CRDF Board update on Bayer Agreement

CRDF Board meeting
Bonita Springs

June 13, 2018 / Denise Manker
CRDF Bayer agreement

Developing a **Discovery Platform** that targets both **Plant Host** and **Pathogen**

- **Discovery**: capitalizing on Bayer’s experience in discovery and development of crop protection products, a **validated screening process** will be designed to identify leads effective against Liberibacter, starting with in vitro lab testing to greenhouse plant tests to field trials
  - Develop high throughput in vitro bacterial assays, including Liberibacter crescens
  - Greenhouse assays for Liberibacter in tomatoes and citrus will be developed/run by academic collaborators at UC Davis and U of Florida.

- **Plant Host Defense**: An existing synthetic plant defense platform is being modified to identify compounds adapted to citrus defense.

- **Targeting the Pathogen**: Bayer’s massive microbial strain library will be searched for strains with activity against Liberibacter. This approach could achieve a faster registration through EPA’s microbial pesticide process.
CRDF Bayer Objectives

Three key areas

1) 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

2) Discover synthetic plant defense leads to target health of citrus trees

Rapidly identify chemical classes that can indirectly rid the plant of bacterial diseases from existing screening process

3) Identify natural microbes that can attack Liberibacter in plants for leads that could quickly be moved to market

Exploit Bayer’s vast, well-characterized microbial collection to find microbes to target bacterial plant diseases
CRDF Bayer agreement

Developing a **Discovery Platform** that targets both **Plant Host** and **Pathogen**

- **Discovery**: capitalizing on Bayer’s experience in discovery and development of crop protection products, a **validated screening process** will be designed to identify leads effective against Liberibacter, starting with in vitro lab testing to greenhouse plant tests to field trials
  - Develop high throughput in vitro bacterial assays, including Liberibacter crescens *Developed in Morrisville, now being transferred and validated in Sacramento*
  - Greenhouse assays for Liberibacter in tomatoes and citrus will be developed/run by academic collaborators at UC Davis and U of Florida. *Agreements in place, assays under development.*

- **Plant Host Defense**: An existing synthetic plant defense platform is being modified to identify compounds adapted to citrus defense. *One chemical class has already been identified for further development*

- **Targeting the Pathogen**: Bayer’s massive microbial strain library will be searched for strains with activity against Liberibacter. This approach could achieve a faster registration through EPA’s microbial pesticide process. *Advanced lead characterized; 1000 strains selected for screening, 429 strains screened*
Progress towards Milestones

**Milestone 1: Q4 2017 (~ 6 months): COMPLETED**
- Setup of staff completed according to Budget
- Selection / prioritization
  - PDM chemistry starting points
  - AMs to be screened first

**Milestone 2: Aug 2018 (~ 15 months)**
Screening Cascade
- Cell test: workflow established -- *Validation in process*
  - Lsol tomato test: cooperation / workflow established -- operational
  - GH citrus test: identification of collaborator, contract setup and method development initiated – Ahead of schedule
- PDM + AM Screening results of first examples in initial tests obtained -- UNDERWAY
- Activity of advanced strains in *Lsol* system known – FIRST LEADS BEING TESTED
CRDF Collaboration

Summary of progress in first thirteen months

- Plant Defense Modulator (PDM) research
  - Process for selecting hits and optimizing chemistry in place
  - Seven chemical families identified as “hits under review”
  - One chemistry class promoted to Hit Exploration Phase 0.1
    - Foliar activity in plants on bacterial disease confirmed for three analogs of Phase 0.1 class
    - Initial toxicology tests completed to approve for field/greenhouse testing
    - Samples being prepared for greenhouse tests vs. Liberibacter

- Anti-bacterial Microbes (AM)
  - In vitro assays, including Liberibacter crescens, established in Morrisville, 429 of first 500 strains screened
  - Total of 1000 strains selected for screening using genomic information
  - Activity in one advanced strain identified

- Research agreement with UCD* in place, assay and application methods being validated, advanced microbe lead extract in test
- Research agreement with UF** in place, trees infected, assay being validated, advanced microbe extract lead ready for shipping

*UCD : University of California Davis
**UF : University of Florida
Plant Defense Modulator Screening cascade

