Detection and Management of *Acidovorax citrulli*

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Disease Management

- A few infected seedlings can lead to wide spread of disease in greenhouse transplants due to high temperature and over head watering
- Similar spread can occur in the fields if the temperature and the humidity are elevated
- *Acidovorax citrulli* is a high temperature pathogen, there is little or no disease below 30 C

Disease Management

- Watermelon fruit blotch should be closely managed:
- Use bacterial free seeds
- Strict seed testing is key to control
- Pathogen free transplants
- Immunostrips are useful for detection in the field
- Eliminating culls and volunteers to minimize pathogen proliferation
- 2 years rotation free of cucurbit crops is important to Watermelon Fruit Blotch management

Seed treatments

- Several chemical treatments are available but none work 100%
- Treated seeds must be assayed

Effect of seed treatments on germination and infection of watermelon seeds

Treatment	% germination after 14 days	% infected
untreated	69	4
Water at room temp 20 min	82	4.75
ACA (0.25% cupric acetate) 50 C 20 min	93	0
Clorox diluted 1:10) 50 C 20 min	55	0
Acidic electrolyzed water 30 min	90	0

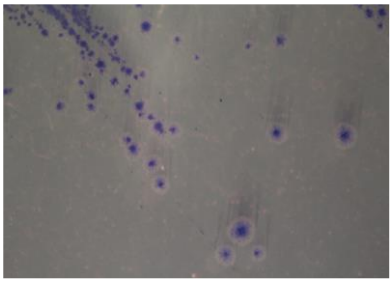
4 replicates of 100 seeds spiked with 5 seeds infected with *Acidovorax citrulli* (Feng et al.)

Methods for Seed Assays

- Soak or grind seeds to extract bacteria
- Agar plating on semi selective media (WFB44, WFB08 and EBBA)
- Polymerase chain reaction (PCR)
- Agar plating/PCR
- Immunomagnetic Separation/PCR

Technical Challenges with Agar Plating

- Antibiotics produced by saprophytic bacteria act synergistically with antibiotics in medium
- Seeds may have large numbers saprophytes
- Extracting infected seeds; grinding, breaking
- Seeds treated with chemicals
- Seeds harvested from fruit under field conditions



Acidovorax citrulli colonies growing on EBBA medium
After 5-7 days of growth at 28C colonies have a clear margin with blue-purple center

Greenhouse seedling grow out

- Grow seeds under favorable conditions
- Inspect seedlings daily for 18 days
- Collect suspect plants for confirmation testing by serology-based immunostrip assays
- Isolate from plants with suspects symptoms
- Look for putative *A. citrulli* colonies based on colony morphology

Sweatbox Assay

- Treat potting mix with fungicide and sow seeds in transplant plastic boxes under favorable growing conditions
- Observe seedlings symptoms and check with immunostrips
- Confirm with PCR and isolation on YDC and EBBA media

Serology Assays

- ELISA
- Immunofluorescence
- Immunostrips, available commercially, (kits are useful for field testing)

These assays are not very sensitive but are very useful for presumptive identification and reliable when combined with isolation on semi-selective media

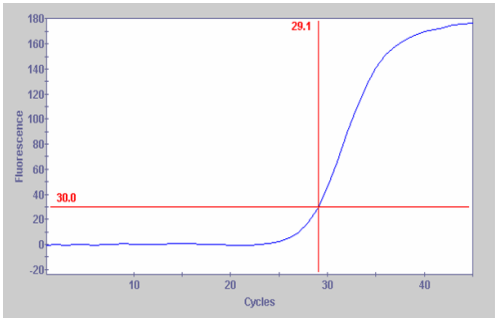
Immunomagnetic separation/PCR

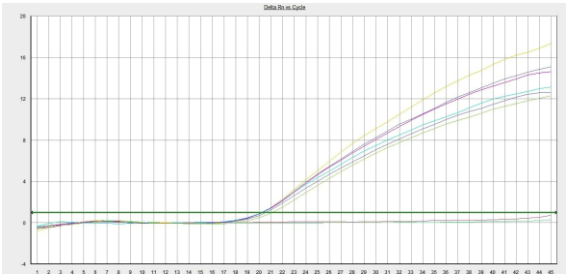
- Place seeds in buffer, vacuum extract with agitation for 1h, filter and centrifuge
- Suspend pellet in buffer and add immunomagnetic beads (IMBs) coated with anti AC antibodies
- Wash in buffer using a magnetic particle concentrator and suspend IMBS in water
- Incubate at 100C to release bacterial DNA
- Use suspect *Acidovorax citrulli* DNA for PCR

PCR

- PCR is most specific and sensitive
- Several primers and probes for identification of *Acidovorax citrulli* are available
- BIO-PCR combines enriching the target bacterium in liquid or agar medium before performing PCR

PCR Cycle threshold (ct)





real time PCR assay

Assay sensitivity using extracts of 1,000 healthy seeds spiked with cells of *A. citrulli*

Ac added (cfu/ml)	cfu detected	cfu detected	Direct PCR	BIO PCR Ct value	BIO PCR Ct value
	EBB	EBBA		EBB	EBBA
0	0	0	**0**	0	
0.1	0	0	0	0	
1.1			0	**0**	**36.63**
11	**10**	**13**	0	26.4	26.56
110	140	167	0	24.0	23.47
1100	**1450**	**1370**	**0**	**23.92**	**21.98**

Seed sanitation in Thailand

Conclusion

- *Acidovorax citrulli* is a high temperature pathogen
- The risk of infection is increasing with global warming
- To control the disease it is very important to have access to sensitive detection and efficient seed treatments.