ERVIEW OF SEED-BORNE BACTERIAL DISEASES

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THE OHIO STATE UNIVERSITY

Outline

- Important seed-borne bacterial pathogens
- Economic importance
- Bacterial detection in seeds
- Seed chain ï bacterial disease management

Seed-borne Bacterial Diseases

- The majority of bacterial diseases of plants are seed-borne
  - True seed
  - Vegetative planting material
- Most bacterial diseases cannot be managed adequately using currently available bactericides, especially in the tropics

Black rot of cauliflower in Bangladesh

Bacterial Pathogens Borne in True Seed

Crop	Pathogen(s)
Wheat	*Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris* pv. *translucens*
Maize	*Pantoea stewartii* subsp. *stewartii*, *Clavibacter michiganensis* subsp. *nebraskensis*
Rice	*X. oryzae* pv. *oryzae, X. oryzae* pv. *oryzicola, Acidovorax oryzae*
Bean	*P. syringae* pv. *phaseolicola, Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Xanthomonas campestris pv. phaseoli and X. fuscans var. fuscans*
Soybean	*P. syringae* pv. *glycinea*
Chickpea	*Rhodococcus fascians*
Cereals, grasses	*Rathayibacter* sp.
Alfalfa	*C. michiganensis* subsp. *insidiosus*

Bacterial Pathogens Borne in True Seed

Crop	Pathogen(s)
Tomato Pepper	*Pseudomonas syringae* pv. *tomato (*tomato*)*, *P. syringae* pv. *syringae, Xanthomonas spp., Clavibacter michiganensis* subsp. *michiganensis*
Carrot	*Xanthomonas campestris* pv. *carotae*
Onion	*Pantoea ananatis, Burkholderia cepacia*
Crucifers	*Xanthomonas campestris* pv. *campestris*, *P. syringae* pv. *alisalensis* (broccoli), *Pseudomonas* spp. (crucifers)
Cucurbits	*P. syringae* pv. *lachrymans, Acidovorax citrulli*
Lettuce	*Xanthomonas campestris* pv. *vitians*

Bacterial Pathogens in Vegetative Planting Material

Crop	Pathogen(s)
Potato	*Clavibacter michiganensis* subsp. *sepedonicus, Ralstonia solanacearum, Streptomyces scabies, Candidatus Liberibacter sp., Erwinia/Dickeya spp.,*
Citrus	*Candidatus Liberibacter asiaticus, Xylella fastidiosa subsp. pauca, Xanthomonas citri*
Strawberry	*X. fragariae*
Grape, almond	*Xylella fastidiosa* subsp. *fastidiosa*,
Pear, apple, quince	*Erwinia amylovora*
Sugarcane	*Leifsonia xyli* subsp. *xyli, Xanthomonas albilineans*
Cassava	*Xanthomonas campestris* pv. *cassavae*
Banana	*X. campestris* pv. *musacearum*

Seed Production Practices Encourage Bacterial Diseases

- True seed should be produced in dry climates to minimize bacterial disease risk
- Seed now produced globally in environments conducive to bacterial diseases
- Hybrid seed produced where labor costs are low
- Global trade in seeds and vegetative material results in potential for introduction of new pathogens/races from source to recipient regions

Economic Importance of True Seed-borne Bacterial Diseases

- Bacterial canker ï tomato
  - Most destructive disease of tomatoes
  - $ Millions in losses: yield and management costs in greenhouse tomatoes
  - 70-80% losses reported in field tomatoes
- Black rot ï crucifers
  - Most destructive disease of crucifers
  - Untold losses in yield and quality

Seedling Production Environment: Ideal for Bacterial Multiplication and Spread

- Many vegetable crops initiated transplants
  - Greenhouse-grown
  - Seedbeds
- Transplant production systems offer ideal conditions for bacterial reproduction and spread

Economic Importance of Vegetatively Propagated Bacterial Diseases

- Ring Rot ï potato
  - Highly destructive
  - Managed by seed certification programs
- Banana Xanthomonas Wilt
  - Highly destructive in East Africa
  - Major food crisis in Uganda in 2000s


British Columbia Ministry of Agriculture

Detection of True Seed-borne Bacterial Pathogens

- Seed health testing
  - Important means of reducing disease risk
  - Direct testing
    - Symptoms/grow-outs
    - Isolation of pathogen
    - Identification
    - Proof of pathogenicity
  - Indirect testing
    - Detection of proteins (serological)
    - Detection of nucleic acids (PCR, isothermal amplification, etc.)

