

**PS 16-879****MOLECULAR MECHANISMS INVOLVED IN THE PSEUDOMONAS FLUORESCENS STRAIN MKB 158-INDUCED FUSARIUM RESISTANCE OF BARLEY SEEDLINGS**

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The *Pseudomonas fluorescens* strain MKB 158 induces systemic barley resistance to Fusarium seedling blight disease caused by the pathogen *Fusarium culmorum*. Microarray analyses determined that the growth of barley seedlings in bacterium-amended soil, as compared to in bacterial growth medium-amended soil, resulted in the up-regulation of 7 and 27 barley genes in pathogen-inoculated stem base tissue at 4 and 24 h post-pathogen inoculation, respectively. These up-regulated genes are involved in diverse processes, including signal transduction, transportation, carbohydrate metabolism and synthesis of defence-related compounds. Down-regulated genes included those involved in jasmonate- and pathogenesis-induced defence. Promoter analyses and/or the function of the bacterium-responsive barley genes or their rice homologs suggested that auxin, abscisic acid and/or brassinosteroids may play a role in this induced systemic resistance mechanism. *In vitro* test indicated that the *Pseudomonas fluorescens* strain MKB 158 is an auxin producer (5.7 µg ml<sup>-1</sup> of culture media). Soil amendments with either the auxin indole acetic acid or the brassinosteroid 24-epi-brassinolide reduced the severity of Fusarium seedling blight disease on *Fusarium culmorum*-inoculated barley stem base tissue (25% and 28% reductions, respectively, relative to disease levels on *F. culmorum*-inoculated plants grown in non-amended soil;  $P \leq 0.050$ ). A preliminary study showed that *F. culmorum* inoculation of stem base tissue resulted in 91% less Fusarium seedling blight disease symptoms on a brassinosteroid insensitive barley mutant derivative (uzu) as compared to on its normal barley line (Akashinriki) ( $P < 0.010$ ). This suggests that either alternative brassinosteroid signalling pathways or a brassinosteroid mediated feedback mechanism influences the response of barley to Fusarium seedling blight disease.

**PS 16-880****MOLECULAR AND BIOCHEMICAL ANALYSIS OF ANTAGONISTIC BACTERIA INVOLVED IN SOIL SUPPRESSIVENESS TO RHIZOCTONIA ROOT ROT**

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*Rhizoctonia solani* is a major pathogen of a variety of crops worldwide. Recently, a soil naturally suppressive to Rhizoctonia root rot of sugar beet was identified in the Netherlands. Bioassays revealed that this soil suppressiveness has a microbiological basis as it was eliminated after heat treatment (50°C) of the soil. The overall objective of this study is to identify key microorganisms, genes and traits involved in disease suppressiveness. Following a classical approach, approximately 600 aerobic bacteria were isolated from the rhizosphere of sugar

beet seedlings grown in the *Rhizoctonia* suppressive soil. A total of 107 isolates was obtained that strongly suppress *R. solani* growth *in vitro*. BOX-PCR and 16S-rDNA sequencing resulted in 13 different bacterial groups, all belonging to the genus *Pseudomonas*. Isolates belonging to one major group produce the well-known antibiotic 2,4-diacetylphloroglucinol (DAPG). Site-directed mutants defective in DAPG production no longer inhibit *R. solani* growth. For each of the two other major genotypic groups that do not produce a known antibiotic, random mutants that do not inhibit *R. solani* were generated. Sequence analysis of the disrupted genes indicated that in both groups non-ribosomal peptide synthetases (NRPS) involved in the production of putative novel cyclic lipopeptide antibiotics (CLPs) were targeted. Sequence analysis of several mutants further indicated a potential role for extracellular glucose in regulation of the identified CLP genes. The CLPs and corresponding gene clusters are currently being analyzed. The effect of the different *Pseudomonas* isolates and their respective mutants on *R. solani* disease suppression will be discussed.

**PS 16-881****CHARACTERIZATION OF AN ANTIFUNGAL GENE CLUSTER ISOLATED FROM PLANT RHIZOSPHERE METAGENOME**

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Soil metagenome comprises total microbial DNA from the majority of unculturable soil microorganisms. The soil metagenome constitutes a valuable resource to search for novel antimicrobial compounds and their genes simultaneously. A soil metagenome library was constructed in *Escherichia coli* using a fosmid vector and DNA directly extracted from pine tree rhizosphere soil. The library consisted of approximately 33,700 clones with an average DNA insert size of 35-40 kb. We screened the library by double agar layer method to select clones with antifungal activity using *Saccharomyces cerevisiae* as a target fungus. A clone named EAF66 active against *S. cerevisiae* was isolated and characterized by DNA sequence analysis and *in vitro* mutagenesis. Genetic analysis of the antifungal clone revealed that maximum 20 open reading frames (ORF) are probably involved in the biosynthesis of antifungal secondary metabolites. The ORFs included genes encoding enzymes, membrane proteins for secretion, and regulatory proteins involved in antifungal compound biosynthesis. Gene organization of EAF66 for antifungal compound biosynthesis was partially similar to a gene cluster in *Pseudomonas putida* KT2440. Deduced amino acids identities between genes of EAF66 and *P. putida* KT2440 were from 29 to 53%. The antifungal compound was not identified since we could not purify the active fraction, although the genes in the cluster were similar to the genes for polyketide biosynthesis. Introduction of one of genes encoding a regulatory protein into *E. coli* with original EAF66 clone increased the antifungal inhibition zone against *S. cerevisiae*, indicating that IclR family response regulator may be a positive regulator for the biosynthesis