also recovered from a part of strawberry plants inoculated with strains isolated from raspberry indicating a possibility for *E. amylovora* to grow on non-wooden plants. Monitoring of *E. amylovora*, *E. billingiae* and *E. tasmaniensis* by real-time PCR allows a description of their populations in infected apple or pear flowers.

(DPG AK Phytobakteriologie)

Effects of bacteriophages and bacteriocins on Erwinias

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Bacteriocins and bacteriophages can be used as specific inhibitors of bacteria. For control of fire blight, they may be applied instead of the antibiotic streptomycin to reduce plant colonization by *Erwinia amylovora*. Partially characterized *E. amylovora* phages were used to interfere with growth of several *E. amylovora* strains, which reacted differently in phage drop tests. PCR primers were designed from the EPS depolymerase gene to distinguish the viral genomes. Analysis of *E. tasmaniensis* strain Et1/99 revealed klebicin-like toxin-genes on one of its five plasmids. By treatment with mitomycin, the bacteriocin was induced, and tested against several strains of the species *E. amylovora*, *E. billingiae* and *E. tasmaniensis*. Growth inhibition was observed for the South African *E. tasmaniensis* strains Esa13 and Esa41 and FLA03 from Germany. They do not carry the immunoprotein gene and are therefore sensitive to the bacteriocin. Application of cell extracts with the induced bacteriocin to an Australian *E. tasmaniensis* strain induced phage plaques. The genome of this novel bacteriophage was sequenced (with M. Kube, Berlin) and is unrelated to described *E. amylovora* phages. An important factor of pathogenicity of *E. amylovora* is synthesis of the capsular EPS amylovoran. It can be cleaved by viral EPS polymerase and determined by a turbidity assay with CPC. This assay was applied not only to amylovoran but also to EPS of *E. billingiae* and *E. persicina*. It can be expected that their EPS is similar to amylovoran. The use of bacteriocins, bacteriophages and EPS degradation can add to the control of fire blight.

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Upstream sequence analysis of the levansucrase gene in *Pseudomonas syringae*

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Plant pathogenic bacteria *Pseudomonas syringae* pv. glycinea PG4180, are cold weather pathogen - effectively displaying the virulence factors at low temperature and causing chlorosis and necrosis in soybean plant. The focus of our research work encompasses the study of levan biopolymer synthesizing enzyme - levansucrase (EC 2.4.1.10). This enzyme is present in three copies namely *lscA*, *B* and *C* where the former is not transcribed in the native host but the latter two code for LscB and C enzymes. Our current effort is to map the transcriptional start site for *lscB* and *C*. With the help of nested deletion strategy, we have generated an entire array of deletion constructs where upstream sequence of the *lscB* is available in various lengths. Such a construct is transconjugated into the lsc mutant namely PG4180.M6 and the phenotype is observed as the slime formation on mannitol-glutamate agar media containing the substrate sucrose. We have predicted the promoter of *lscB* gene between -440 and -330 position upstream to the translational start site of the open reading frame. Further experiments are being conducted where the exact transcriptional start site will be mapped by primer extension method.

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Screening for *Erwinia billingiae* and *E. tasmaniensis* in field isolates, differentiation by sequence analysis and effects as antagonists of fire blight

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In assays with pear slices and with apple flowers, bacteria of the species *Erwinia billingiae* and *E. tasmaniensis* are antagonistic to colonization of plant tissue with *E. amylovora*, the causative agent of fire blight. PCR primers were designed from the *wbdN* gene of both species to detect them in bacterial populations of field samples. The signals were confirmed with primers for real-time PCR and positive bacteria were isolated. Most showed a close homology of the 16S rRNA and the *wbdN* gene sequences. Base changes were visualized by SSCP analysis. A recently isolated *E. tasmaniensis*-like strain from Germany showed a signal with *wbdN* primers but not with primers from the *hrpL* region. Other strains were isolated in Australia, in South Africa and in the area of Heidelberg and were positive with *hrpL* primers. *E. billingiae*-like strains were first isolated in England and recently in Poland. They were closely related for their 16S rRNA and the *wbdN* genes. Their EPS could be degraded with an EPS depolymerase, specific for degradation of amylovoran and supported their coclassification with described *E. billingiae* strains. Two additional strains were isolated from apple leaves in an orchard of the BBA Dossenheim (now Julius Kuehn Institute). PCR amplification of the house keeping genes *recA* and *rpoS* and subsequent analysis of the DNA cleavage pattern may add to their classification within the species *Erwinia*.

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Genome-wide analysis of multidrug efflux in *Pseudomonas syringae* pv. *tomato* DC3000

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Multidrug efflux (MDE) transporters are major contributors to bacterial resistance towards antibiotics. In contrast to the well-understood role of MDE in clinically relevant microbes, only little is known about MDE transporters in environmental bacteria. In this project we aim to identify and characterize all MDE pumps in the plant pathogen *Pseudomonas syringae* and to gain in-depth knowledge about their regulation and natural functions. MDE pumps may play an important role in the adaptation of *P. syringae* to its respective host plants by protecting them against plant antimicrobials. The available genome sequence of *P. syringae* pv. *tomato* DC3000 was used to develop a microarray containing genes of all members of transport protein families that include MDE systems. The microarray will be used to identify transporters that are expressed after treatment with antibiotics, antimicrobial plant metabolites, and in *planta*. A second strategy to identify actice MDE pumps in *P. syringae* pv. *tomato* DC3000 involves the complementation