Detection of True Seed-borne Bacterial Pathogens

- Issues
  - Identification of appropriate causal agent (specificity)
  - Low levels of infestation (sensitivity)
  - Dead vs. live bacteria
  - Inhibitors of ELISA/PCR in seed extracts
- Potential solutions
  - Enhanced specificity of detection method
  - Concentration of seed extract; enrichment; enhanced assay sensitivity
  - Enrichment
  - Separation of bacteria from extract
    - Immunomagnetic separation
    - Filtration

Direct Testing: Grow-outs to Detect Bacterial Pathogens in Seed Lots

- Longest-standing method
- Highly selective
- Sensitivity varies
- 30,000 seeds per seedlot need to be tested to meet infestation threshold (I in 10,000)
  - Very high labor and infrastructure costs

Direct Testing: Grow-outs to Detect Bacterial Pathogens in Seed Lots

- Environmental conditions must be conducive
  - High relative humidity
  - Temperature optima
- Avoid cross-contamination between seedlots
  - Barriers
  - Space
  - Limit passive contamination by vectors
- Follow up with isolation and testing

Direct Testing: Isolation from Seed Lots

- Extraction
  - Seed soak (passive)
  - Stomacher (crushing)

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- Direct testing or biological amplification to increase sensitivity

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- Isolation on semi-selective and diagnostic media

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- Identification

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- Pathogenicity

Seed

Mesh Stomacher bag

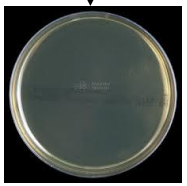
Extraction Buffer

Stomacher

Extract

PCR, ELISA, LAMP

Selective media



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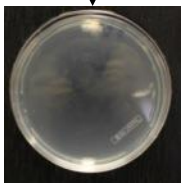

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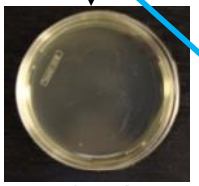


