Oral Presentations
Plant Pathogen Interactions I

O PPI I-1
A biochemical engineering approach to identify priming compounds
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Introduction: An alternative crop protection strategy is needed since the resistance of pathogens and pests to agrochemicals is increasing. Moreover, the extensive use of pesticides, fungicides. A promising alternative crop protection strategy is defense priming [1]. This way of defense exploits the immune system of plants, and thus enhances the stress tolerance to biotic and abiotic stress [1].
Objective: The demand for new compounds for an effective crop protection is increasing and so is the need for an effective screening system. Until now, the identification of priming compounds relied on invasive approaches [2], or required the detection of secreted furanocoumarin phytoalexins in parsley cell cultures [3] resulting in prolongation of the experiment. In this work, a simple, fast, and noninvasive technique is established for identifying new priming-inducing compounds for plant protection based on the oxygen consumption of parsley suspension cell cultures.
Materials and methods: The respiration activity monitoring system (RAMOS) [4] was used to on-line identify priming compounds. The impact and dose-dependency of well-known priming compounds and the impact of non-priming compounds on the oxygen transfer rate (OTR) was evaluated with the parsley model system. 2D-fluorescence measurements of the furanocoumarin phytoalexins [3] served as validation.
Results: Treatment of parsley suspension cells with the known priming compound salicylic acid (SA) resulted in a dose-dependent increase in OTR. The addition of putative priming-active and priming-inactive compounds confirmed that the presence of priming correlates with an increase in OTR. The results were underlined by the determination of the furanocoumarin phytoalexins via 2D-fluorescence spectroscopy.
Conclusion: The OTR was assessed to identify priming compounds immediately after addition of the compounds to parsley cell cultures. The online signal enabled a robust and fast determination of priming compounds and can be applied to other plant cell suspension cultures.

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O PPI I-2
Comparative analysis of *Acholeplasmataceae* genomes highlights the particular genetic repertoire of '*Candidatus* Phytoplasma' strains
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Introduction: *Acholeplasmataceae* comprises the genera *Acholeplasma* and '*Ca*. Phytoplasma'. Acholeplasmas are described as saprophytic bacteria in general, while phytoplasma strains are characterized as obligate intracellular parasites of the plant phloem associated to diseases in >1,000 plant species. Genome research enables the identification of effector proteins and the reconstruction of the metabolism. The complete genomes of 5 phytoplasma strains and 4 *Acholeplasma* spp. have been analysed (1,2).
Objectives: Comparative genome analyses provide insights into the evolutionary split of these two genera and the obligate parasitism of phytoplasmas in comparison to the acholeplasmas.
Methods: Different technologies ranging from clone-based Sanger sequencing, pyro-sequencing, sequencing by synthesis and single molecule real time sequencing were applied. Annotation included functional reconstruction and comparative analyses accomplished by gene expression studies.
Results: The conserved gene core of phytoplasmas is also encoded by the analysed 4 acholeplasmas in majority. Phytoplasmas are separated by a particular carboxylic acid metabolism, membrane proteins involved in host interaction and virulence factors.

Conclusion: Particularities of the phytoplasmas such as the symporter for the uptake of carboxylic acids and their conversion to pyruvate should be interpreted with respect to the Gram+ origin in contrast to genes encoding effectors, which may be derived from horizontal gene transfers.

References

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O PPI I-3

Pathogenicity of *Erwinia amylovora* on Host and Non-Host Plants

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Erwinia amylovora causes fire blight disease, a necrotic and invasive disease responsible for considerable economic losses in host plants and to elicit the HR in nonhost plants depends on the presence of a functional type III secretion system (TTSS) encoded by the *hrp* gene cluster. The TTSS effector DspA/E is an essential pathogenicity factor of *E. amylovora*. In the study, a wild-type and a TTSS mutant strains of *E. amylovora*, seeds of the *A. thaliana*, Col-5, Ler-0, Wseds, Ler-Fls2 ecotype and mutants, and two year-old 'Gala' apple seedlings, and eight week-old tobacco plants, *Nicotiana tabacum* cvs. Xanthi, Benthamiana and White Burley, were used. The leaves of *A. thaliana*, apple and tobacco seedlings were infiltrated with bacterial suspensions at a concentration of 10^8 CFU ml^{-1} using a needleless syringe. Bacterial counting was performed at 24 hpi on NA medium. Symptom severity was scaled as described in our previously studies. Callose was stained with aniline blue and the leaf disks were examined by fluorescence microscopy. Enzyme extractions and measurements of peroxidase, catalase, glutathione-S-transferase activities and of total protein contents were assayed. We confirmed that DspA/E acts as a major cell-death inducer during disease and HR, because the *dspA*/*E* mutant is severely impaired in its ability to induce electrolyte leakage in apple, *A. thaliana* and tobacco leaves. Bacterial populations of the *dspA*/E mutant decreased immediately following inoculation. Wild type and mutant strains of *E. amylovora* activated the antioxidative enzymes and activation reached to maximum level after 24 h later. DspA/E repressesed protein synthesis and triggered a defence response. Mutant *dspA*/*E* strain induced a HR in tobacco cvs. with different types and at 12-36 hours. In addition, wild-type strain led to a significant increase in electrolyte leakage in both host and nonhost plants. This study showed that *E.amylovora* wild type and mutant strains played role at different levels in the induction of cell death, activation of defense pathways, protein and ROS accumulation, the multiplication and survival in host and nonhosts, apple, *A. thaliana* and tobacco. Knowing of further details on the pathogenicity of *E. amylovora* and of defense mechanisms of the host and non host plants will be crucial for fire blight control strategies.

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O PPI I-4

Molecular characterization of the prehaustorial resistance against wheat leaf rust in Einkorn

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Leaf rust of wheat caused by *Puccinia triticina* f. sp. *tritici* causes yield losses worldwide. *Triticum monococcum* accessions are valuable sources for improving leaf rust resistance in wheat. In screening programs accession *Pi272560* has been identified showing prehaustorial resistance (PHR). The race non-specific PHR prevents the infection by *P. triticina* prior to the formation of haustorial mother cells (HMC).

Goals of our studies were (i) to analyze the biochemical background of this resistance by microscopy, the detection of H_2O_2 and the peroxidase and chitinase acitivity in leaves and (ii) to determine the molecular background by genome wide expression studies using the massive analysis of cDNA ends (MACE) and a BlastX to identify gene ontology (Go) terms.

Microscopy was performed using a 1,3 Diaminobenzidine stain of H_2O_2 and Uvitex 2B for the specific stain of fungal cells. Concentration of H_2O_2 was measured by a xylenol orange assay, peroxidase and chitinase activity was determined using assays from Sigma-Aldrich (Taufkirchen). Within the first 24 hours after inoculation (hai) MACE was performed by the GenXpro GmbH (Frankfurt), for Go-term identification "Blast2Go" software package was used.

Microscopy showed an inhibition of fungal growth after the generation of very few HMC in Pi272560 accompanied by auto-fluorescence around infection sites and a reduced fluorescence of fungal cell walls. An increased concentration of H_2O_2 24-48