

Bayer and the Citrus Research & Development Foundation announced their partnership to combat citrus greening

Bayer and the Citrus Research & Development Foundation team up



THE WALL STREET JOURNAL

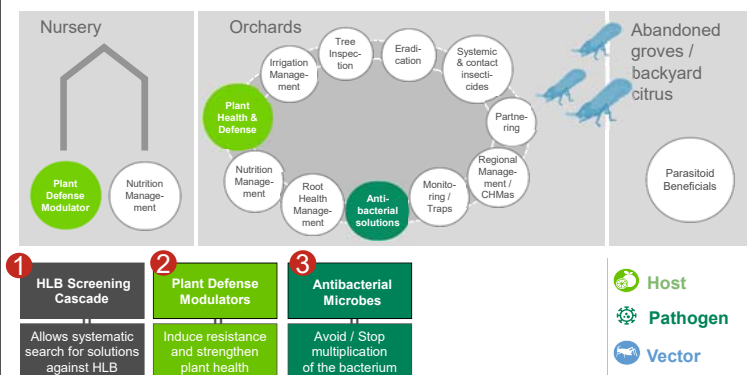
Bayer and the Citrus Research and Development Foundation (CRDF), a non-profit organization in Florida supporting citrus growers, have signed a research collaboration agreement to find solutions to Citrus Greening disease, which currently threatens the global citrus production and juice industry.



<https://www.thestreet.com/story/14273593/1/bayer-and-the-citrus-research-and-development-foundation-team-up-to-find-solutions-against-citrus-greening.html>

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The Project Will Pursue Three Key Elements to Find a Solution for HLB



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What is unique about this approach?



1) HLB Screening Cascade

Focus on developing a validated screening cascade

- Purpose is to have a systematic approach to identify and characterize hits with confirmed in planta HLB activity
- Develop in house high through-put in vitro *Liberibacter crescens* assay
- Collaborate with academic lab to optimize *Liberibacter solanacearum* in planta assay greenhouse for screening leads
- Fund development of a validated in planta HLB citrus greenhouse assay to select leads for field testing



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What is unique about this approach?



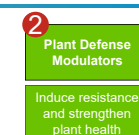
2) Plant Defense Modulators (PDM)

Develop pool of candidates that target both **Pathogen** and **Host** and would have relatively shorter regulatory path to market

- Host Approach:** Screen synthetic compound libraries for their ability to control bacterial diseases indirectly via induction of plant defense pathways

Advantages:

- Indirect activity – not a pesticide/bactericide, low risk of resistance
- Bayer has a screen in place for discovering Plant Defense Modulators (PDM) for other bacterial diseases so can rapidly identify chemical classes



Induced systemic response

- Broad spectrum
- Long lasting
- Low risk of resistance

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What is unique about this approach?

3) Antibacterial Microbes (AM)

Develop pool of candidates that target both **Pathogen** and **Host** and would have relatively shorter regulatory path to market

- Pathogen Approach:** Screen microorganisms that can directly inhibit *Liberibacter* through production of anti-bacterial compounds

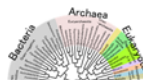
Advantages:

- Active microbes can be registered through EPA's BPPD in an expedited process
- Bayer has a collection of >140,000 microbial strains and sophisticated in-silico screening capabilities to identify strains that are likely to be anti-bacterial based on their genome