CNS

Samples

WASH

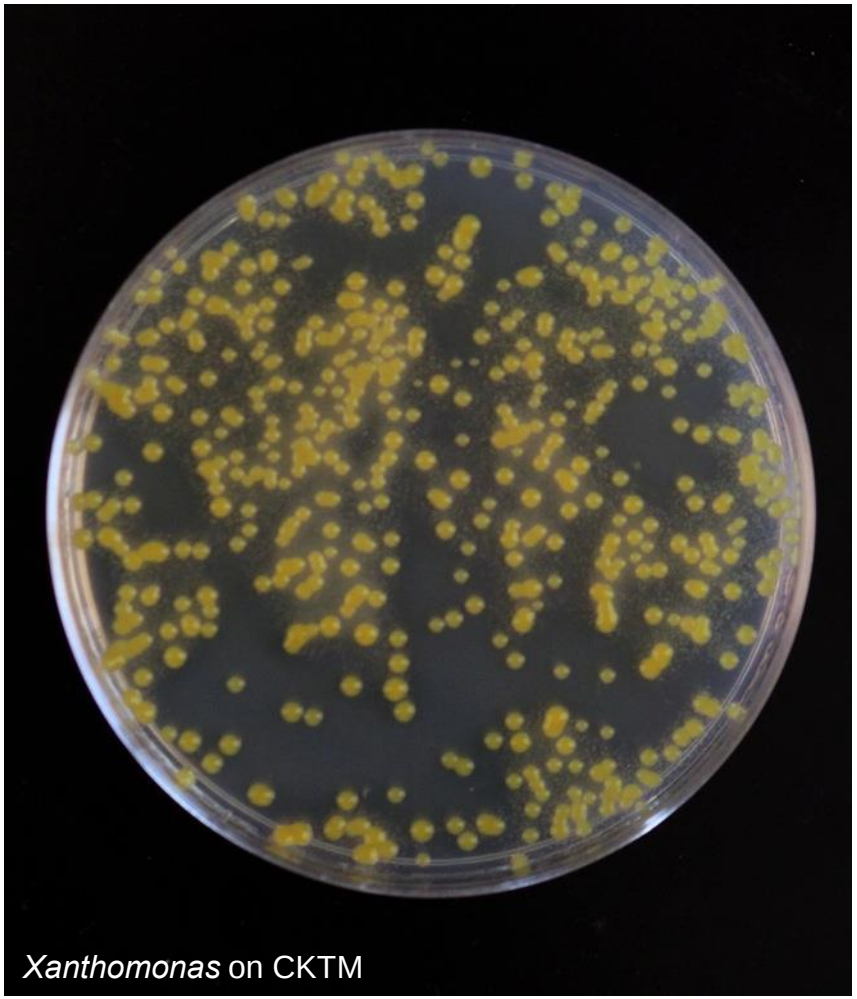
COLONY PICK

Bio-PCR, Bio-LAMP

Modified from Jarred Yasuhara-Bell, University of Hawai`i at MŪnoa, MBBE, Alvarez Lab, jarredyb@hawaii.edu

Pathogen Isolation ï Semi-selective Media

- Media that favor the growth of target bacteria over other microorganisms
- Based on nutritional requirements and physiological tolerances of target bacteria
  - Utilizable carbon, nitrogen sources
  - Antibiotics, dyes
  - pH, high sucrose

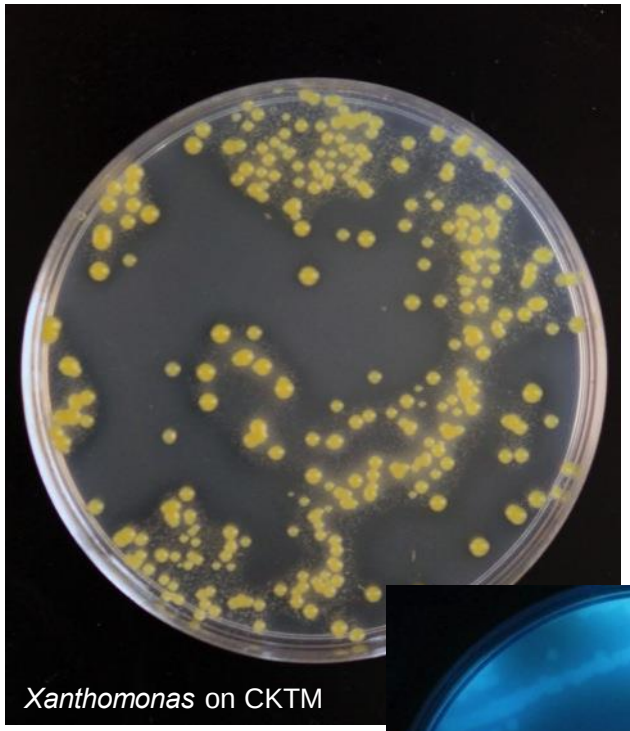


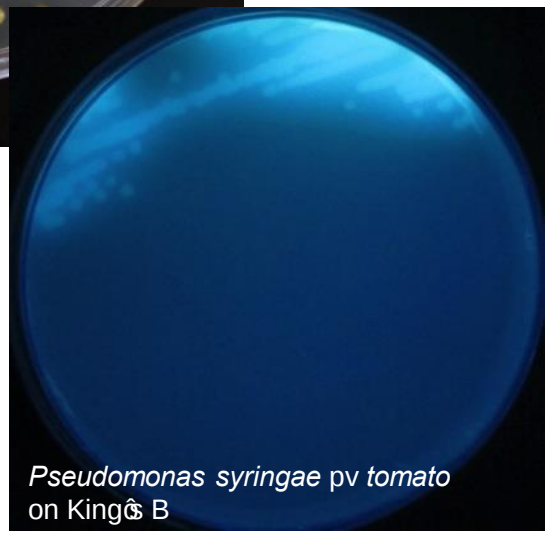
Xanthomonas on CKTM

Pathogen Isolation ï Diagnostic Media

- Media that differentiate target bacteria based on colony appearance or changes in medium

