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Project ACHILLES

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Zusammenfassung und Highlights

Die Pflanzenkrankheit Feuerbrand, ausgelöst durch das Bakterium *Erwinia amylovora*, befällt Kernobstgewächse, dazu gehören Apfel, Birne oder Quitte. Sie verursacht jedes Jahr beträchtliche Schäden und die Überwachung und Bekämpfung sind eine grosse Herausforderung im Obstbau. Die Stärke des Befalls und damit die Höhe der Schäden ist von den klimatischen Bedingungen während der Blütezeit abhängig. In dieser Zeit können sich die Bakterien auf den Blüten vermehren und die Pflanze schliesslich invadieren. Um Infektionen zu verhindern, muss dieses Bakterienwachstum gehemmt werden, denn sobald das Bakterium in der Pflanze ist, hilft nur noch ein Rückschnitt oder, im fortgeschrittenem Stadium der Krankheit, die Rodung der Pflanze. Eine Strategie der Feuerbrandbekämpfung, die in der Schweiz als Reaktion auf das verheerende Feuerbrandjahr 2007 erlaubt wurde, ist der restriktive Einsatz von Streptomycin. Der Einsatz dieses Antibiotikums ist allerdings umstritten. Es wird befürchtet, dass sich resistente Feuerbranderreger bilden und dass so auch das Auftreten von Resistenzen in der Humanmedizin gefördert wird. Deshalb sind alternative Bekämpfungsmassnahmen notwendig.

Bakterielle Antagonisten sind eine vielversprechende Alternative. Sie besiedeln ebenfalls Kernobstblüten und konkurrieren so mit *E. amylovora* um Platz und Nährstoffe. Beispiele solcher Antagonisten sind Bakterien der Gattung *Pantoea*. Einige *Pantoea* Stämme produzieren zusätzlich einen antibakteriellen Wirkstoff, der das Wachstum vom Feuerbranderreger direkt hemmt. Solche Antagonisten sind in einigen Ländern bereits kommerziell erhältlich, ihre Wirkungsweise ist allerdings noch nicht vollständig geklärt.

Agroscope hat in vergangenen Forschungsprojekten wichtige neue Erkenntnisse über den Feuerbrand gewonnen. Erwähnt seien hier die Entschlüsselungen der Genome vom Feuerbranderreger (*E. amylovora* CFBP1430) und eines seiner Antagonisten (*Pantoea vagans* C9-1). Diese Arbeiten geben einen Einblick in die „Arsenale“ des Feuerbranderregers und seiner Gegenspieler und ermöglichen weiterführende Erkenntnisse über Krankheitserreger und Wirkungsweise der Antagonisten zu erlangen.

Das Ziel des Projektes „Achilles“ war es, die von Agroscope-Forschern erarbeiteten Grundlagen zu nutzen, um vorhandene Bekämpfungsstrategien verbessern und neue entwickeln zu können. In zwei Arbeitspaketen wurden der Erreger bzw. die Antagonisten untersucht.

Im ersten Teil (Erreger) wurden *Erwinia* Genomsequenzen verwendet, um Spezies- und Stamm-spezifische DNS-Sequenzen, sogenannte molekulare Marker, zu identifizieren. Damit konnten Methoden entwickelt werden, die die Erkennung des Feuerbranderregers bzw. einzelner Stämme ermöglichen. Mit einem sogenannten LAMP-Schnelltest (LAMP; loop-mediated isothermal amplification) kann *E. amylovora* innerhalb von 15 Minuten im Feld identifiziert werden. Die Sensitivität dieses Tests ist um einiges höher als diejenige des AgriStrip® Schnelltests, es können also kleinere Zellzahlen nachgewiesen werden. Die Auflösung der Stamm-spezifischen Methode basierend auf VNTR (VNTR; variable number tandem repeat) ist so hoch, dass es

möglich ist, einzelne Stämme aufgrund ihrer Herkunft zu gruppieren. Dies ist für epidemiologische Untersuchungen bei einem Feuerbrand-Ausbruch wichtig. Des Weiteren kann bestimmt werden, ob ein *E. amylovora* Stamm *Rubus*-Gewächse (z.B. Brombeeren) oder Pflanzen der Unterfamilie Spiraeoideae (z.B. Apfel) befällt.

Durch vergleichende Genomik mit Sequenzen anderer *Erwinia* Spezies konnte ein Typ VI Sekretionssystem (T6SS) in *E. amylovora* als möglicher Virulenzfaktor identifiziert werden. Experimentelle Untersuchungen zeigten jedoch, dass das T6SS eher einen Einfluss auf Stoffwechsel und Beweglichkeit des Feuerbranderregers hat.

Ein wichtiger Teil des ersten Moduls war eine Transkriptomanalyse des Erregers und des Wirtes. Die Sequenzierung eines Genoms bestimmt die darin vorhandenen Gene. Durch die Transkriptionsanalyse kann gezeigt werden, welche dieser Gene exprimiert, also während eines Prozesses aktiviert werden. Um herauszufinden, welche Gene des Feuerbranderregers bei der Infektion wichtig bzw. welche Gene des Apfelbaums bei der Abwehrreaktion aktiviert sind, wurde eine solche Transkriptomanalyse durchgeführt. Die Identifizierung von Genen/Prozessen, die bei der Infektion bzw. Abwehr des Feuerbanderregers wichtig sind, bildet eine wichtige Grundlage bei der Entwicklung von Bekämpfungsmassnahmen mit direkter Wirkung auf diese Gene/Prozesse. Es zeigte sich, dass durch eine Infektion mit *E. amylovora* viele Gene des Apfelbaums aktiviert werden, die mit Stressreaktionen und Abwehr von Pathogenen verbunden sind. Die Funktion einiger aktivierter Gene konnte, aufgrund fehlender Informationen im Apfelgenom, nicht ermittelt werden. Die Transkriptomanalyse bildet eine wichtige Grundlage für die weitere Erforschung der Interaktion zwischen *E. amylovora* und seinem Wirt. In Nachfolgeprojekten sollten die Reaktionen von *E. amylovora* resistenten/toleranten Pflanzen mit Sensitiven verglichen werden. Zusätzlich sollte die Reaktion einer sensitiven Pflanze auf verschiedene Bakterien analysiert werden, um Gene mit einer spezifischen Abwehrfunktion gegen Feuerbrand weiter eingrenzen zu können. Dieses Wissen könnte direkt in die Züchtung feuerbrandresistenter Apfelsorten transferiert werden.

Modul 2 beschäftigte sich mit den Antagonisten. Die in diesem Modul erarbeiteten molekularen Grundlagen ermöglichten die Entwicklung von Methoden zur Erkennung bestimmter Antagonisten. Molekulare Marker erlauben es, *Pantoea* Spezies schnell und vor allem zuverlässig zu identifizieren. Damit kann die Ausbreitung von Antagonisten nach der Applikation im Feld verfolgt werden. Dies ist wichtig, um abschätzen zu können, welchen Einfluss die Freisetzung von Bakterien auf die Umwelt hat. Zudem wurden im *P. vagans* Stamm C9-1 sowie in *Pantoea agglomerans* E325 Gene identifiziert, die diese Stämme zur Produktion eines Antibiotikums befähigen. Die Identifizierung dieser Gene kann dazu genutzt werden, vielversprechende Antagonisten gezielt zu suchen. Die Bedeutung dieser Gene für die antagonistische Wirkung wurde experimentell gezeigt. Einer in diesen Genen mutierter Stamm von *P. vagans* C9-1 war in seiner Wirkung gegen den Feuerbrand deutlich reduziert.

Dank der Kenntnis der für die Antibiotika-Produktion von *P. agglomerans* E325 verantwortlichen Gene konnten neuartige Biosensoren entwickelt werden. Mit Hilfe dieser Biosensoren kann die

Produktion des Antibiotikums nun einfach quantifiziert werden. Damit kann die Produktion der antibakteriellen Substanz unter verschiedenen Bedingungen getestet und mit gezielter Formulierung optimiert werden.

