



# *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**

**1**  
**HLB Screening Cascade**  
Allows systematic search for solutions against HLB

### 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**

**2**  
**Plant Defense Modulators**  
Induce resistance and strengthen plant health

### 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**

**3**  
**Antibacterial Microbes**  
Avoid / Stop multiplication of the bacterium



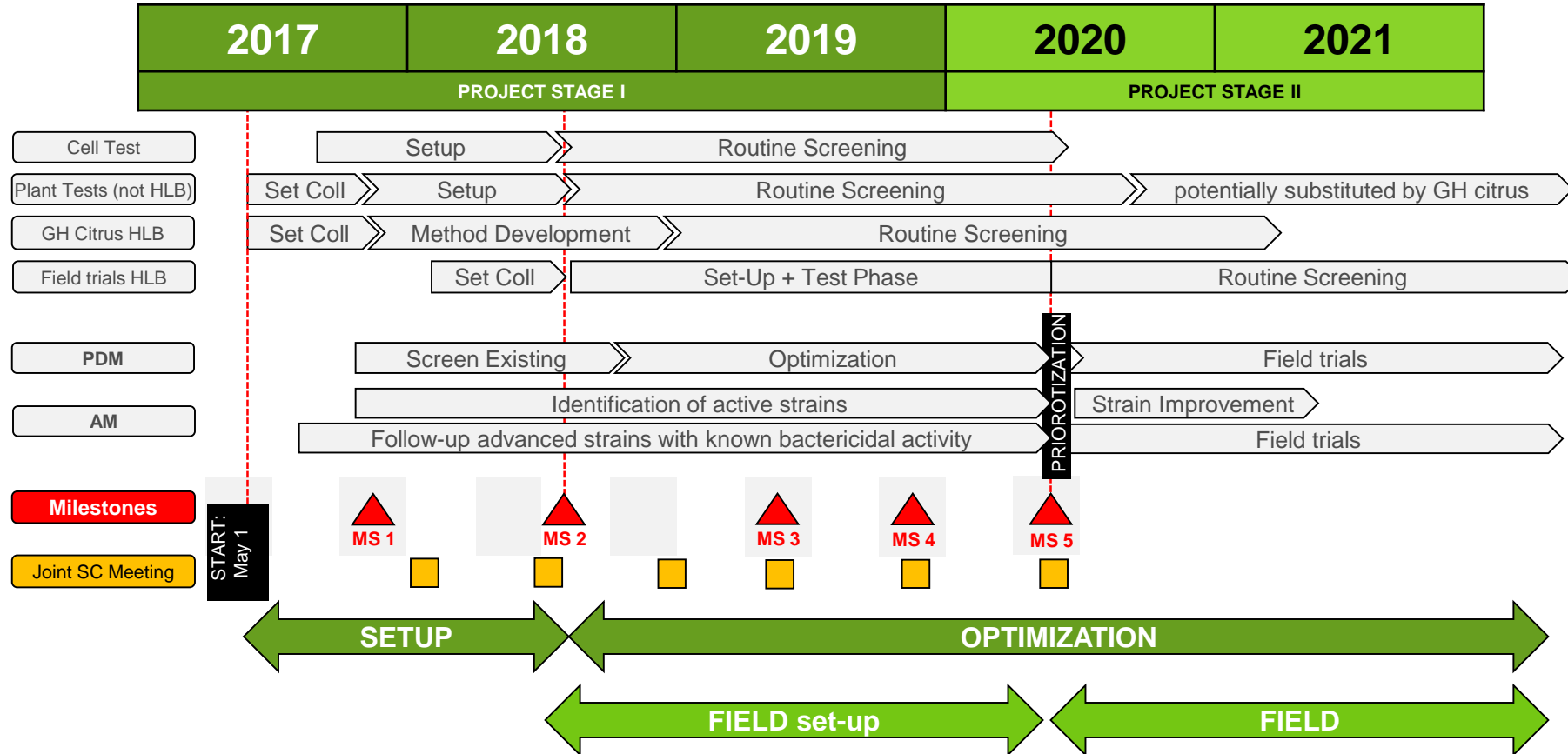
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- **Discovery:** capitalizing on Bayer's experience in discovery and development of crop protection products, a **validated screening process** will be designed to identify leads effective against Liberibacter, starting with in vitro lab testing to 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*