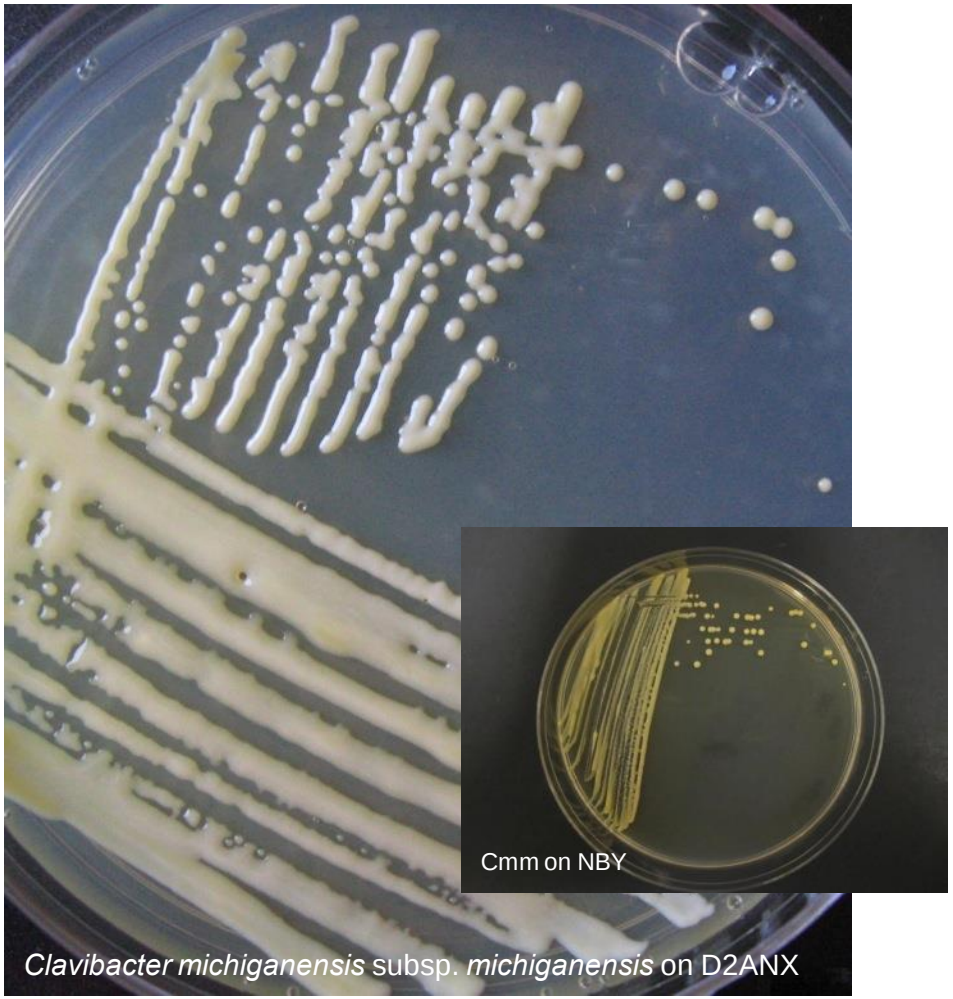
- Examples
  - CVP- pits in medium differentiate pectinolytic bacteria
  - CKTM/Tween B ï white precipitate and clear zones differentiate *Xanthomonas* spp.
  - Kingôs B/PF ï fluorescent colonies