Analog zu Modul 1 wurde eine Transkriptionsanalyse mit dem Antagonisten *P. agglomerans* E325 durchgeführt. Die Datenauswertung ist noch nicht abgeschlossen. Sie wird einen Einblick in diejenigen Gene geben, die bei der antagonistischen Wirkung gegen Feuerbrand eine wichtige Rolle spielen. Bei diesen Experimenten wurde zusätzlich mit einer quorum-sensing (QS) Mutanten gearbeitet. QS spielt eine wichtige Rolle bei der bakteriellen Kommunikation; so können Prozesse abhängig von der Zelldichte gesteuert werden. Im Falle von *P. agglomerans* ist die Bildung des Antibiotikums, das bei der Feuerbrand-Bekämpfung wichtig ist, möglicherweise QS-abhängig.

Es gab Berichte über *Pantoea* Spezies, die in menschlichem Gewebe gefunden wurden. Solche Berichte sind dem Einsatz dieser Bakterien als Feuerbrand-Antagonisten nicht zuträglich. Im Genom der Antagonisten konnten jedoch keinerlei Virulenzfaktoren identifiziert werden und es konnte eindeutig gezeigt werden, dass viele als *Pantoea* eingeordnete Stämme falsch klassifiziert worden. Verwechslungen in der Klassifizierung sollten nun der Vergangenheit angehören.

Summary

Fire blight caused by the Gram-negative pathogen *Erwinia amylovora* is a serious disease affecting *Rosaceae* species such as apple and pear. It is the major threat to pome fruit production worldwide with high financial losses every year. The currently most reliable control option is the application of antibiotics, mainly streptomycin. Resistance development and legal restrictions forward the search for alternative control measures. A promising option is the application of bacterial antagonists, which interfere with *E. amylovora* growth on open flowers. This stage of epiphytic growth is crucial for the progress of the disease. Due to competition for nutrients and the production of antimicrobial substances, antagonists of the genus *Pantoea* are able to inhibit epiphytic growth of *E. amylovora*, thus preventing infection of the host.

In past projects, researchers from Agroscope completely sequenced the genomes of the pathogen *E. amylovora* CFBP1430 and *Pantoea vagans* C9-1, a commercially available antagonist. These projects gave an important insight into the “arsenals” of the pathogen and the antagonist, and offer a solid basis for the development of novel tools for detection and control.

The aim of the ACHILLES project was to exploit our acquired molecular knowledge to identify *E. amylovora* weaknesses to develop novel control compounds directly targeting survival and virulence genes, and to improve efficacy of biocontrol using bacterial antagonists.

By applying comparative genomics and sequence analysis, gene regions specific for certain species or strains were identified, so-called molecular markers. With these markers, a series of tools to detect certain species or strains was developed. *E. amylovora* can be detected in the field within 15 min using a LAMP-assay (loop-mediated isothermal amplification). Variable regions in the genomes of *E. amylovora* strains (VNTR, variable number tandem repeat) were the basis for strain-specific detection, facilitating epidemiological studies. We also developed PCR-based methods targeting molecular markers of antagonists. These tools allow specific detection of *Pantoea agglomerans*, pantocin A-, and herbicolin I-producing strains. This is of use for tracking antagonists after their release into the environment and the isolation of new biocontrol strains with high potentials.

Genes actually expressed during infection were identified by transcriptome analysis of both *E. amylovora* and apple. Virulence determinants of the pathogen can now be identified to develop biosensors to rapidly screen conditions that suppress these genes. In the apple genome, general resistance and stress response pathogens were up-regulated. Genes involved in the specific response to *E. amylovora* could be identified by comparing the response to different bacteria. The transcriptome of *P. agglomerans* strain E325 was also analyzed in order to identify important biocontrol determinants. Analysis of the sequencing data is in progress and will be finished soon.

To improve biocontrol efficacy of strain E325, a biosensor tool was developed. The production of the antimicrobial substance can now be quantified under different conditions to optimize formulation.

1 Introduction

Fire blight, the first described bacterial plant disease, is the major threat to global pome fruit production, causing high economic losses every year due to reduced yield, control measures and compensation costs. The causative agent, *Erwinia amylovora*, is a member of the *Enterobacteriaceae* [1-3]. As a major breakthrough, genome sequencing of the pathogen (strain CFBP1430) was accomplished in a prior BLW project [4]. It was the starting signal for a comprehensive study of *Erwinia* genomes. Comparative genomics within *Erwinia* species could help to detect *E. amylovora* weaknesses to develop strategies, which directly target the pathogen's Achilles' heel by interfering with important survival and virulence genes.

The molecular understanding of the host also advanced recently as the complete genome of the economically most important susceptible plant, the domesticated apple, was published [5]. However, the molecular basis of resistance and susceptibility of apple to *E. amylovora* is largely unknown. Only transcriptome analyses can reveal induced and repressed pathways in *E. amylovora* – apple interactions [6]. The molecular knowledge acquired in past projects offers a solid basis for transcriptome analyses. Genes actually expressed during infection could be analyzed to identify important virulence determinants (pathogen) and resistance genes (host). This knowledge could be used to improve the efficacy of control compounds and it could be transferred to resistance breeding.

Fire blight can reliably be controlled by the prophylactic application of antibiotics (mainly streptomycin). Nevertheless, alternative control measures are required due to resistance development of the pathogen, health concerns of the consumers and regulatory restrictions [7-9]. Infection mainly occurs during the flowering period of *E. amylovora* hosts. For infection, the pathogen needs to multiply on the flower stigmas and reach a certain cell density [10]. Bacterial antagonists, which are able to suppress the epiphytic growth of *E. amylovora*, offer promising and environmentally sound alternatives for the control of the disease. Strains of *Pantoea vagans* and *Pantoea agglomerans* are bacterial antagonists able to suppress the epiphytic growth of *E. amylovora* due to a dual mode of action. They compete for the same niche and nutrients (competitive exclusion) and produce antimicrobial compounds active against *E. amylovora* [11-16]. Researchers from Agroscope contributed to the better understanding of the antagonist mode of action by sequencing the genome of *P. vagans* strain C9-1 [17]. Sequencing of *P. agglomerans* E325 is finished, but not published yet. So far, only a few biosynthesis genes of antibiotics produced by *Pantoea* spp. have been identified [18-21]. One antibiotic gene cluster (herbicolin I) of *P. vagans* C9-1 and the cluster of *P. agglomerans* E325, both commercially used antagonists, were previously unknown. The latter strain produces a unique compound [12, 22]. The identification of antibiotic biosynthesis gene clusters of antagonist allows the screen for potential antibiotic producing strains and the development of molecular marker tools enabling monitoring of biocontrol agents in the environment and isolation of new biocontrol strains. Transcriptome analysis of antagonists could reveal important biocontrol determinants and the development of

biosensor tools quantifying the production of these biocontrol determinants could help to optimize formulation of these strains.

Our molecular understanding of *Erwinia* spp. and *Pantoea* spp. improved in the past with the availability of complete genome sequences. Sequencing gave insights into potential virulence factors, biocontrol determinants and evolutionary aspects. In this project, we aimed to further exploit our knowledge for the development of molecular markers and biosensors, and for transcriptome analyses to improve current and to lay the foundation for novel (bio)control strategies.

2 Module 1 – Pathogen

2.1 Objectives

The aim of module 1 was to deepen our molecular understanding of *Erwinia* species in general and of *E. amylovora* in particular. The focus, however, was set on applied genomics, i.e., using the solid basis of past sequence analyses to provide solutions for the management of fire blight.

Module 1 was divided into the following subprojects:

a) Genomics and diversity
b) Pathogen and host transcriptomics

Sequencing and comparative genomics were a major topic in past projects. The knowledge gained in these studies was aimed to be exploited to develop novel tools for the detection and control of fire blight. Virulence determinants of *E. amylovora* could be identified by comparative genomics of *Erwinia* spp. For example, a type VI secretion system was identified in *E. amylovora* [4]. Type VI secretion systems fulfill versatile functions, e.g., in bacterial communication, as anti-bacterial toxin delivery system, and in host-specificity [23]. However, the function and potential contribution of the *E. amylovora* type VI secretion system was unknown and therefore investigated in this study. In addition, comparative genomics can be applied for a clear identification of this pathogen, and it allows discrimination between different strains of *E. amylovora*, which is of use for tracking purposes and evolutionary analyses. Transcriptome analyses also require an in-depth study of the genomes including a reliable annotation. By analyzing the transcriptome of *E. amylovor*a and its host, the apple tree, genes important for infection and resistance, respectively, could be identified, improving the molecular understanding of the infection process and plant defense determinants. This knowledge in turn could then be used to develop control methods suppressing these genes critical for infection and to improve resistance breeding.