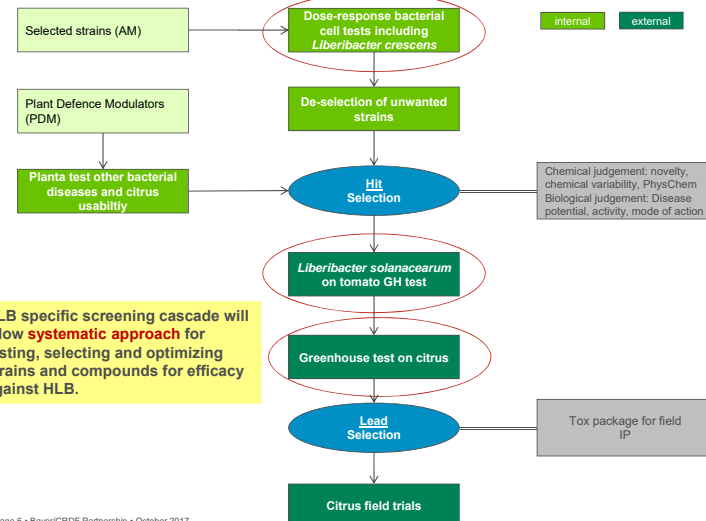
3
Antibacterial
Microbes

Avoid / Stop
multiplication
of the bacterium



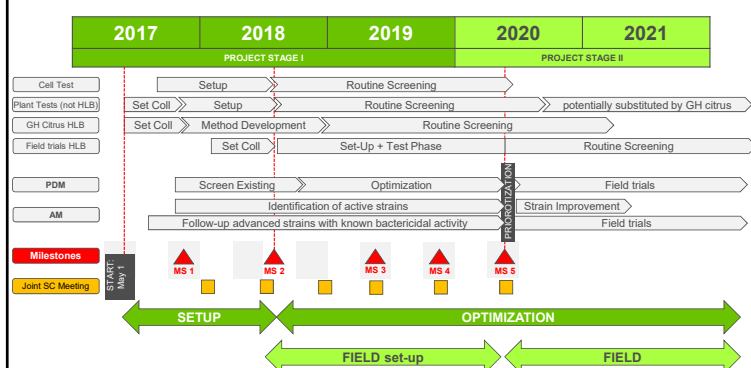
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HLB Specific Screening Cascade: from Cell Test to Field



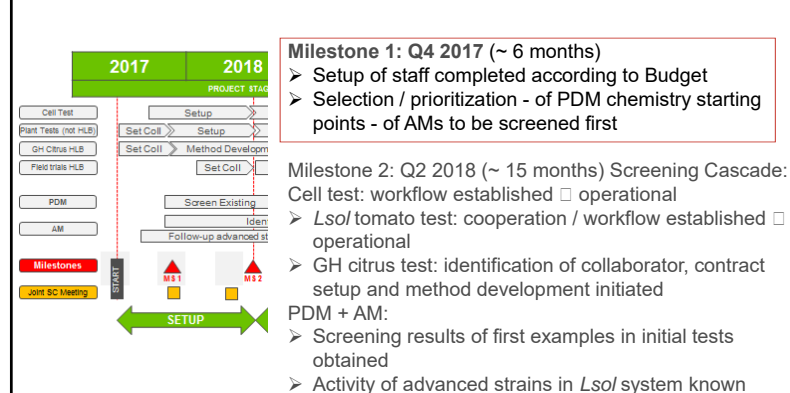
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Project Milestones



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Progress towards Milestones



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Bayer HLB Project Team and hiring progress



Project Leader:
Denise Manker – Responsible for oversight of project and coordinating external collaborations

Antibacterial Microbes (AM) Technical Lead:
Jean Broadhvest, W. Sacramento CA
Three FTE's hired, Plant Pathology, Chemistry, Bioinformatics

Plant Defense Modulators Technical Lead:
Thomas Knobloch, Lyon FR
Four FTE's hired, Chemistry, Biology, Biochemistry
One FTE Candidate identified, offer pending

Hiring process nearing completion with seven of eight positions already on the job

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Progress on HLB Screening Cascade: In vitro assay



Liberibacter crescens in vitro assay:

- Strain obtained from public strain collection
- Communications initiated with E. Triplett, UF for transfer of method
- Face to face meeting in W. Sacramento with CRDF team members from Morrisville, NC to map out logistics for microbial extract production and testing



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Progress on HLB Screening Cascade: In planta Zebra chip



Liberibacter solanacearum in planta assay:

- Discussions underway with Bryce Falk at UC Davis for validation of assay in tobacco/tomato and plans for screening up to 200 leads/year. Assay has been run previously and infected psyllids are maintained onsite
- Research agreement to be put in place between Bayer and UCD



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Progress on HLB Screening Cascade: HLB GH assay




Candidatus Liberibacter asiaticus in planta assay:

- **Collaborator identified, Ozgur Batuman, Citrus Pathologist, UF, IFAS**
- **Research collaboration signed between Bayer and UF (9/24/17)**
 - Research technician to be hired
 - Best inoculation method to be determined
 - Development of quantitative PCR method
 - Optimization of tissue sampling (type, quantity, frequency)
 - Determine method for introducing leads to be screened (drench, injection...)
 - Preparation for screening 5-10 leads per year in validated GH assay
- **Discussions underway with Fundecitrus researcher (S. Lopes) for further refinement of in planta HLB Citrus assay**



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Progress on Plant Defense Modulators




Plant Defence Modulators (PDM)

Planta test other bacterial diseases and citrus usability

Hit Selection

Liberibacter solanacearum on tomato GII test

Greenhouse test on citrus




Citrus plants in Lyon greenhouse, Bayer

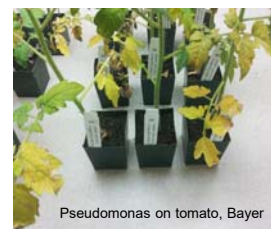
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Progress on Plant Defense Modulators


Planta test other bacterial diseases and citrus usability



- Xanthomonas cabbage assay has been optimized
- Two Pseudomonas assays are under development (bean and tomato)
- Citrus established in greenhouse for phytotoxicity tests
- In planta citrus PDM assay under development
- One chemistry class has been identified which can protect plants from bacterial disease via plant defense mechanism**
 - A library of compounds in this class will be synthesized for further testing/optimization activity




Pseudomonas on tomato, Bayer



Citrus plantlets, Bayer

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Progress on Antibacterial Microbes

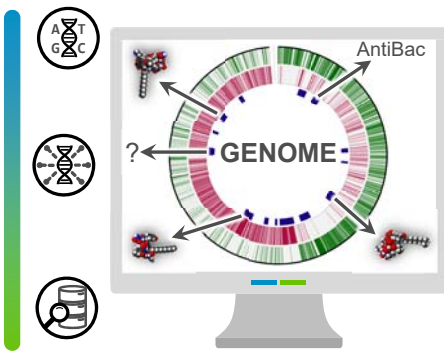


In-silico screening of Bayer's microbial library

Sequencing

Functional Genomics


Microbial Genome Portal



The genetics of microbial isolates are used to select strains predicted to have anti-bacterial biological chemistry

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Progress on Antibacterial Microbes



- Selection/prioritization of microbes to be screened
 - Bioinformatics selection -- In silico screening
 - Literature search led to two gene clusters with activity vs. *Liberibacter* spp.
 - Eleven strains in Bayer collection contain these genes
 - Screen for genes that make antibacterial compounds
 - After removing closely related strains, 462 strains were selected representing high diversity of microbes
- These 473 strains will be fermented and extracted in preparation for screening in the *Liberibacter crescens* in vitro assay

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Review of external technologies to include in validated screening cascade



Once the screening cascade has been validated..

In addition to leads developed from Plant Defense Modulator and Anti-bacterial Microbe efforts, external technologies will be included

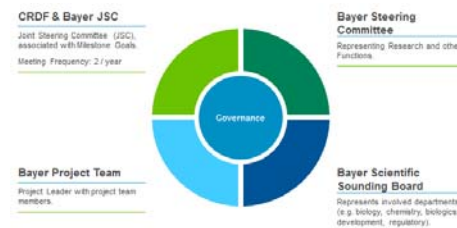
Review and analysis of external technologies will continue as an on-going activity

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Governance activities



- First Quarterly Report submitted to CRDF, September 2017
- Visit to W. Sacramento from CRDF/CC/PepsiCo scheduled for Nov 15, 2017
- Plan for Scientific Sounding Board meeting, Q4 2017
- Scheduled first Joint Steering Committee, January 17, 2018



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Next 100 Days....Next Steps



What we plan to achieve....