Xanthomonas on CKTM


Pseudomonas syringae pv *tomato* on Kingôs B

Semi-selective Media Issues

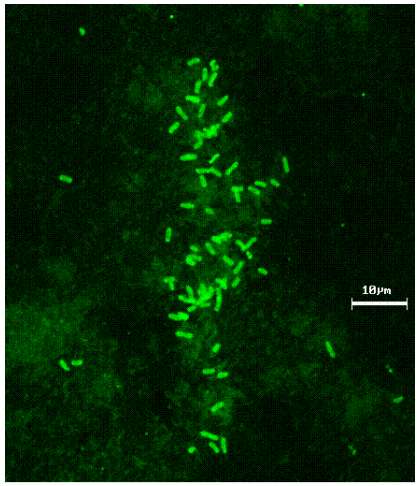
- Strain variation
  - Some strains don't grow ï recovery rates vary
  - Strains differ in appearance
- Source of medium ingredients
  - Brand, lot
- Post-harvest seed treatments
  - Chemical, biological
- Age of seedlot
- Microflora may inhibit growth ï natural antibiotic production
  - Spiked control (marked strain) needed

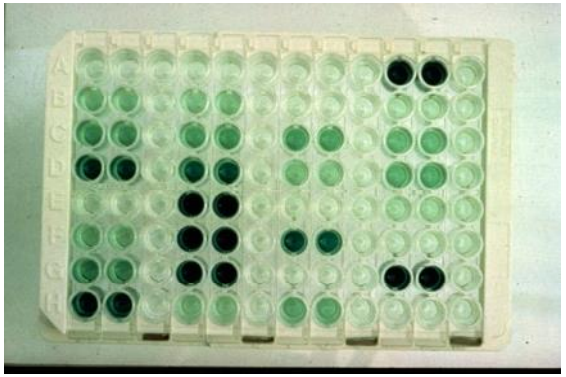


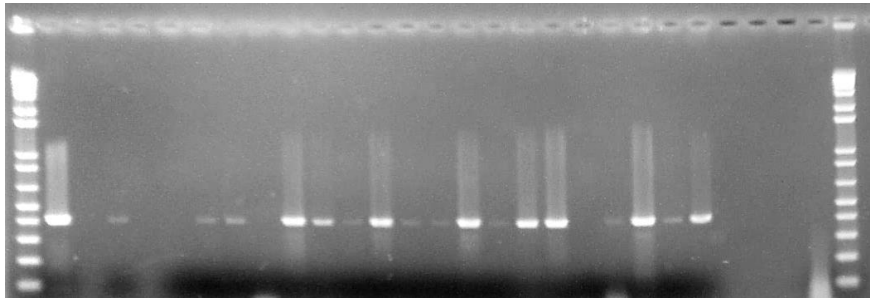
Clavibacter michiganensis subsp. *michiganensis* on D2ANX

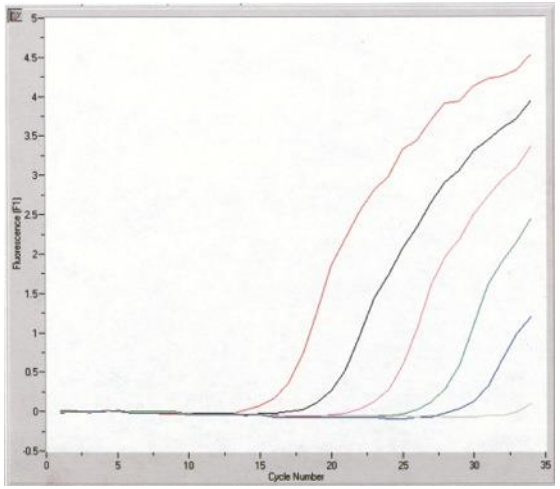
Identification of Isolated Bacteria

- Immunoassays
  - ELISA
  - Immunofluorescence
- DNA Amplification
  - End-point PCR
  - Quantitative PCR (real time)
  - Isothermal amplification (e.g. LAMP)
- Sequencing