2.2 Results

2.2.1 Module 1a: Pathogen genomics and diversity

Comparative genomics has become a powerful tool for the study of bacterial evolution thanks to the advent of next-generation sequencing and advances in informatics. With the help of this approach, an unprecedented degree of genetic uniformity among strains of *E. amylovora* could be evidenced, suggesting that the pathogen has undergone a recent genetic bottleneck [6, 24, 25].

Comparative genomic analysis of twelve strains representing distinct populations of *E. amylovora* was used to describe the pan-genome of this major pathogen. The pan-genome is highly conserved relative to other phytopathogenic bacteria with an average 89% of conserved core genes. The chromosomes of Spiraeoideae-infecting strains were highly synthenous, while greater genetic diversity was observed between Spiraeoideae- and *Rubus*-infecting strains (and among individual *Rubus*-infecting strains) [26]. Data obtained suggest that *Rubus*-infecting *E. amylovora* strains underwent a process of adaptation to the new host by replacement of the central region of their lipopolysaccharides (LPS) biosynthetic gene cluster [24]. This cluster may be used as a molecular marker to distinguish between *Rubus*- and Spiraeoideae-infecting strains of *E. amylovora* using the primers designed in the study.

Historically, the Hrp pathogenicity island of *E. amylovora* did not only include the Hrp type III secretion system (T3SS) and other genes required for pathogenesis on host plants, but also included the so-called island transfer (IT) region, which was shown now to originate from an integrative conjugative element (ICE). Comparative genomic analysis of the IT regions of Spiraeoideae- and *Rubus*-infecting strains revealed that the regions in Spiraeoideae-infecting strains were highly conserved in length and content, whereas in *Rubus*-infecting strains they were more variable, showing a mosaic structure of genes that are commonly known to be present in ICEs [27]. However, none of the ICE were complete, as in the ICE of *Erwinia piriflorinigrans* CFBP 5888^{T} [28]. This study gave first evidence that the Hrp T3SS was independently inserted into *E. amylovora* from the IT region. Overall, the data support the hypothesis of reductive genome evolution in *E. amylovora* during adaptation to their present hosts.

A novel target on the *E. amylovora* chromosome was identified by applying a comparative genomic analysis pipeline. By designing appropriate primers on that region, a fast and highly specific method for on-field diagnosis of fire blight that uses LAMP (loop-mediated isothermal amplification) of DNA was developed. Using this approach, it is possible to detect consistently down to 10 colony forming units (CFU) of *E. amylovora* per reaction in 15 minutes, either directly in orchards or in the plant diagnostic laboratory [29].

Epidemiology tools based on genomic biomarkers (VNTRs) were developed to monitor phylogeography and population structure of *E. amylovora* under field conditions [30]. This result provides the tools for further studies on the epidemiology of the pathogen at different geographic levels, as for example dissemination between orchards, thus setting the foundation for the improvement of the phytosanitary measures to prevent fire blight spread.

A chromosomal mutation in the *rpsL* gene conferring high-level streptomycin resistance [31] was found in two Mexican strains of *E. amylovora,* whereas a single nucleotide polymorphism was detected in all three sequenced genomes in effector gene *avrRpt2* that overcomes fire blight disease resistance in *Malus* × robusta 5 [32, 33].

Furthermore, we sequenced the complete genome of the closely related *E. piriflorinigrans* type strain CFBP 5888^{T}, a necrotrophic pathogen of pear reported from Spain that destroys flowers but does not progress further into the host, clarifying its phylogenetic position within the genus *Erwinia* that indicates a position between its closest relative, the epiphyte *Erwinia tasmaniensis* and other plant pathogenic *Erwinia* spp. such as *E. amylovora* and the Asian pear pathogen *Erwinia pyrifoliae* [28]. *E. piriflorinigrans* plasmids pEPIR37 and pEPIR5 were sequenced and pEPIR37 was determined to have a similar role in pathogen virulence as the one that pEA29 holds in *E. amylovora* [34].

Significance of the acquired knowledge for an improved fire blight management

Comparative genomics enabled the development of biomarker-based tests for detection of *E. amylovora* and epidemiological studies. A new test (LAMP) is now available, which allows detection of low numbers of *E. amylovora* in the field within 15 min. The performance of this test regarding sensitivity, specificity, speed and simplicity is equivalent or greater than real-time PCR (TaqMan), immunoassays (e.g., AgriStrip®) and plating. Its practical utility needs to be assessed by implementation into routine testing.

Using the VNTR-based method, specific *E. amylovora* strains can now be detected. This method is beneficial for epidemiological studies at different geographic levels and allows tracking of fire blight spread. The knowledge gained in such studies will be important for prevention of further dissemination of the disease.

2.2.2 Module 1b: Pathogen and host transcriptomics

Type VI secretion systems (T6SS) are widespread among Gram-negative bacteria and are essential factors to maintain virulence or to establish symbiotic interaction. Three T6SS gene clusters are present in the genome of *E. amylovora* CFBP 1430. To assess the contribution of T6SSs to virulence of *E. amylovora*, single and double mutants in two structural T6SS genes were generated. Plant assays showed that the different mutants displayed only slightly altered virulence in apple shoots and on apple flowers compared to the wild type strain (not shown). Comparison of transcriptomes indicated that the *E. amylovora* T6SS influences metabolic and motility/chemotactic processes rather than being a virulence factor [35].

The genome of apple, economically the important host of *E. amylovora*, has also been sequenced [5], creating new opportunities for the study of interactions between host and pathogen during fire blight development and the identification of resistance genes. These can be achieved, e.g., by the implementation of genome-level transcriptomics studies [6]. The reactome of susceptible apple flowers in response to fire blight infection was analyzed by RNA-sequencing. In total, 1,080 differentially expressed genes were detected 48 h post-inoculation (Figure 1). Of these, 208 were down-, 872 were up-regulated, respectively. The complete list of significantly differentially expressed genes of apple in response to *E. amylovora* infection can be found in the appendix of ref. [35]. To 240 of the differentially expressed genes, no homologies to annotated open reading frames in the apple genome could be found. In general, reads that mapped to regions where no genes were assigned could be used to identify potential novel genes involved in this mechanism. Transcripts differentially expressed in infected flowers included putative disease resistance, stress, pathogen-related, general metabolic, phytohormone and -related genes. The data showed that, upon *E. amylovora* attack, apple elicits an array of defense responses, reflected in the expression of genes of the jasmonic acid, ethylene, and phenylpropanoid pathways as well as putative resistance genes. Since a compatible host-pathogen interaction was analyzed, the induction of these pathways does not seem to limit disease progression. Either the pathogen modulates/delays the responses directly by secreted effectors or susceptible plants might not accumulate/produce inhibitory compounds to the level needed to stop pathogen growth. If the observed transcriptional changes were specific to *E. amylovora* or common stress responses, was analyzed by comparing the reaction to different bacteria using RT-PCR. Genes expressed in all flowers inoculated with bacteria indicated a general reaction on these bacteria or stress response. The RT-PCR results indicated that specific apple responses to *E. amylovora* can be detected by comparing the gene expression patterns of the plant inoculated with different bacteria. The approach could be expanded to whole transcriptome analysis to identify candidate genes conferring resistance or susceptibility in a given cultivar [35].