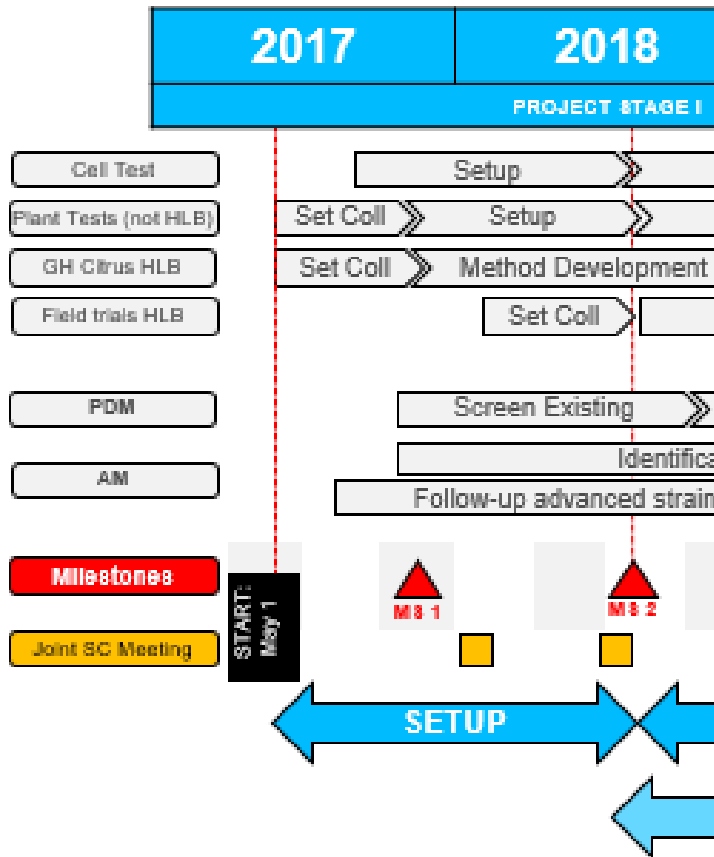


# Project Milestones





# 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

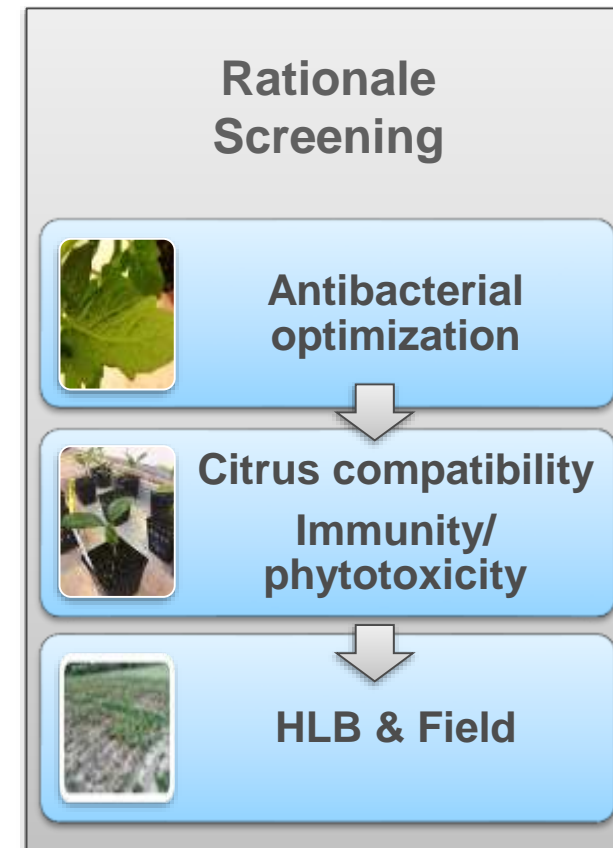
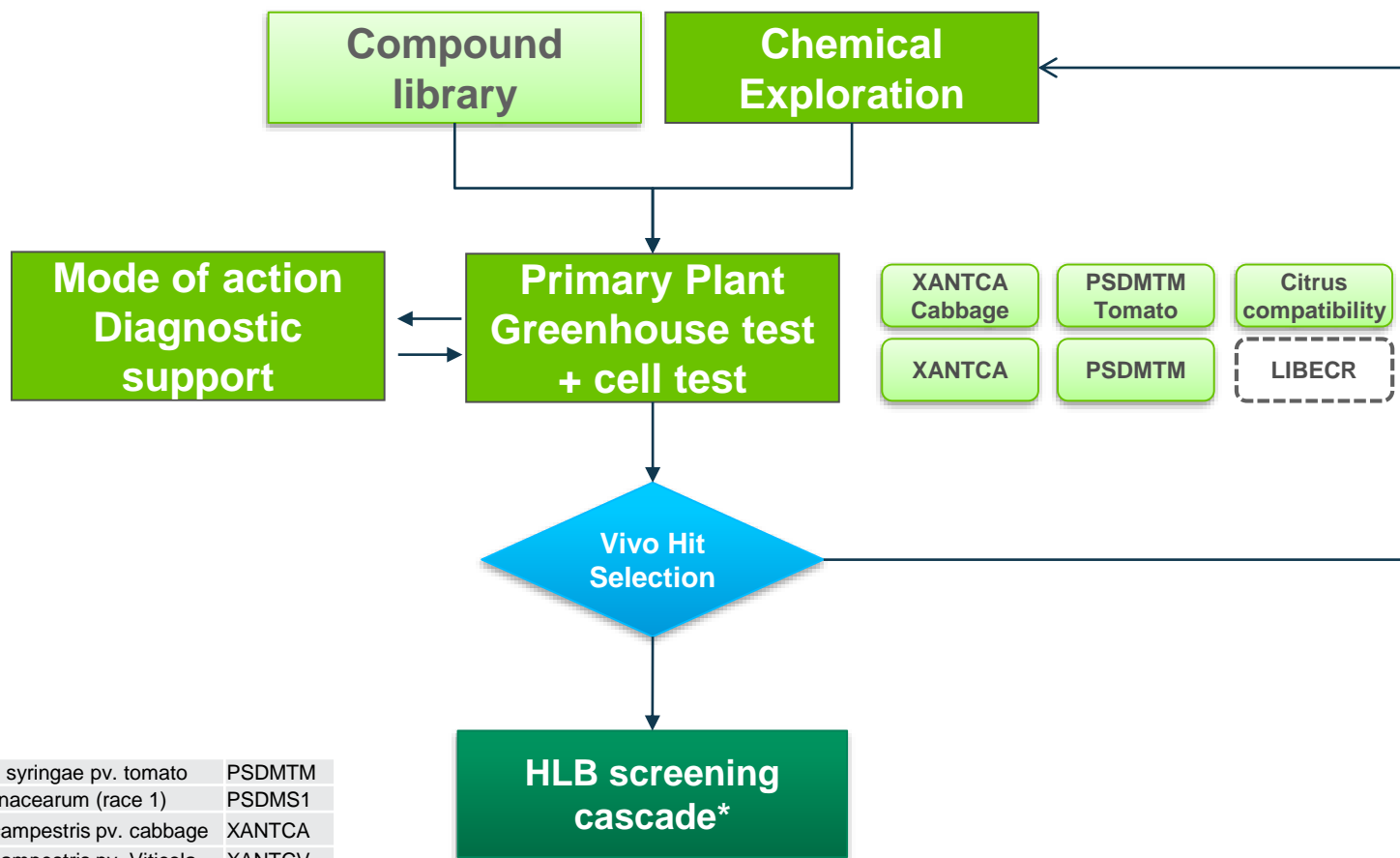
\*UCD : University of California Davis

\*\*UF : University of Florida



# Plant Defense Modulator Screening cascade

High throughput screen for synthetic compounds that can induce host defense



















<i>Pseudomonas syringae</i> pv. tomato	PSDMTM
<i>Ralstonia solanacearum</i> (race 1)	PSDMS1
<i>Xanthmonas campestris</i> pv. cabbage	XANTCA
<i>Xanthmonas campestris</i> pv. Viticola	XANTCV
<i>Liberibacter crescens</i>	LIBECR
<i>Agrobacterium tumefaciens</i>	AGRBTU