High throughput screen for synthetic compounds that can induce host defense

Mode of action
Diagnostic support

Primary Plant
Greenhouse test
+ cell test

Vivo Hit
Selection

HLB screening
cascade*

Rationale
Screening

Antibacterial optimization

Citrus compatibility
Immunity/phytotoxicity

HLB & Field

* PDM leads enter the HLB screening cascade in the plant test assays as they have no direct activity on pathogen.
# PDM Primary Test Status

## Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Xanthomonas</th>
<th>Pseudomonas</th>
<th>HLB disease free</th>
<th>Cell tests</th>
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<tr>
<td>Pathogen selection</td>
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<td>Plant feasibility</td>
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### Rationale Of Screening
- Hit identification
- Antibacterial optimization
- Citrus compatibility
- Immunity / phytotoxicity
- HLB & Field

**Bayer Partnership with CRDF**

- Routine screening SAR support
- Routine screening Jan-2018
- Screening LTS Q2-2018
- Routine screening
Plant Defense test in Citrus plantlets HLB disease free test

Citrus Molecular Diagnostic – HLB disease free test

• Proof of concept of multiple marker tracking
• Low Throughput Screen to be routine in Q3-2018
# Biology Lead compound

## Plant test

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<thead>
<tr>
<th>Test object</th>
<th>Xanthomonas</th>
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## Cell test

### Lead

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<td>ED95 (ppm)</td>
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Pseudomonas Efficacy demonstrated in plants

Untreated

Lead

Bayer Partnership with CRDF
Chemistry Pipeline Status

Six Hit classes identified; 1 lead family advanced to Phase 0.1

Sources
- Patent
- PDM vRS « 50 clusters »
- Hit Vivo team « 2 »
- Opportunistic « 1 natural product »

Phase 0.1

Hits class under review

1
2
3
4
5
6

Gate 1

Hit Class Exploration

Lead family 108

Gate 2

Bayer Partnership with CRDF
Toward citrus induce resistance

Chemistry optimization / SAR

Induce plant immune system

Enviromental & human Safety

Efficient against Pseudomonas

Compatibility to citrus

Efficient against Xanthomonas

Healthy citrus

HLB

Bayer Partnership with CRDF
CRDF Collaboration

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*UCD : University of California Davis
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Bayer and Monsanto will create a global leader in sustainable agriculture
Focus on Sustainable Horticulture through Innovation

Why citrus matters to us…

Focus

• With 25%, horticulture accounts for the largest share of our business.
• Leader of the global citrus crop protection segment.
• Citrus is our No. 1 horticulture crop in the US and Brazil.
• Leading company in crop protection research and technology.

Commitment for sustainable horticulture:

• The current Citrus Greening management practices are not sustainable.
• To secure future citrus fruit supply, the way of cultivation needs to change.
• Sustainable agriculture is the only way to overcome the challenge.

Innovation

• Our company mission is the foundation of what we strive to achieve within agriculture.
• We help to secure the supply of high-quality, affordable food.
• We invest about 10 percent of our annual sales in R&D, and a significant part into horticulture.
How will divestment affect Bayer’s CRDF project?

Bayer to sell off $9 billion in assets as part of Monsanto takeover

Bayer offered $66 billion for Monsanto

By AARON SMITH AND JESSICA SCHNEIDER

Posted: 7:08 AM, May 30, 2018
Updated: 7:06 AM, May 30, 2018
Divestment announcement

Effect on CRDF Agreement

The divestment of Morrisville Traits research affected CRDF agreement by:

-- Loss of one FTE, transferred to BASF

-- Loss of site/access to High Throughput Screening (HTS)

Activities to mitigate these effects:

Replacement FTE hired, internal post-doc transferred to project allowing for quick start

Transfer and validation of HTS currently underway
   Taking opportunity to modify process to enhance discovery of active microbial metabolites

RESULT: Approximately two months behind in screening AM’s in vitro

Long term gains: More efficient process – no shipping of strains and extracts between CA and NC
   Media optimized for metabolite production
Workflow for microbial library primary screening at Morrisville, NC

**Primary screen**: modify established fungal cell test protocols to quantify activity of microbes against panel of target bacteria.