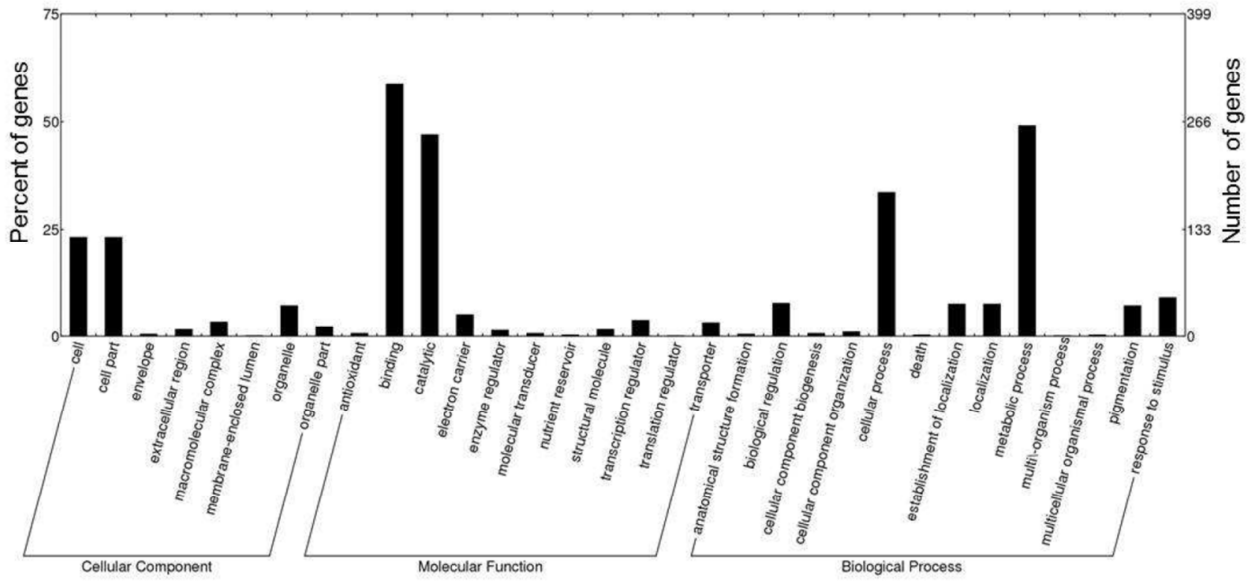

Figure 1. Differentially expressed apple genes in response to *E. amylovora*. Transcripts were assigned to cellular component, biological processes and molecular function according to the gene ontologies. From [35].

Significance of the acquired knowledge for an improved fire blight management

Transcriptome analysis of both *E. amylovora* and apple identified genes that are expressed during the infection process. The identified virulence genes of *E. amylovora* may serve as new marker genes and biosensors could be developed to monitor their expression *in planta* and to screen new fire blight products with a suppressive activity.

In the apple genome, pathogen resistance and general stress response were up-regulated. Genes involved in the specific response to *E. amylovora* could be identified by comparing transcriptomes of apple challenged with different bacteria. The results gained in these studies could be used to improve resistance breeding.

3 Module 2 – Biocontrol

3.1 Objectives

In the past years, we have obtained a solid genome sequence data set for *Pantoea* spp. Module 2 was aimed to further deepen our molecular understanding of *Pantoea* spp., which could be applied for the development of molecular markers and biosensors as well as transcriptome analysis *in planta*.

Module 2 is divided into the following subprojects:

a) *Pantoea* genomics and diversity
b) Molecular markers
c) Quorum sensing-mutants and *Pantoea* transcriptomics
d) Biosensors

Comparative genomics allow the development of molecular markers specific for species, strains or biocontrol traits. Strain-level detection methods are needed as “tracking devices” in order to better understand the ecology and biology of the introduced organism and to estimate its environmental impact on natural microbial communities. Ecological studies on the establishment and multiplication of antagonists may answer questions regarding impact of formulation, application rate and timing to get sufficient efficacy against the pathogen. The identification of antibiotic biosynthetic gene clusters facilitates streamlining the screening process for new biocontrol agents by rapid selection of environmental isolates that produce antibiotics. By transcriptome analysis genes could be identified that are actually expressed in flowers and critical for biocontrol activity. QS-mutants will be included into these experiments to identify genes, which are under the control of this bacterial communication system. Finally, biosensors for optimization of biocontrol compound production during the formulation and application in orchards will be developed.

3.2 Results

3.2.1 Module 2a: *Pantoea* genomics and diversity

Deeper understanding of biocontrol mechanisms could be achieved by performing the genome analysis of different *Pantoea* genomes. Major factors that contribute to biocontrol activity of isolates of this genus are competition for the same ecological niche and for limiting substrates, as well as antibacterial metabolite production. *P. vagans* C9-1 was sequenced previously [17]. There was no evidence of potential virulence factors that could enable an animal or phytopathogenic lifestyle and no indication of any genetic-based biosafety risk in the antagonist [13]. Other genomes currently in preparation include biocontrol strains *P. agglomerans* E325 and *P. agglomerans* P10c, as well as clinical isolates for the assessment of potential pathogenicity determinants (Smits, T. H. M. et al., in preparation). In the case of *P. agglomerans* E325, we could determine the position of the E325-specific antibiotic biosynthesis cluster on plasmid pPag4 based on mutational analysis. With this information, a biosensor was developed (see Module 2d). In *P. vagans* C9-1, the biosynthetic genes for the antibacterial peptide pantocin A were found on a 28-kb chromosomal genomic island. The second antibiotic known for this strain, herbicolin I [16], was purified from culture filtrates of *P. vagans* C9-1 and determined to be 2-amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine [11, 36]. A mutant library was screened for colonies that had lost the ability to produce herbicolin I and, as a consequence, had reduced biocontrol efficacy in immature pear assays (Figure 2).

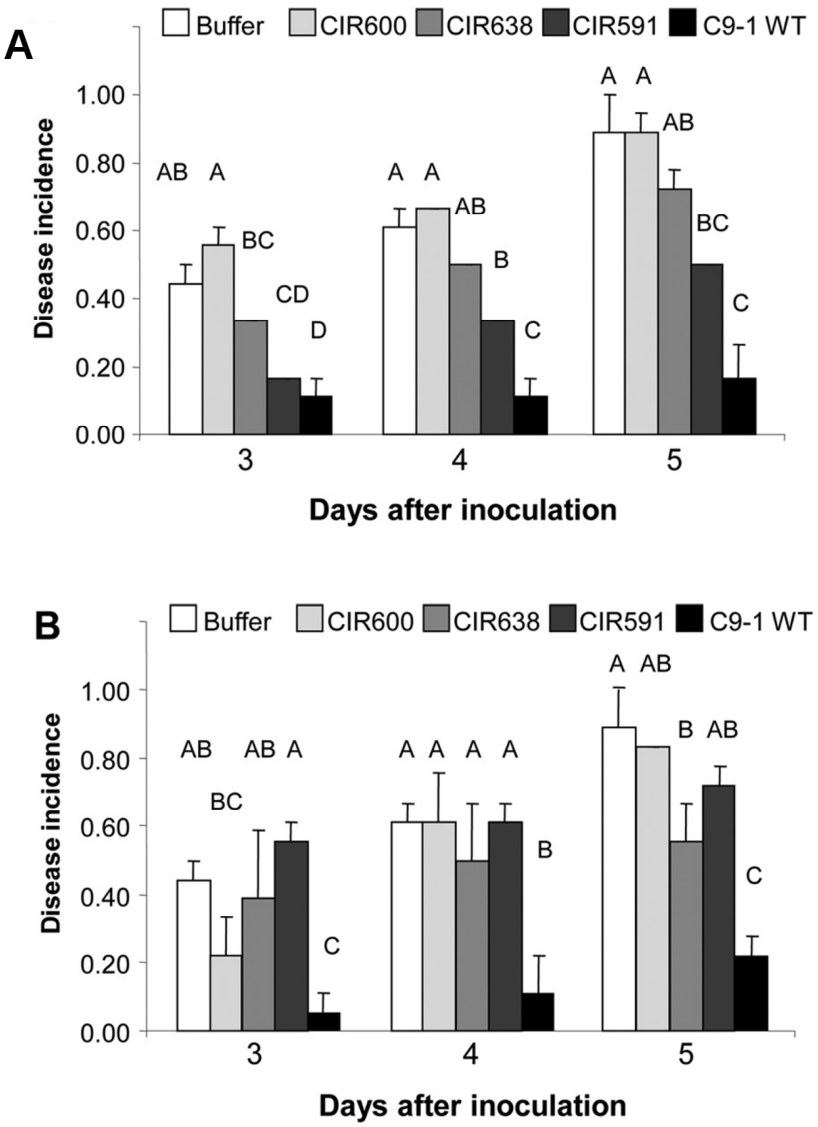

Figure 2. Reduction of incidence of disease symptoms (necrosis and/or bacterial ooze) in immature pear fruit by *P. vagans* C9-1 and antibiotic deficient derivatives CIR600 (herbicolin I^-/pantocin A^-), CIR638 (herbicolin I^+/pantocin A^-), and CIR591 (herbicolin I^-/pantocin A^+). Immature pear fruits were treated with (**A**) buffer or 5 $\times 10^6$ CFU/ml wild type C9-1 or derivatives or (**B**) buffer or 5 $\times$ 10^5 CFU/ml of wild type C9-1 or antibiotic deficient mutants, and subsequently challenged with 5 $\times 10^5$ CFU/ml *E. amylovora* Ea110. Vertical lines indicate standard error of the mean. Similar letters above bars for a time point indicate that the transformed incidence of symptoms between treatments is not significantly different according to Fisher's protected least significant difference at P =0.05. From [11]

