strongly exceeded (1,35–2,72 times). Apparent intragenomic variation was present within several orders in both datasets. There were found considerable differences at the lower taxonomic scale and similar coverage at the higher taxonomic level. Remarkably there were clear logarithmical distributions of reads to OTUs which prevents using the numbers of reads for quantification OTUs. In conclusion the *RPB2* was found applicable as a barcode for fungal community studies and can serve as a marker supplementary to ITS.

Selected references from own group

Kostovcik M, Bateman CC, Kolarik M, Stelinski LL, Jordal BH, Hulcr J, 2014. The ambrosia symbiosis is specific in some species and promiscuous in others: evidence from community pyrosequencing. ISME Journal. 1 August 2014 (doi:10.1038/ismej.2014.115)

Větrovský T., Baldrian P. (2013). Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. Biology and Fertility of Soils 49, p: 1027-1037.

Pažoutová, S., Follert, S., Bitzer, J., Keck, M., Surup, F., Šrůtka, P., Holuša, J. and Stadler, M. (2013). A new endophytic insect-associated *Daldinia* species, recognised from a comparison of secondary metabolite profiles and molecular phylogeny. Fungal Diversity 60, p. 107-123.

Abstract List of Participants

SEBASTIÀN LÓPEZ-FERNÁNDEZ	juan.lopezfernandez@fmach.it	ITALY/GERMANY
FADILA MOHAMED MAHMOUD	m-fadhela@netcourrier.com	ALGERIA
MC SOTO-BARAJAS	carlos.soto@irnasa.csic.es	SPAIN
IÑIGO ZABALGOGEAZCOA	i.zabalgo@irnasa.csic.es	SPAIN
FRANK SURUP	frank.surup@helmholtz-hzi.de	GERMANY
MOHAMED MAALEJ	maalejmohamed@yahoo.fr	TUNISIA
AKILA BERRAF-TEBBAL	berraf.a@hotmail.fr	ALGERIA
TEJESVI MYSORE V.	mvtejesvi@gmail.com	FINLAND
ANNA MARIA PIRTTILA	am.pirttila@oulu.fi,	FINLAND
RIMVYS VASAITIS	Rimvys.Vasaitis@slu.se	SWEEDEN
TEZERJİ RAHELEH SHEIBANI	rahela.sheibani@univie.ac.at	AUSTRIA
LUTFI FKI	lotfikfi@yahoo.fr	TUNISIA
DALIA MUFTAH ALKHAYAT	d.muftahalkhayat@stud.uni-goettingen.de	GERMANY
BARBARA SCHULZ	b.schulz@tu-bs.de	GERMANY
ANDREA CAMPISANO	andrea.campisano@fmach.it	ITALY
ULF TTRANE	ut@bio.dtu.dk	DENMARK
BERNA TUNALI	btunali@omu.edu.tr	TURKEY
SIEGRID WIDHALM	Siegrid.widhalm.fl@ait.ac.at	AUSTRIA
MIROSLAV KOLARIK	mkolarik@biomed.cas.cz	CZECH REPUBLIC
ROBERT WILLIAM JACKSON	r.w.jackson@reading.ac.uk	UK
MICHAEL SCHMID	michael.schmid@helmholtz-muenchen.de	GERMANY
ADAM SCHIKORA	Adam.Schikora@agrار.uni-giessen.de	GERMANY
TOMAS ZELENKA	zelenkt@gmail.com	CZECH REPUBLIC
HATICE OZAKTAN	hatice.ozaktan@ege.edu.tr	
LALEHAN YOLAGELDI	lalehan.yolageldi@ege.edu.tr	TURKEY
AYŞE GÜL	ayse.gul@ege.edu.tr	TURKEY
MUSTAFA AKBABA	mustafa.akbaba@ege.edu.tr	TURKEY
SERCAN PAZARLAR	sercan.pazarlar@ege.edu.tr	TURKEY
BİRSEN ÇAKIR	birsen.cakir@ege.edu.tr	TURKEY
KALLIOPE PAPAPOULOU	kalpapad@bio.uth.gr	GREECE
BIRGIT MITTER	birgit.mitter@ait.ac.at	AUSTRIA
CAROLINE SCHNEIDER	schneider@pflanzenkultur.de	GERMANY
BELLIS KULLMAN	belliskullman@gmail-com	ESTONIA
BAYRAM KANSU	kansu_bayram@yahoo.com	TURKEY