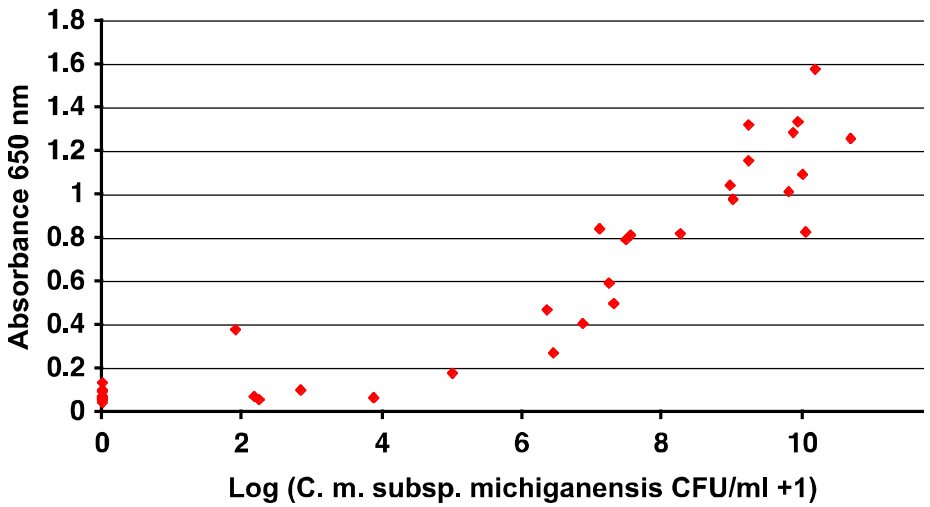






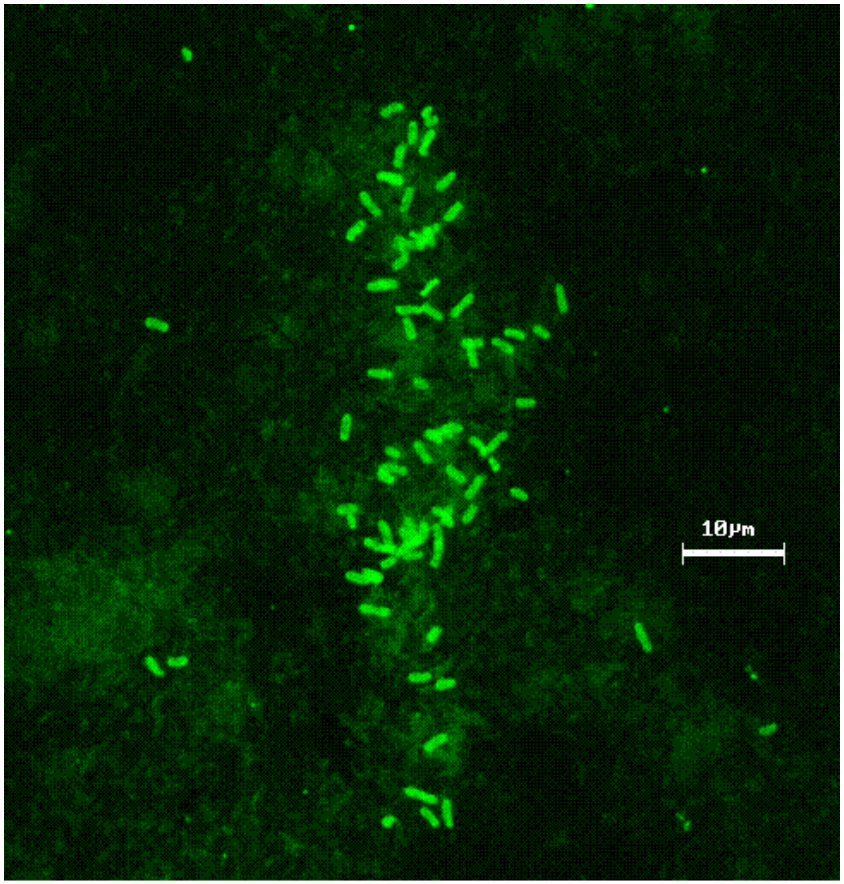


Detection Methods: Immunoassay



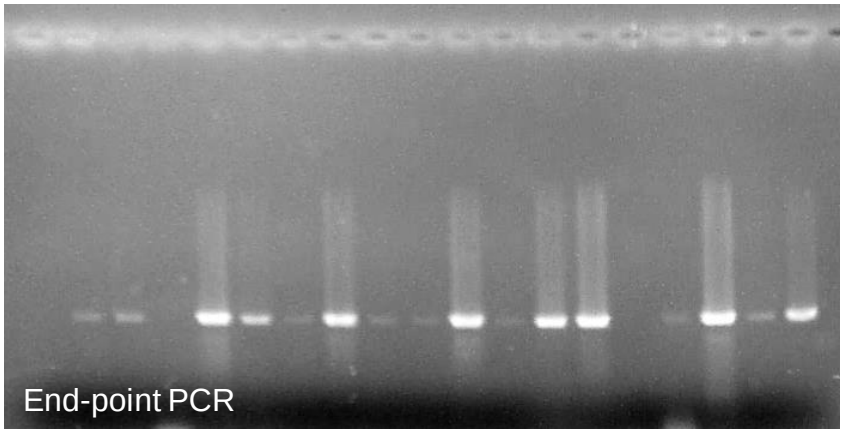
- ELISA is rapid, easy to use and relatively inexpensive
- Kits are widely available commercially
- Monoclonal and/or polyclonal antibody-based
- Sensitivity generally no less than ~10^6 CFU/ml
- False positives of concern

Detection Methods: IF

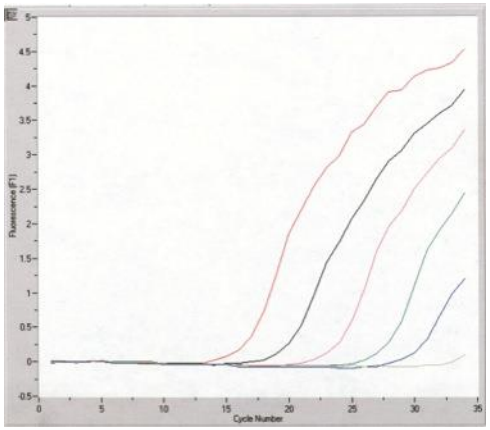


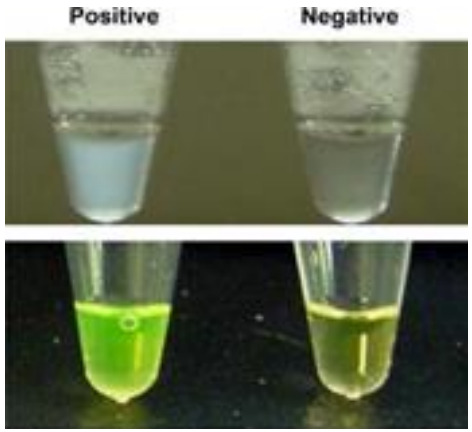
- IF-tagged (conjugated Mabs and Pabs available commercially)
  - Direct IF
  - Indirect IF
  - Immunofluorescent tagging combined with morphology increases specificity, but false positives common
- IF colony staining (IFC) ï only viable pathogens detected

DNA Amplification Assays


End-point PCR

- Primers published for major bacterial pathogens
- Relatively easy to develop primers for emerging pathogens


Q-PCR output



http://www.markergene.com WebNewsletter3.11.htm
LAMP Isothermal Assay

- Sensitivity usually greater than ELISA
- Real-time PCR, isothermal amplification: improvements in sensitivity, portability