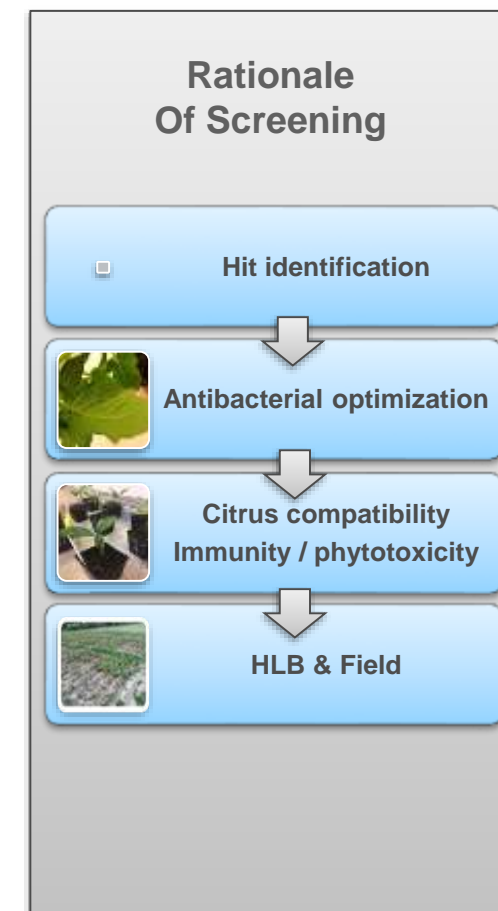
\* PDM leads enter the HLB screening cascade in the plant test assays as they have no direct activity on pathogen





# PDM Primary Test Status

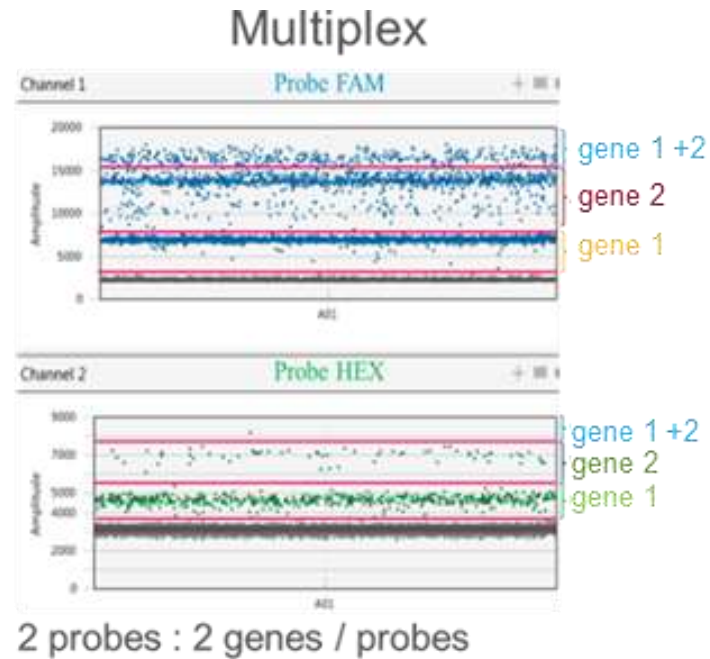
Criteria	<i>Xanthomonas</i>	<i>Pseudomonas</i>	HLB disease free	Cell tests
• Pathogen selection	 Strain ✓	 Strain ✓		 Strains ✓
• Plant feasibility	 Cabbage ✓	 Tomato ✓	 Citrus ✓	
• Disease test development	 Test conditions ✓	 Test conditions ✓		
• Automation	 Screening ✓	 Screening	 Screening	 Screening ✓
	 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



QX200™ Droplet Digital™ PCR System



- Proof of concept of multiple marker tracking
- Low Throughput Screen to be routine in Q3-2018



