

ent activities against plant pathogens into one organism can improve the performance of biocontrol agents (BCAs). The 5.8-kb *prnABCD* operon encoding the strong antifungal antibiotic pyrrolnitrin [Prn, (3-chloro-4-(2'-nitro-3'-chlorophenyl) pyrrole)] from *Pseudomonas fluorescens* Pf-5 was cloned into the broad host-range plasmid vector pUCP26 under control of the *tac* promoter. The construct was employed to enhance the antifungal activity and broaden the range of target pathogens controlled by the closely related strain *P. fluorescens* Q8r1-96. The latter strain produces 2,4-diacetylphloroglucinol (DAPG), a broad-spectrum antibiotic responsible for the natural suppressiveness of some soils to *Gaeumannomyces graminis* var. *tritici*, which causes take-all disease of wheat. Strain Q8r1-96 is an aggressive rhizosphere colonizer able to maintain a high population density in the wheat rhizosphere. However, Q8r1-96 is relatively inefficient against some plant pathogens including *Rhizoctonia solani*, which infects many economically important crops. Strain Q8r1-96 derivatives carrying the hybrid plasmid pUCP26/*tac-prnABCD* produced Prn in addition to DAPG. The recombinant clones protected beans against *Rhizoctonia* root rot under greenhouse conditions significantly better than did the parental strain. Strain Q8r1-96 and its Prn-producing derivative showed similar persistence in natural rhizosphere soil under greenhouse conditions. Moreover, the plasmid containing the *prn* genes was stably maintained in vitro and in soil for at least 15 days during the greenhouse assay.

This research was supported by the United States - Israel Bina-tional Agricultural Research and Development Fund (BARD), Grant No. US-3789-05.

PS 16-870

MOLECULAR AND FUNCTIONAL ANALYSIS OF MASSETOLIDE A BIOSYNTHESIS IN THE BIOCONTROL BACTERIUM *PSEUDOMONAS FLUORESCENS*

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Cyclic lipopeptides (CLPs) are biosurfactants produced by a variety of microorganisms, including plant-pathogenic and beneficial *Pseudomonas* species. The CLP massetolide A is produced by antagonistic *Pseudomonas fluorescens* strain SS101 and consists of a 9-amino acid cyclic peptide linked to a 10-C fatty acid tail. Molecular analysis revealed that massetolide A biosynthesis is governed by three large non-ribosomal peptide synthetase (NRPS) genes, designated *massA*, *massB* and *massC*. *MassA*, *B* and *C* contain in total nine modules, each responsible for the incorporation of one amino acid in the peptide ring. The massetolide A biosynthetic gene cluster also includes regulatory genes in the LuxR family and genes with predicted roles in transport. In contrast to other CLP biosynthetic gene clusters described to date, the physical organization of the *mass* genes is different with *massA* being fully disconnected from *massBC*. Massetolide A is produced by strain SS101 in the early exponential growth phase and appears not to be regulated by *N*-acylhomoserine lactones. Mutants deficient in massetolide biosynthesis are impaired in biofilm formation and surface motility. Massetolide A has activity against a wide range of plant pathogens, including *Pythium* species, *Phytophthora infestans* and *Rhizoctonia solani*. The discovery of the genes and antimicrobial activities of massetolide A is an essential step toward re-

solving the complexity of biosynthesis and regulation of CLPs, and to expand the potential application of these compounds for plant protection.

PS 16-871

FLUORESCENT REPORTER-BASED MONITORING OF BIOCONTROL GENE EXPRESSION IN *PSEUDOMONAS FLUORESCENS* ON ROOTS OF DIFFERENT PLANT SPECIES AS WELL AS HEALTHY AND PATHOGEN-ATTACKED PLANTS

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Plant roots release a vast diversity of compounds and some of them are likely to influence the growth and activity of microorganisms inhabiting the rhizosphere. The abundance and diversity of these compounds may significantly change among different monocotyledonous and dicotyledonous plant species as well as when the plant is stressed or attacked by a pathogen. We suppose that such a modification of root exudates might have a positive or negative influence on the production of secondary metabolites in root-associated pseudomonads. In this study, we focus on the effect of the plant health status on the expression of important biocontrol genes in *Pseudomonas fluorescens* strain CHA0. This bacterium produces the antimicrobial compound 2,4-diacetylphloroglucinol (DAPG) which is major determinant of its disease suppressive activity. Our specific aim is to monitor the influence of plant infection by phytopathogens on the expression of DAPG biosynthetic genes in the rhizosphere of different crop plant species. The plant-pathogen systems used include the leaf-pathogens *Glomerella lagenaria* and *Botrytis cinerea* on cucumber, *Puccinia recondita* on wheat as well as the root pathogen *Pythium ultimum* on different plant species. For monitoring effects on DAPG gene expression, we rely on GFP-based reporters fused to the biosynthetic gene *phlA* of CHA0. The in situ expression of the GFP-based reporter fusions on roots is quantified using a FACSCalibur flow cytometer. First results are presented.

PS 16-872

FUSARIUM RESISTANT WHEAT PLANTS- DO THEY CULTIVATE INSIDE THE FLORET THEIR OWN MERCENARIES FIGHTING AGAINST HEAD BLIGHT?

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The development of microbial products to prevent fungal diseases on agricultural crops has become an important issue to reduce the application of conventional fungicides. The target to search for antagonistic microorganisms was the gene pool on the infection site of Fusarium causing Fusarium head blight (FHB). Two wheat genotypes (CM82036 and Remus) with different FHB resistance were grown under the same conditions in the field. At anthesis they were artificially inoculated with the same amount of a deoxynivalenol-producing *Fusarium graminearum* strain. Microbial DNA was extracted directly from the infection site, the surface of anthers collected from wheat florets. Microbial community fingerprint patterns were generated with the help of T-RFLP. The results showed that there exist