Milestone 2: Q2 2018 (~ 15 months) Screening Cascade:

Cell test *L. crescens*: workflow established & operational

- ***Lso*/tomato test: cooperation / workflow established** -- operational
- **GH citrus test: identification of collaborator, contract setup and method development initiated**
- PDM + AM:
 - Screening results of first examples in initial tests obtained
 - Activity of advanced strains in *Lso*/system known

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Bayer-CRDF Research Agreement on Solutions for Citrus Greening Disease

Quarterly report, September 13, 2017

Prepared by Denise Manker, Project Manager, Crop Science Division, Bayer

Excellent progress has been made to fill the positions funded by the CRDF collaboration and practical work is underway for identifying Plant Defense Modulators, establishing a screening process for selecting antibacterial microbes and developing external collaborations for greenhouse testing.

For the Plant Defense Modulator research in Lyon, three technicians have been hired in Biology and Biochemistry and will start in September and October. Work on this project is ongoing with permanent staff in Lyon. Interviews are in process for two additional positions including a PhD chemist. Processes for selecting hits and optimizing compounds are in place and the screening cascade is being adapted to suit the citrus target. A *Xanthomonas* test has been optimized and two *Pseudomonas* tests are being developed. A bacterial cell test is now running. Citrus trees were ordered and are in the greenhouse and a propagation regime is being developed. Regarding lead chemistry, in July, one hit was promoted to “exploration class” based on promising results in both plant tests, indicating plant defense, and cell based tests showing no direct effect on bacterial pathogens, thereby confirming activity is via a PDM mechanism. The team has a plan for moving forward with this chemistry class and orders of library compounds based on selected hits have been placed. Monthly functional meetings have been established.

The hiring process for filling positions for the antibacterial microbe screening has been completed. Offers have been made and accepted for a plant pathologist (start date Aug 29), a bioinformatics scientist (start date October 11) and a natural product chemist (start date October 9). A biologist has been hired for development of the cell test in Morrisville (start date September 25). The *Liberibacter crescens* strain has been obtained and culturing has been initiated in the first step toward achieving a plate test that can be developed into a high throughput assay to screen preselected microbes. For selection of strains, a thorough literature search has been started and is still ongoing with the aim of identifying genes and secondary metabolite gene clusters that are known to inhibit or kill *Liberibacter* species. Based on this literature search, two specific new secondary metabolite gene clusters were identified that have been attributed with activity against *Liberibacter* spp. The DNA reference sequences of these two compounds were extracted from the literature and public databases and added to our proprietary database. Subsequent searches revealed 11 strains in our sequenced collection that contained one of these compounds. No strains containing the second compound were found. These eleven strains will be used to develop and test the bioassays and other downstream activities.

Besides these specific selections, a larger number of strains were selected using Bayer’s established *in-silico* screening pipeline. Out of Bayer’s large sequenced strain collection, strains were selected that contained one or multiple predicted antibacterial secondary metabolite gene clusters. From that list, strains that showed high overall genome similarity towards each other were eliminated to increase the

diversity in the strains being tested. This has resulted in identification of an additional 462 strains for the first batch of screening, once methodologies are in place. Monthly functional meetings have been established.

Plans are underway for knowledge exchange between the AM and PDM groups.

External collaborations: Bayer and UF are very close to signing a research agreement to fund development of an in planta citrus HLB greenhouse assay with Dr. Ozgur Batuman at UF's SWFRE site in Immokalee. This will include development of a quantitative PCR assay for tracking levels of HLB in citrus seedlings for the screening assay. Several visits have been made to this facility to discuss the plan for developing this assay. Several discussions with Dr. Silvio Lopez of Fundecitrus in Brazil have taken place. Bayer and Fundecitrus are currently working to determine how further research on developing an in planta HLB greenhouse assay can be funded by this collaboration. Dr. Lopes and Dr. Batuman are both open to collaborating and sharing best practices to develop a robust greenhouse screening assay.

Several discussions have taken place at UC Davis with Dr. Bryce Falk. A plant assay is in place there for testing *Liberibacter solanacearum* and has been run in a variety of solanaceas crops including tomato and tobacco. They have potato psyllid in culture with *Liberibacter* and can see symptoms within three weeks. The assay has not been actively running in recent months, but Dr. Falk was interested in screening leads and has reinitiated the assay to make sure that it will be running smoothly when compounds are available for testing. A research agreement needs to be put into place for this eventual screening. Proximity to the West Sacramento Bayer site and the experience of the UC Davis research team will simplify transfer of test materials and obtaining results in a timely manner.