# Biology Lead compound

Plant test	Test object	Xanthomonas
Lead	Structure	Dosage and Unit
	500 PPM	
	125 PPM	
	31 PPM	
	500 PPM	
	500 PPM	
	125 PPM	
	31 PPM	
	31 PPM	71
	16 PPM	96
	8 PPM	91
	4 PPM	91
1 PPM	35	
Analog 1	500 PPM	0
	125 PPM	63
	31 PPM	75
	500 PPM	W 80
	31 PPM	96
	16 PPM	66
8 PPM	47	
Analog 2	500 PPM	0
	125 PPM	0
	31 PPM	50
	500 PPM	W 80
	40 PPM	94
	8 PPM	93

## Cell test

Lead	Result	Dosage	Pseudomonas	Xanthomonas
	Eff.	0.625	1	0
	Eff.	1.25	19	0
	Eff.	2.5	5	3
	Eff.	5	7	5
	Eff.	10	5	2
	Eff.	20	3	2
	ED50 (ppm)		>20	>20
	ED95 (ppm)		>20	>20

Pseudomonas Efficacy demonstrated in plants



Untreated



Lead



# Chemistry Pipeline Status

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

## Sources

Patent

PDM vRS

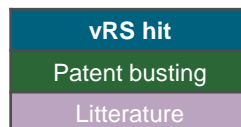
« 50 clusters »

Hit Vivo team

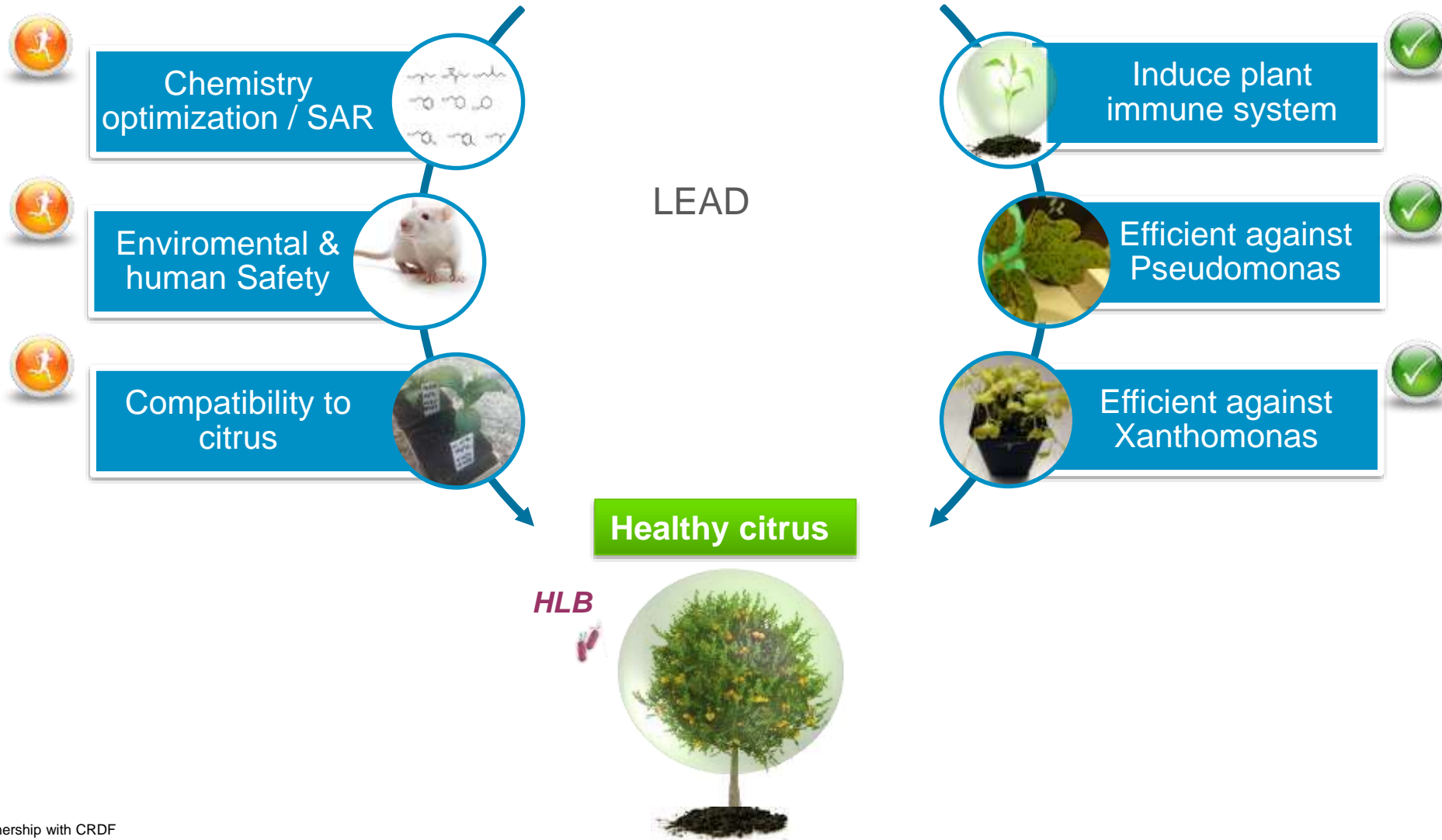
« 2 »