Biosynthetic genes of *P. vagans* C9-1 for herbicolin I were identified in ten coding sequences located on the 166-kb plasmid pPag2 [13]. These genes are not ubiquitous in biocontrol *Pantoea* spp., but BLAST analysis revealed that they are present also in the genomes of *P. agglomerans* CU0119 and *Serratia proteamaculans* 568, most likely indicating acquisition by horizontal gene transfer [11]. Preliminary information is available that the E325 antibiotic is only present in E325, but not in other *Pantoea* biocontrol strains. Owing to their species or even strain-specificity, such genes could be used as molecular markers for identification of biocontrol-strains (see Module 2b).
Pantoea spp. were isolated from a wide range of ecological niches, but only some strains are adapted to the phyllosphere and display biocontrol activity. Adaptation to this lifestyle occurs by genotypic diversification, amongst others by the acquisition and maintenance of plasmids. Comparative analysis of the large universal *Pantoea* plasmid LPP-1 present in all currently sequenced *Pantoea* spp. shows that this plasmid contributes to a wide range of *Pantoea* phenotypes, including colonization and persistence in the host and environment, plant pathogenesis and antibiosis [37].

Significance of the acquired knowledge for an improved fire blight management

Antibiotic biosynthesis clusters of *Pantoea* with impact on the biocontrol efficacy were identified. The results are prerequisites for the development of the molecular markers in Module 2b.

3.2.2 Module 2b: Molecular markers

Based on comparative genomic information, species- and strain-specific markers for qPCR as well as molecular markers targeting genes that are critical for biocontrol performance (e.g., those responsible for antibiotic biosynthesis) were developed. The targets of the highly specific qPCRs (96-98%) were *P. agglomerans* strain E325, *P. agglomerans* species, and pantocin A producers, respectively (Table 1). These assays will facilitate monitoring the environmental behavior of biocontrol *P. agglomerans* after orchard application for disease protection, proprietary strain-tracking, and streamlined screening for discovery of new biocontrol strains [38].

The biosynthetic gene cluster of herbicolin I in *P. vagans* C9-1 was identified by sequencing the flanking regions of the mutated genes in herbicolin I-deficient mutants and consists of ten coding sequences located on the 166-kb plasmid pPag2. Test with PCR showed absence of this cluster in a broad collection of *Pantoea* species tested [11].

Biosafety concerns on the use of *P. agglomerans* and related species as biological control agents are still thriving because of frequent misidentifications that take place in clinical diagnostics due to inadequate characterization protocols (Figure 3). A set of molecular and biochemical determinants for the correct identification of *P. agglomerans* was thus defined to prevent such misidentifications which occur mainly with isolates belonging to species of the genus *Enterobacter* [39-41].

Table 1. Results of the qPCR specificity assays for *P. agglomerans* and other *Pantoea* species from worldwide collections. E325: *P. agglomerans* E325 strain specific qPCR; *pagR2*: *P. agglomerans* specific qPCR; *paaA*: qPCR specific for pantocin A producers. From [38].

Species	Strain	qPCR results		
		E325	*pagR2*	*paaA*
Pantoea agglomerans	Eh318	(+)[a]	+	+
	E325	+	+	−
	P10c	−	+	+
	Eh 1087	−	+	−
	Eh 239	−	+	−
	Eh 454	−	+	−
	Eh 460	−	+	−
	EPS125	−	+	−
	ATCC 27987	−	−	−
	ATCC 27155^T	−	+	−
	CIP A181	−	+	−
	EM21cb	−	+	−
	EM22cb	−	+	−
	VA21971	−	+	−
	CIP 82.100	−	+	−
	LMG 2557	−	+	−
	LMG 2595	−	+	−
	LMG 2941	−	+	−
	P1SAA	−	+	−
	P2SAA	−	+	−
	P3SAA	−	+	−
	P4SAH	−	+	−
	P5WAM	(+)	+	−
	P7NSW	−	+	−
	P9QLB	−	+	−
P.agglomerans pv. *gypsophilae*	ATCC 43348	−	+	−
	CFBP 4342	−	+	−
Pantoea sp.	Eh252	−	−	+
Pantoea sp.	P6WAL	−	−	−
Pantoea ananatis	ATCC 27995	−	−	−
	ATCC 27996	−	−	−
	LMG 5342	−	−	−
	LMG 20103	−	−	−
	LMG 2665^T	−	−	−
	LMG 2676	−	−	−
Pantoea conspicua	EM17cb	−	(+)	+
Pantoea dispersa	LMG 2770	−	−	−
	LMG 2603^T	−	−	−
	LMG 2605	−	−	−
	CIP 102701	−	−	−
Pantoea brenneri	ATCC 29001	−	−	−
	LMG 5343^T	−	−	+
Pantoea septica	LMG 5345	−	−	−
Pantoea anthophila	EM13cb	−	−	−
Pantoea stewartii subsp. *indologenes*	CFBP 3614^T	−	−	−
P. stewartii subsp. *stewartii*	CFBP 3517^T	−	−	+
Pantoea vagans	C9-1	−	−	+
	C9-1W	−	−	+
	LMG 24196	−	−	−
	LMG 24199^T	−	−	−
Pantoea sp.	P8SAA	−	(+)	−
Pantoea sp.	P10QLC	−	(+)	−
Pantoea sp.	EPS 486	−	−	−
Pantoea sp.	EPS 595	−	(+)	−

[a] (+) indicates a weak positive fluorescence signal at higher C_T values (>30).