- **Grow microbial strains in liquid culture, filter supernatant. Aqueous and organic extracts are also screened**
- **Setup 96 well plates with target inoculum and microbial strain filtrates**
- **Incubate plates and measure OD**
- **Calculate % inhibition of target in presence of strain extract**
- **Identify strains that cause inhibition for secondary screen**

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<th>Bayer Partnership with CRDF</th>
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| 0.4% | 55.0% | 49.8% | 46.3% | 34.7% | 49.6% | 0.6% | 32.2% | 31.9% | 31.2% | 42.0% | 76.9% |
| 2.9% | 56.4% | 45.8% | 41.3% | 30.7% | 53.8% | -7.9%| 34.0% | 32.2% | 30.3% | 46.8% | 72.4% |
| 2.2% | 28.2% | 47.7% | 42.0% | 36.2% | 39.9% | 2.0% | 34.3% | 34.7% | 33.8% | 45.3% | 70.6% |
| -6.5%| 38.0% | 42.0% | 39.0% | 28.9% | 36.9% | 4.8% | 44.4% | 28.9% | 42.0% | 40.4% | 74.8% |
Divestment of Morrisville, NC group to BASF: Transfer/optimization of primary screen for HLB project

**Primary screen:** Opportunity to increase efficiency and improve outcome by optimizing system for HLB project rather than fitting it to the Traits approach

Previously, strains shipped to CA for extraction, extracts shipped back for screening. Improvement: NO SHIPPING

Screen media was developed for proteins. New media will be optimized for chemistry

CLS strain selection
NO CHANGE

Incubate plates and measure OD

Calculate % inhibition of target in presence of strain extract

Identify strains that cause inhibition for secondary screen
In-silico screening of Bayer’s microbial library

Using this method, 100 strains selected to validate assays and first 1000 strains selected for screening

The genetics of isolates are used to select strains predicted to have anti-bacterial biological chemistry
Antibacterial Microbe screening cascade
Using Microbes from the environment to target the disease

- **Strain Collection**
  - Genomic/knowledge
  - Hypothesis based selection of strains

- **Cell test**
  - XANTAV
  - PSDMTM
  - LIBECR
  - AGRBTU

- **In vitro test**
  - XANTAV
  - PSDMTM
  - LIBECR

- **In planta test**
  - XANTSO
  - Tomato
  - PSDTM
  - Tomato

**Hit Selection**

**Knowledge loop**
optimization of strain selection

**Hit Selection Criteria:**
Disease potential, activity, mode of action

**Strain Collection:**
- Pseudomonas syringae pv. tomato (PSDMTM)
- Ralstonia solanacearum (race 1) (PSDMS1)
- Xanthomonas campestris pv. cabbage (XANTCA)
- Xanthomonas campestris pv. Viticola (XANTCV)
- Liberibacter crescens (LIBECR)
- Agrobacterium tumefasciens (AGRBTU)
Advanced microbial lead chemically characterized

Enriched active fraction has been prepared for first plant testing in tomato and citrus tests

Several microbial strains from advanced projects were previously identified to be active in vitro on Liberibacter crescens

AQ713 – antibacterial activity identified, concentrated samples prepared for in planta tests

AQ2808 -- initial chemical characterization is underway with good progress being made

Remaining leads awaiting further chemical analysis
CRDF Collaboration

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CRDF Collaboration

Summary of progress in first twelve months

In planta screening collaborations

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Challenge in these assays and in plant assays in Sacramento is introduction of microbial leads to phloem of plants for access to Liberibacter

*UCD : University of California Davis
**UF : University of Florida
External collaborations for greenhouse screening
Collaborations with University of California Davis and University of Florida

UC Davis, Bryce Falk, Sandra Thuy Vu. *Liberibacter solanacearum* assay on tomato. Psyllid inoculated. qPCR analysis. No containment required. First samples of advanced microbial lead are in test.

Univ. of Florida, Southwest Florida Research and Education Center, Ozgur Batuman has developed an HLB in planta greenhouse assay on citrus. Psyllid inoculated. qPCR analysis. First samples for testing to be shipped in June.
Bayer CRDF agreement -- 13 month summary

- On track to produce leads for 2019 field trials vs HLB
- Screening cascade expected to be ready for routine testing in Q3
- Good progress on collaborator in planta assays vs Liberibacter (CA, FL)
- Promising PDM chemical class to be tested in greenhouse Liberibacter assays in July
- Validation and optimization of primary in vitro screen in process in Sacramento
- Activity identified in advanced microbial lead with concentrated material in test in tomato Lsol assay
- 1000 strains selected through genomic analysis for in vitro screening
Thank You!