Seed Chain ï Managing Seed-borne Bacterial Diseases

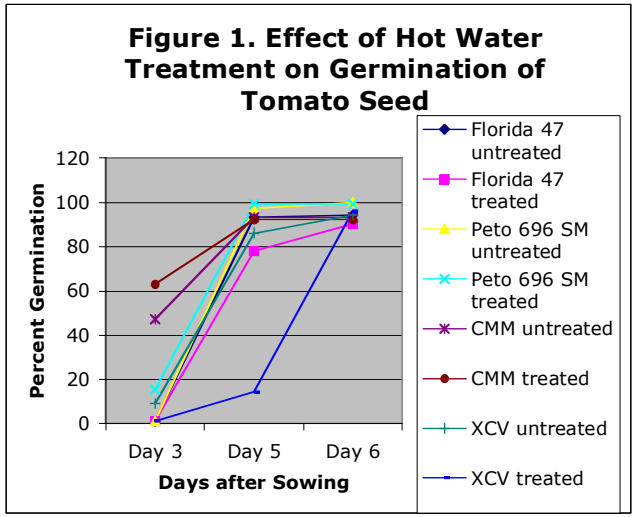
- Reduce or eliminate bacterial diseases from seed production fields
  - Zero tolerance in seed production
    - Ring rot - potato
    - Bacterial spot, speck and canker
    - Rigorous scouting
    - Cultural practices ï sanitized seed, crop rotation, protected culture, strict sanitation
- Test seed for known high impact pathogens
- Sanitize seed
  - Often done for positive-testing lots only
  - Sometimes done for all lots ï e.g. black rot of cabbage

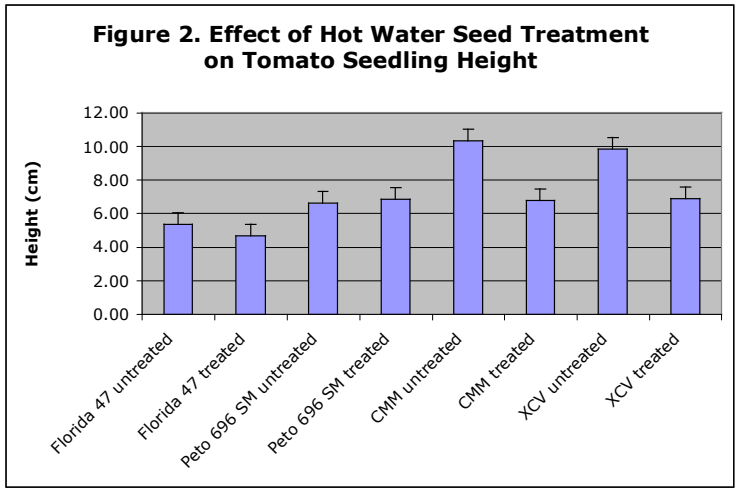
Seed Treatment to Eliminate Seed-borne Bacterial Pathogens

Hot water treatment

Seed	Water temperature		Minutes
	° F	° C	
Brussels sprouts, eggplant, spinach, cabbage, tomato	122	50	25
Broccoli, cauliflower, carrot, collard, kale, kohlrabi, rutabaga, turnip	122	50	20
Mustard, cress, radish	122	50	15
Pepper	125	51	30
Lettuce, celery, celeriac	118	47	30

Hot Water Treatment - Concerns





- May delay or reduce seed germination, especially in old or poor quality seed lots
- May reduce seedling vigor
- May reduce seed longevity
- Not compatible with other seed treatments (pelleting, priming etc.)
- May be damaging to large-seeded vegetables

Alternative Seed Treatments

- Sodium hypochlorite
  - Lettuce bacterial leaf spot
    - Sahin and Miller Plant Dis. 81:1443-46
    - 0.52% sodium hypochlorite/5 minutes
    - Highly effective; no effect on germination
- Peroxyacetic acid
  - Watermelon fruit blotch
    - Hopkins et al. Plant Dis. 87:1495-1499
- Hydrochloric acid
  - Bacterial canker of tomato
    - 1.24% HCl for 30 min
    - Highly effective; no effect on germination

More Information

- Gitaitis, R. and Walcott, R. 2007. The epidemiology and management of seedborne bacterial diseases. Annu. Rev. Phytopathol. 45:371-397.

- International Seed Health Initiative website:
  http://www.worldseed.org/isf/ishi.html

- International Seed Testing Association website:
  http://www.seedtest.org/en/home.html

- Fatmi, Walcott and Schaad (eds) (to be published in late 2014/early 2015). *Detection of Plant Pathogenic Bacteria in Seed and Planting Material*. APS Press.