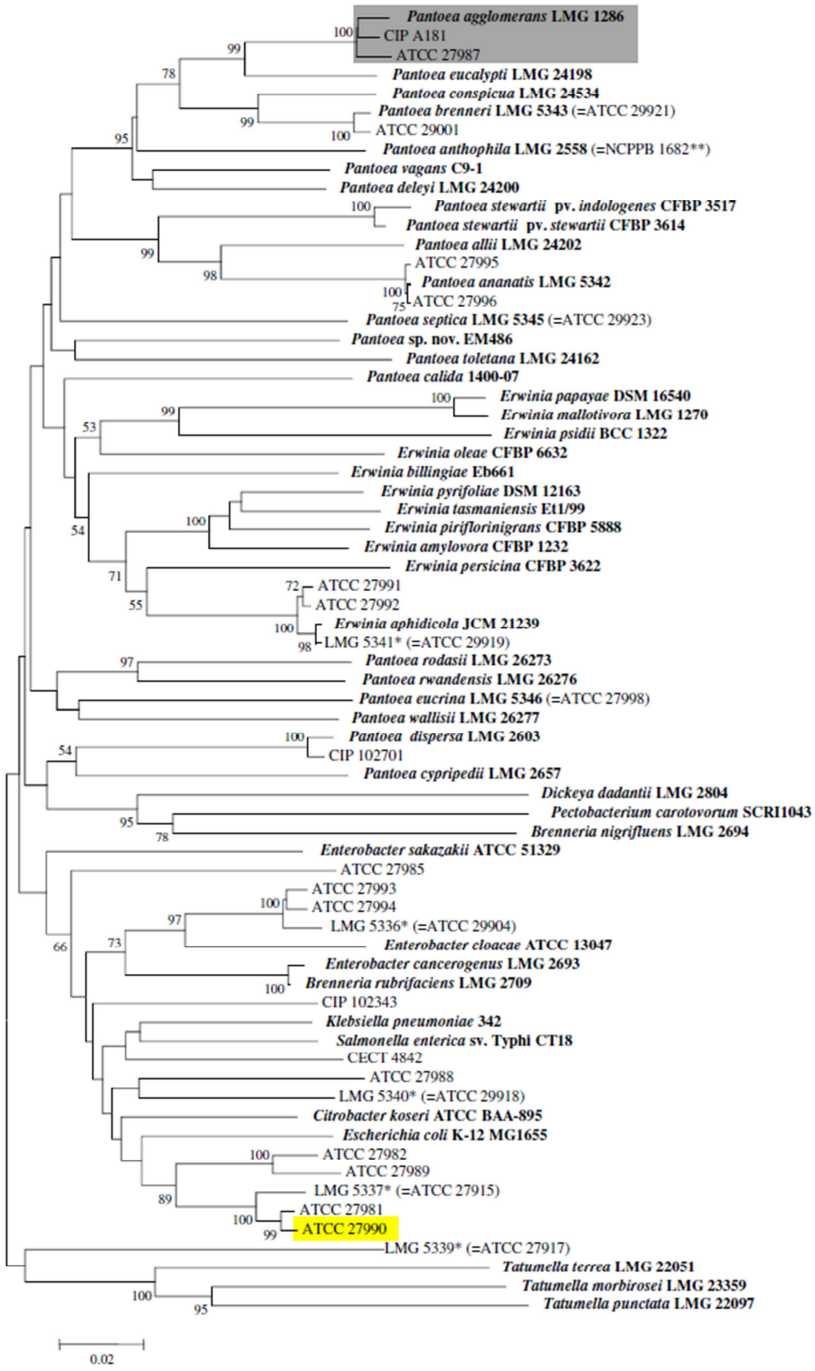

Figure 3. Phylogenetic position of ATCC 27990 (yellow box) used as the reference to identify a Brazilian outbreak attributed to *P. agglomerans*, compared with true *P. agglomerans* (grey box) and other clinical isolates received from culture collections as *P. agglomerans* or basonyms *Erwinia herbicola* and *Enterobacter agglomerans*. From [39].

Significance of the acquired knowledge for an improved fire blight management

Molecular markers were used to develop tools for tracking and detection of biocontrol strains and clarify misidentification of *Pantoea* spp. The environmental behavior of biocontrol strains after their release can now be studied, which helps to optimize application rate and timing to get sufficient efficacy against the pathogen. The available methods identifying biocontrol determinants (e.g., antibiotic synthesis clusters) facilitate isolation of new biocontrol strains with high potential. Finally, *Pantoea* spp. can reliably be identified to avoid wrong classification.

3.2.3 Module 2c: QS-mutants and *Pantoea* transcriptomics

The LuxRI system of *Vibrio fisheri* is the model for cell-density dependent bacterial communication (quorum sensing; QS). This system is widespread among Gram-negative bacteria. LuxR is a regulator known to bind to *lux*-boxes preceding QS-regulated genes. LuxI is a synthase of *N*-acyl-homoserine lactone (AHL) signal molecules. Growth of bacteria causes production and secretion of the signal molecule. Once a certain concentration is reached, the AHL bind to LuxR and activate expression of LuxR-dependent proteins [42].

In *Pantoea* species, the expression of important genes was found to be regulated by QS. EanRI, the LuxRI-ortholog of *Pantoea ananatis*, regulates EPS-biosynthesis, biofilm formation and plays a role in infection of onion leaves. However, in contrast to the LuxRI-model, EanR is a negative regulator, which represses expression of target genes by binding to *ean*-boxes. This repression can be derepressed by AHLs [43]. Pathogenicity, polysaccharide-production and bacterial adhesion are also AHL-dependent in *Pantoea stewartii*. Likewise, EsaR was found to act as a repressor [44-47]. LuxRI-orthologs were also identified in *P. agglomerans*. *pagRI* single and double-mutants of *P. agglomerans* pv. *gypsophilae*, a gall-forming pathogen of gypsophila, showed a reduced virulence [48].

We identified *pagRI* genes in the draft genome of *P. agglomerans* E325. To assess their influence on the expression, we included QS-mutants into our transcriptional analyses. For this purpose, the QS-genes encoding a putative AHL-synthase (*pagI*) and regulator (*pagR*), respectively were replaced by an Ω-cassette carrying a kanamycin-resistance determinant. Complementation of the single mutants Δ*pagR* and Δ*pagI* as well as the double mutant Δ*pagRI* was obtained by complementation with *pagRI*-bearing plasmid pBBR1MCS-5 [49].

RT-PCR confirmed the lack of expression of *pagRI* in Δ*pagRI*, of *pagR* in Δ*pagR* and of *pagI* in Δ*pagI*, respectively (Figure 4). Expression could be restored by complementation with pBBR1MCS-5::*pagRI*. Mutations did not affect the growth rates in LB and partial stigma-based medium (PSBM) [21] (not shown). AHL-production was visualized by a reporter assay using *Chromobacterium violaceum* strain CV026 [50]. No violet halo indicative of AHL-production was produced by Δ*pagRI and* Δ*pagI*, confirming the function of PagI as an AHL-synthase (Figure 4).

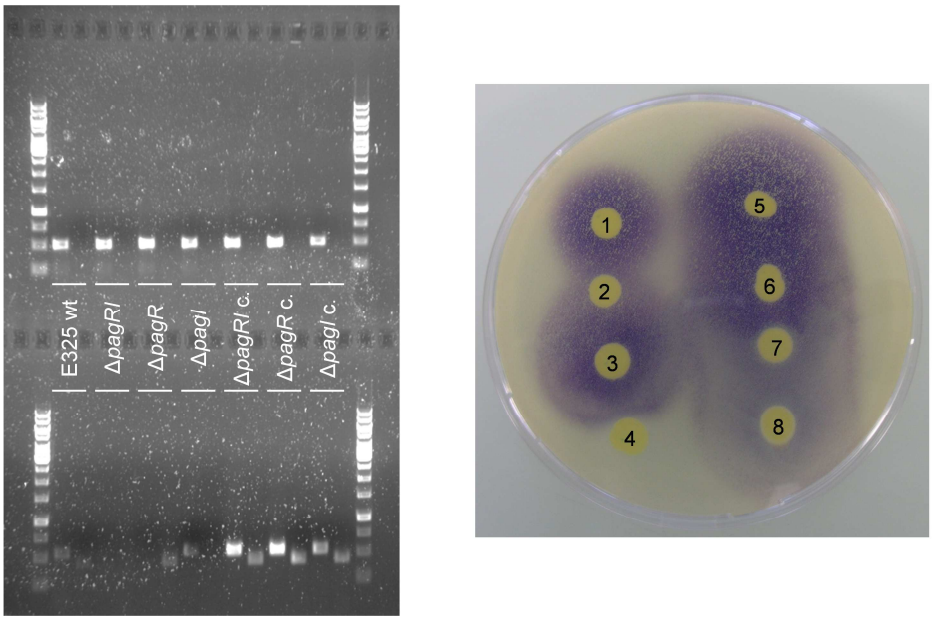

Figure 4. Left: Expression of *pagRI* analyzed by RT-PCR. Top: Control PCR with *gyr* as target. As a negative control, the reverse transcriptase was replaced by H_2O in the second lane of each strain. Bottom: *pagRI*-PCRs. The fragments of the *pagR*-PCRs are in the first, the *pagI*-PCR in the second lane of each strain, respectively. "c." indicates complemented strains. Right: AHL-production visualized by a *C. violaceum* reporter assay. C. violaceum mixed with PSBM soft agar was poured onto an LB plate. *P. agglomerans* strains were dropped on top. 1: E325 wt; 2: Δ*pagRI*; 3: Δ*pagR*; 4: Δ*pagI*; 5: E325 wt complementation (c.); 6: Δ*pagRI* c.; 7: Δ*pagRI* c.; 8: Δ*pagRI* c.