Opportunistic

« 1 natural product »



# Toward citrus induce resistance





# CRDF Collaboration

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# Bayer and Monsanto will create a global leader in sustainable agriculture



Innovation Engine

Commitment to Sustainability

Social Responsibility



# Why citrus matters to us...

Focus on Sustainable Horticulture through Innovation



## 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: 7:08 AM, May 30, 2018



Share Your Opinion

Share

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# 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

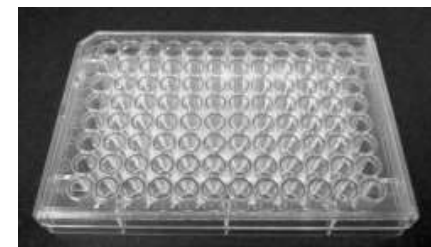
**Primary screen:** modify established fungal cell test protocols to quantify activity of microbes against panel of target bacteria.



CLS strain selection



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

0.4%	56.0%	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%
41.6%	34.7%	29.1%	37.8%	29.6%	35.2%	38.0%	37.8%	26.3%	40.6%	41.3%	-8.6%
48.2%	49.4%	39.2%	47.0%	35.2%	31.4%	33.8%	46.1%	28.4%	49.6%	34.7%	-6.7%
51.9%	51.9%	41.6%	54.5%	29.1%	36.4%	31.7%	28.9%	26.5%	50.8%	41.8%	-0.4%
54.8%	50.8%	37.6%	52.4%	28.4%	37.1%	-36.9%	29.3%	26.7%	47.5%	32.9%	10.5%

Calculate % inhibition of target in presence of strain extract



Identify strains that cause inhibition for secondary screen



# Divestment of Morrisville, NC group to BASF: Transfer/optimization of primary screen for HLB project

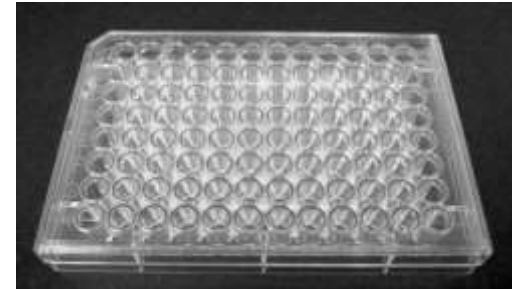
**Primary screen:** Opportunity to increase efficiency and improve outcome by optimizing system for HLB project rather than fitting it to the Traits approach



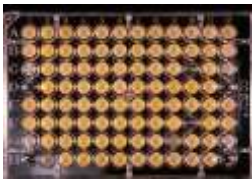
CLS strain selection  
NO CHANGE



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



Incubate plates and measure OD

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-6.5%	38.0%	42.0%	39.0%	28.9%	36.9%	4.8%	44.4%	28.9%	42.0%	40.4%	74.8%
41.6%	34.7%	29.1%	37.8%	29.6%	35.2%	38.0%	37.8%	26.3%	40.6%	41.3%	-8.6%
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Calculate % inhibition of target in presence of strain extract

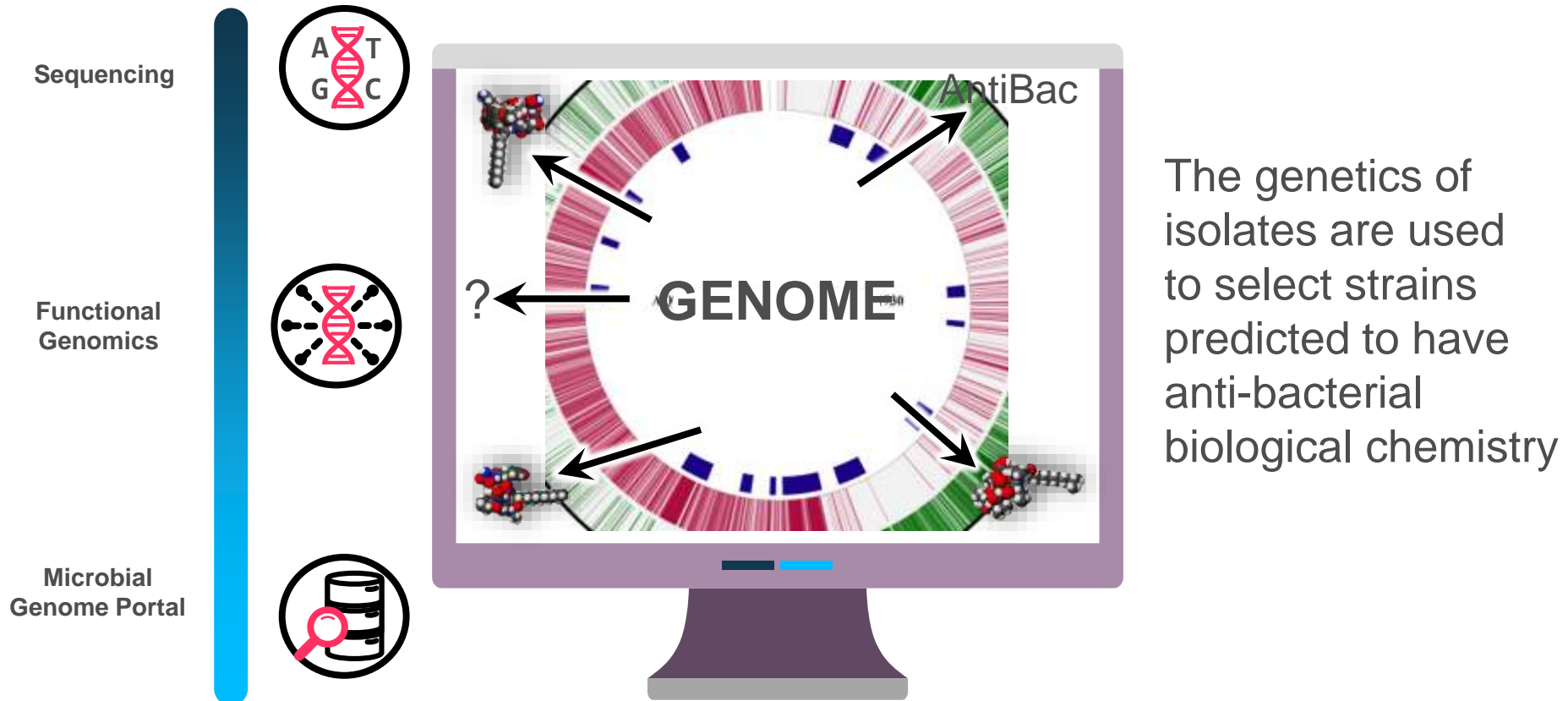


Identify strains that cause inhibition for secondary screen



# In-silico screening of Bayer's microbial library

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

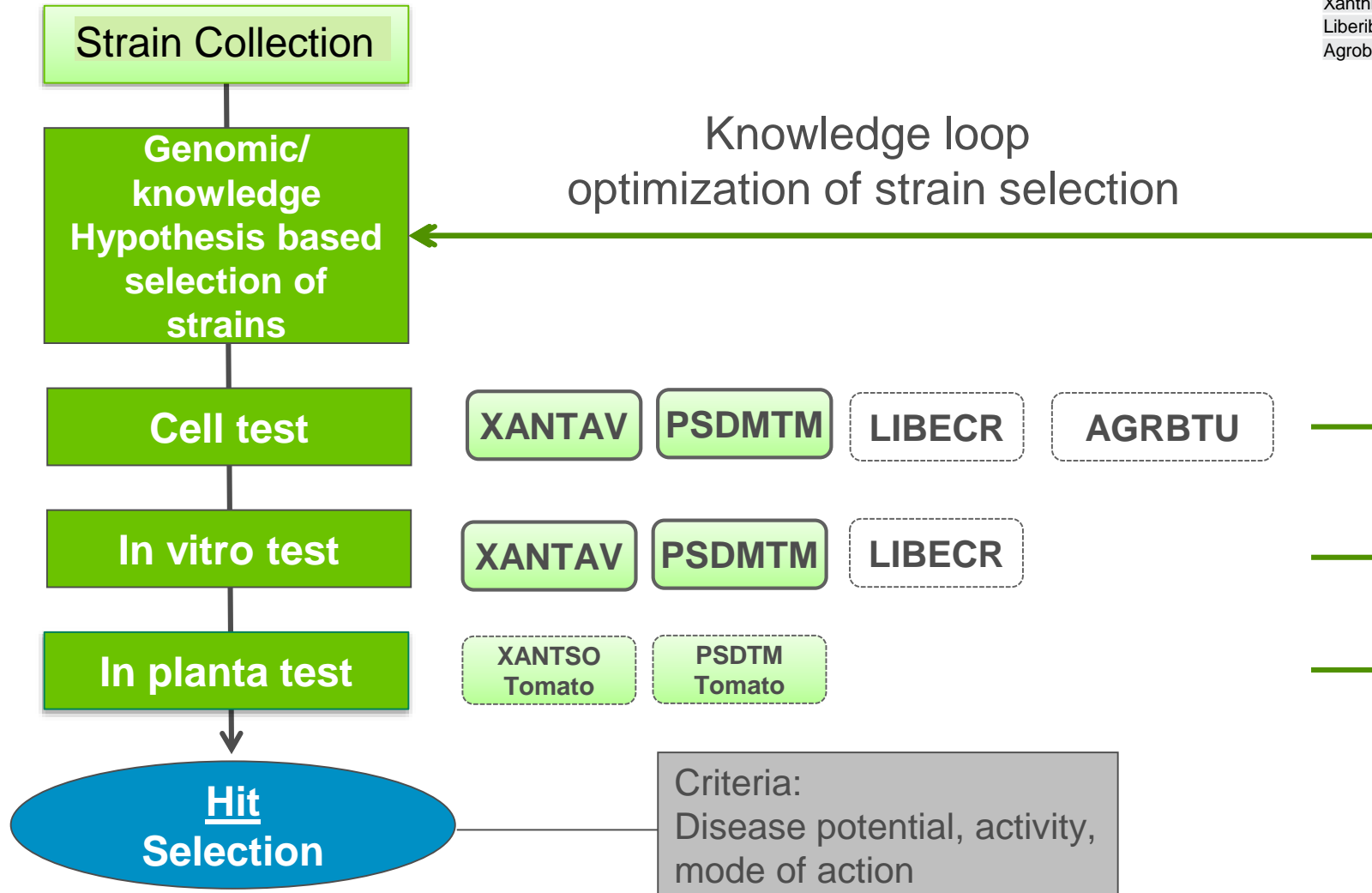




# Antibacterial Microbe screening cascade

Using Microbes from the environment to target the disease

<i>Pseudomonas syringae</i> pv. tomato	PSDMTM
<i>Ralstonia solanacearum</i> (race 1)	PSDMS1
<i>Xanthomonas campestris</i> pv. cabbage	XANTCA
<i>Xanthomonas campestris</i> pv. Viticola	XANTCV
<i>Liberibacter crescens</i>	LIBECCR
<i>Agrobacterium tumefaciens</i>	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



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# CRDF Collaboration

Summary of progress in first twelve months

## In planta screening collaborations

- Research agreement with UCD\* in place, assay and application methods being validated, advanced microbe lead extract in test
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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

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# 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!*