For the transcriptome analysis, E325 wildtype and the Δ*pagRI* mutant were grown in PSBM at 28℃. The composition of PSBM is based on a chemical analysis of stigma exudates from pomaceous flowers. Thus, it simulates in vivo conditions with regard to free sugars, amino acids and phosphate levels [22, 51]. RNA of bacteria sampled after 2, 3, 5, and 9 h was extracted and a cDNA library was generated and sequenced by GATC Biotech AG (Konstanz, Germany). Analysis of the RNA-seq data is in progress and will be finished soon.

Significance of the acquired knowledge for an improved fire blight management

Analysis of the transcriptome data of *P. agglomerans* E325 is ongoing. The results will give insight into QS-controlled processes of the fire blight antagonist. Genes important for biocontrol activity could be identified and used in follow-up projects to construct additional biosensors to improve the biocontrol efficacy (see Module 2d).

3.2.4 Module 2d: Biosensors

The efficacy of *P. agglomerans* strain E325 to suppress *E. amylovora* on flower stigmas was found to be significantly increased compared to other species and strains of *P. agglomerans* [52, 53]. Pathogen inhibition of E325, which is commercially available as Bloomtime® FD (Northwest Agricultural Products, Pasco, WA), is also owing to competition and production of a small peptide antibiotic [12, 22]. The antibiotic produced by E325 differs from those of other *Pantoea* spp. in that it is highly specific to *E. amylovora*, inactive at pH values above 7 as well as at high phosphate levels, and unaffected by amino acids and enzymes known to inactivate antibiotics produced by other *Pantoea* strains [22].

Biosensors were constructed in order to be able to monitor the antibiotic production of *P. agglomerans* strain E325 at different conditions. The *leuS* gene encoded on plasmid pPag4 was selected based on bioinformatics analyses and transposon mutagenesis. The upstream region of *leuS* (approximately 1,000 bp) containing the regulatory sequence information was amplified and cloned into the promoter-probe vector pPROBE-TI [54] to fuse it to the promoterless *inaZ* gene. Electro-competent cells of *P. agglomerans* strain E325 were then transformed with the pPROBE-construct. RT-PCR analysis was applied to test proper expression of *inaZ.* Under the control of the *leuS*-promoter region, *inaZ* was expressed in all growth phases tested (Figure 5).

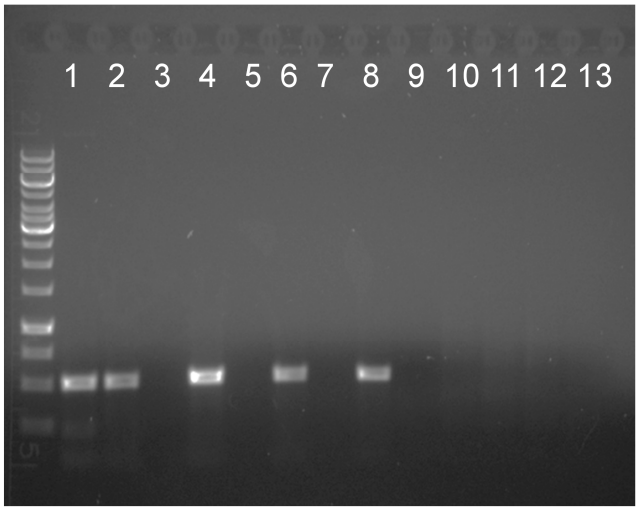

Figure 5. Expression of *inaZ* analyzed by RT-PCR. 1: positive control, 2: *leuS*-reporter (RNA extracted after 2 h), 3: *leuS*-reporter (2 h, negative control without RT), 4: *leuS*-reporter (3 h), 5: *leuS*-reporter (3 h, negative control), 6: *leuS*-reporter (5 h), 7: *leuS*-reporter (5 h, negative control), 8: *leuS*-reporter (9 h), 9: *leuS*-reporter (9 h, negative control), 10: E325 wildtype (2 h), 11: E325 wildtype (3 h), 12: E325 wildtype (5 h), 13: E325 wildtype (9 h).

Expression of *inaZ* in the *leuS*-biosensor was functionally confirmed by the appearance of frozen drops in the ice-nucleation assay as described by Rezzonico et al. [55]. This assay relies on the ability of InaZ to catalyze ice formation at temperatures of -5℃ (Figure 6). Drops of cultures of the wildtype strain never froze.

Figure 6. Ice-nucleation assay. A tenfold dilution series of the *leuS*-biosensor was dispensed on a small aluminium bowl floating on H_2O/ethanol cooled to -3.5℃. The white arrow depicts the concentration gradient (16 drops per dilution, 10^{-3}-10^{-6}). Picture was taken after 5 min of incubation.

It was reported earlier that the E325 antibiotic is more active at lower pH values [12]. To assess if this is caused by instability of the antibiotic at higher pH values or if there is a negative correlation between the pH value and the production of the antibiotic, pH-dependent production was measured. There was no significant difference, confirming that the antibiotic is produced similarly at all pH values tested (Figure 7). It is therefore likely that it is only stable under slightly acidic conditions.

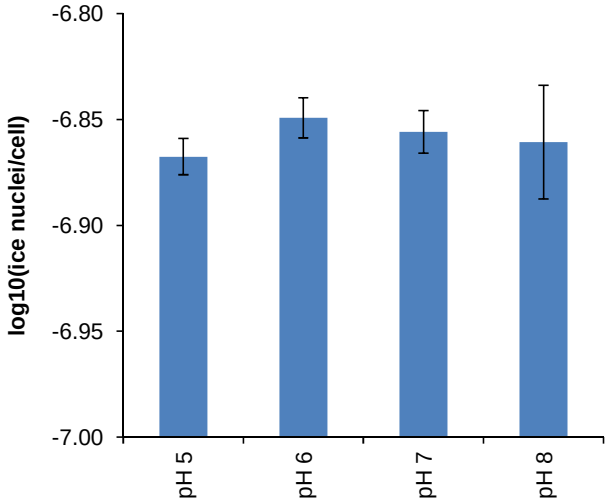

Figure 7. Ice-nucleation at different pH values. The *inaZ*-biosensor was cultivated in PSBM medium at different pH values and ice nucleation was measured after 24 h.

Significance of the acquired knowledge for an improved fire blight management

A biosensor is now available, which enables quantification of antibiotic-production in *P. agglomerans* E325 under different conditions. Optimal conditions can be evaluated and results implemented into the formulation of the biocontrol strain to achieve highest efficacy against *E. amylovora*.

4 Project Deliverables

<table>
<tr><td colspan="2">Module 1</td></tr>
<tr><td>Proposal</td><td>Achievement</td></tr>
<tr><td>Pathogen biosensors will be produced that efficiently report expression of genes involved in flower colonization and invasion.</td><td rowspan="2">In this project, the transcriptome of E. amylovora during colonization and invasion was analyzed. It is now known, which genes are involved in these processes. These results provide the basis for the development of biosensor tools. These biosensors could be used to report the expression of critical genes in planta and for the screening of new fire blight control products.</td></tr>
<tr><td>Tools will be developed that could be used for streamlined screening method for rapid, high-throughput discovery of new fire blight control products (alternative chemicals, organics, biocontrol).</td></tr>
<tr><td>Molecular understanding of plant defense determinants for potentially improving resistance breeding.</td><td>In this project, we showed that transcriptome analysis can be applied to monitor reactions of apple to infection by E. amylovora. Up-regulation of general plant resistance pathways was observed. In order to identify E. amylovora specific reactions, transcriptomes of apple challenged with different bacteria need to be compared. The results gained in these studies could then be used to improve resistance breeding.</td></tr>
</table>

Module 2	
Proposal	**Achievement**
Biocontrol agent biosensors will be produced that could be applied to identify fermentation conditions that increase active ingredient in biocontrol formulations.	*leuS*, a gene of *P. agglomerans* E325 involved in the synthesis of its antimicrobial compound, was fused to *inaZ*. Antibiotic production can now be monitored under different conditions by a simple ice-nucleation assay to identify optimal fermentation conditions.
Molecular tools will be developed that could be applied to monitor biocontrol agents after application in orchards and determine environmental fate and impact.	Strain- (e.g., *P. agglomerans* E325) and species specific (*P. agglomerans*) PCRs were developed. These PCRs enable the tracking of biocontrol agents in the environment after their release.
Molecular tools will be developed that could be applied to streamline screening of biocontrol isolates from Switzerland that have higher-chance efficacy against fire blight	Antibiotic gene clusters of *Pantoea* spp. were identified and specific primers designed. These allow specific detection of new biocontrol isolates with high potential.

5 Publications

5.1 Papers with peer-review

I. Barbé, S., P. Llop, J. Blom, J. Cabrefiga, A. Goesmann, B. Duffy, E. Montesinos, T.H.M. Smits, and M.M. López. (2013) Complete sequence of *Erwinia piriflorinigrans* plasmids pEPIR37 and pEPIR5 and role of pEPIR37 in pathogen virulence. Plant Pathol, 62:786-798.

II. Braun-Kiewnick, A., A. Lehmann, F. Rezzonico, C. Wend, T.H.M. Smits, and B. Duffy. (2012) Development of species-, strain- and antibiotic biosynthesis-specific quantitative PCR assays for *Pantoea agglomerans* as tools for biocontrol mentoring. J Microbiol Methods, 90:315-320.

III. Bühlmann, A., T. Dreo, F. Rezzonico, J.F. Pothier, T.H.M. Smits, M. Ravnikar, J.E. Frey, and B. Duffy. 2013. Phylogeography and population structure of the biologically invasive phytopathogen *Erwinia amylovora* inferred using minisatellites. Environ Microbiol, *in press*, DOI: 10.1111/1462-2920.12289

IV. Bühlmann, A., J.F. Pothier, T.H.M. Smits, M. Andreou, N. Boonham, J.E. Frey, and B. Duffy. (2013) *Erwinia amylovora* loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site diagnosis of fire blight. J Microbiol Methods, 92:332-339.

V. De Maayer, P., W.Y. Chan, J. Blom, S.N. Venter, B. Duffy, T.H.M. Smits, and T.A. Coutinho. 2012. The large universal *Pantoea* plasmid LPP-1 plays a major role in biological and ecological diversification. BMC Genomics, **13**:625.

VI. Kamber, T., T.A. Lansdell, V.O. Stockwell, C.A. Ishimaru, T.H.M. Smits, and B. Duffy. (2012) Characterization of the biosynthetic operon for the antibacterial peptide herbicolin in *Pantoea vagans* biocontrol strain C9-1 and incidence in *Pantoea* species. Appl Environ Microbiol, 78:4412-4419.

VII. Malnoy, M., S. Martens, J.L. Norelli, M.-A. Barny, G.V. Sundin, T.H.M. Smits, B. Duffy. (2012) Fire blight: Applied genomic insights of the pathogen and host. Annu Rev Phytopathol, 50:475-495.

VIII. Mann, R.A., J. Blom, A. Bühlmann, K.M. Plummer, S.V. Beer, J.E. Luck, A. Goesmann, J.E. Frey, B.C. Rodoni, B. Duffy, and T.H.M. Smits. (2012) Comparative analysis of the Hrp pathogenicity island of *Rubus* - and Spiraeoideae-infecting *Erwinia amylovora* strains identifies the IT region as a remnant of an integrative conjugative element. Gene, 504:6-12.

IX. Rezzonico, F., A. Braun-Kiewnick, R.A. Mann, B. Rodoni, A. Goesmann, B. Duffy, and T.H.M. Smits. (2012). Lipopolysaccharide biosynthesis genes discriminate between *Rubus*- and Spiraeoideae-infective genotypes of *Erwinia amylovora.* Mol Plant Pathol, **13**:975-984.

X. Rezzonico, F., T.H.M. Smits, and B. Duffy. (2012) Misidentification slanders *Pantoea agglomerans* as a serial killer. J Hosp Infect, 81:137-139.

XI. Smits, T.H.M., F. Rezzonico, M.M. Lopez, J. Blom, A. Goesmann, J.E. Frey, and B. Duffy. 2013. Phylogenetic position and virulence apparatus of the pear flower necrosis pathogen *Erwinia piriflorinigrans* CFBP 5888T as assessed by comparative genomics. Syst Appl Microbiol, **36**:449-456.

XII. Smits, T.H.M., V. Guerrero-Prieto, G. Hernández-Escarcega, J. Blom, A. Goesmann, F. Rezzonico, B. Duffy, and V.O. Stockwell. 2014. Whole-genome sequencing of *Erwinia amylovora* strains from Mexico detects SNPs in *rpsL* conferring streptomycin resistance and in the *avrRpt2* effector altering host interactions. Genome Announc, 2: e01229-13.

5.2 PhD thesis

I. Kamber, T. 2013. *Characterization of* Erwinia amylovora-*host plant and -biocontrol agent interactions*, Universität Zürich.

5.3 Talks

I. Bühlmann, A., J.E. Frey, and B. Duffy. (2012) LAMP diagnostics based on genomics information. Q-Detect Annual Meeting, Vienna, Austria

II. Bühlmann, A., J.F. Pothier, T.H.M. Smits, N. Boonham, J.E. Frey, and B. Duffy. (2012) Vorstellung des EU-Projektes Q-Detect: Neue Möglichkeiten um QO's schnell und vor Ort zu identifizieren. EPSD Tagung, Wädenswil

III. Bühlmann, A., J.F. Pothier, T.H.M. Smits, J.E. Frey, and B. Duffy. (2013) Summary of ACW work in Q-Detect, Q-Detect Final Meeting, Ljubljana, Slovenia

IV. Duffy, B. (2012) Fire blight pregnancy test for rapid, on-site phytosanitary diagnostics. PhytFire Kick-off Stakeholder Forum, Oeiras, Portugal

V. Duffy, B. and A. Bühlmann. (2012) Communication of Q-Detect project achievements and translational research progress. Q-Detect Annual Meeting, Vienna, Austria

VI. Duffy, B., T.H.M. Smits, T. Kamber, F. Rezzonico, A. Bühlmann, and E. Holliger. (2012) ACHILLES: Angewandte Genomiks für innovative Bekämpfungsprodukte. BegObst-Tagung, Wädenswil

VII. Pflüger, V., F. Rezzonico, J.F. Pothier, T.H.M. Smits, and B. Duffy. (2013) MALDI-TOF as tool to distinguish species in *Erwinia* and related genera. 13th ISHS International Fire Blight Workshop, Zürich

VIII. Rezzonico, F. and B. Duffy. (2012) Fire blight forensics: source-tracking methods to identify inoculum reservoirs. PhytFire Kick-off Stakeholder Forum, Oeiras, Portugal

IX. Rezzonico, F., T.H.M. Smits, and B. Duffy. (2012) Biosafety questions regarding the fire blight biocontrol agent *Pantoea agglomerans*. Swiss Society for Microbiology Annual Meeting, St. Gallen

X. Rezzonico, F., T.H.M. Smits, M. Tonolla, V. Pflüger, and B. Duffy. (2013) Strange case (reports) of Dr. *P. agglomerans* and Mr. *Enterobacter* sp. 13th ISHS International Fire Blight Workshop, Zürich

5.4 Posters

I. Born, Y., L. Fieseler, J. Klumpp, M.R. Eugster, K. Zurfluh, B. Duffy, and M.J. Loessner. (2013) Tail-associated depolymerase of T7-like phage L1 is essential for adsorption to *Erwinia amylovora* and synergistically enhances Y2 phage efficacy. 13th ISHS International Fire Blight Workshop, Zürich

II. Born, Y., L. Fieseler, M.J. Loessner, and B. Duffy. (2012) *Erwinia amylovora* specific bacteriophage as an alternative fire blight control option. BegObst-Tagung, Wädenswil

III. Bühlmann, A., J.F. Pothier, F. Rezzonico, T.H.M. Smits, M. Andreou, B. Boonham, B. Duffy, and J.E. Frey. (2013) *Erwinia amylovora* loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site fire blight diagnosis. 13th ISHS International Fire Blight Workshop, Zürich

IV. Bühlmann, A., J.F. Pothier, T.H.M. Smits, N. Boonham, J.E. Frey, and B. Duffy. (2012) Genomics informed rapid diagnostics of fire blight based upon LAMP for on-site phytosanitary application. BegObst-Tagung, Wädenswil

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