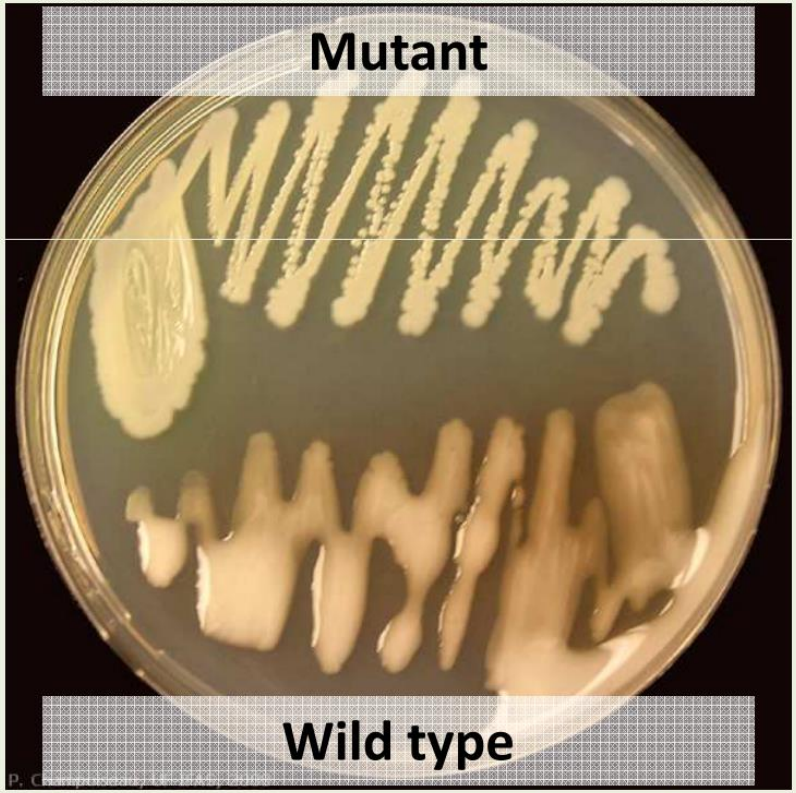
- *Ralstonia solanacearum* is a plant pathogenic bacterium.
- Formerly known as *Pseudomonas solanacearum*.

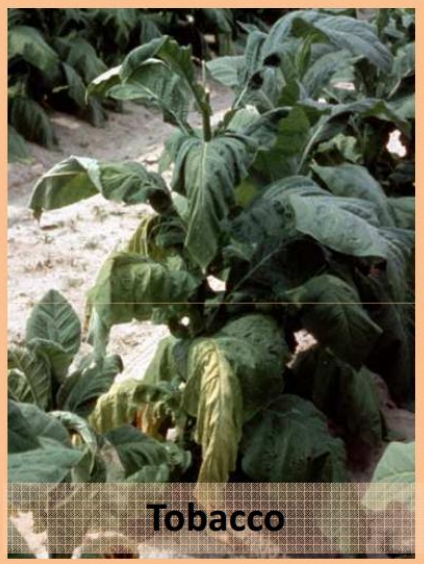


Cultures of virulent (bottom) and non-virulent (top) colonies of *R. solanacearum.*

Culture of *R. solanacearum* on TZC agar medium

Ralstonia solanacearum

- Bacterial wilts: >200 plant species, > 50 families
  (trees, cultivated crops, ornamentals, and weeds).



- Worldwide distribution from tropical to cold-temperate areas (Europe, North Asia).
- Soil-borne and water-borne pathogen.

Table 1. Races and Biovars of *Ralstonia solanacearum*

Race	Host Range	Geog. Distribution	Biovar
1	Wide	Asia, Australia, Americas	3, 4 1
2	Banana Other Musa spp.	Caribbean, Brazil Philippines	1
3	Potato, some other Solanaceae, Geranium; plus a few other species.	Worldwide except US and Canada	2
4	Ginger	Asia	3, 4
5	Mulberry	China	5

Symptom caused by *R. solanacearum* R3b2 on geranium. Photo: C. Allen, U. Wisconsin.

NPDN
National Plant Diagnostic Network

National Plant Diagnostic Network

Standard Operating Procedure for Plant Diagnostic Laboratories

Southern Wilt/Brown Rot
***Ralstonia solanacearum* Race 3 Biovar 2**

CSREES
USDA

VERSION 2.3

Ralstonia solanacearum

Detection, identification

- Step 1: Symptom identification - Early stages

Potato/Tomato

Wilting of the youngest leaves

Geranium

Upward curling

Wilting of lower leaves

Ralstonia solanacearum

Detection, identification

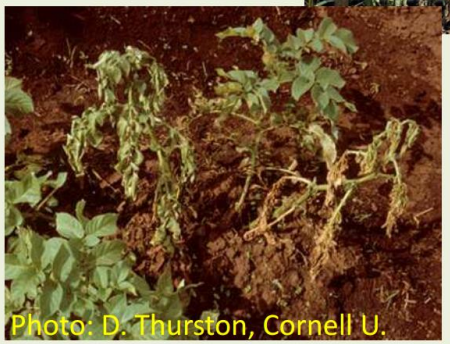
- Step 1: Symptom identification - Late stages

Potato/Tomato

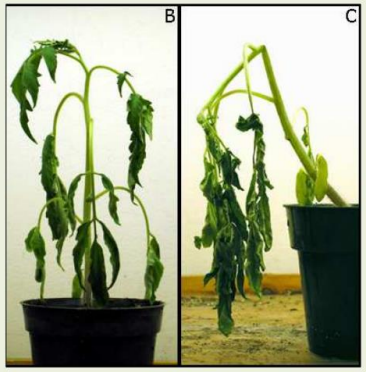
Wilting of foliage
Drying of leaves
Stunting of plants

Plant death

Brown discoloration of vascular tissue



Stem collapse



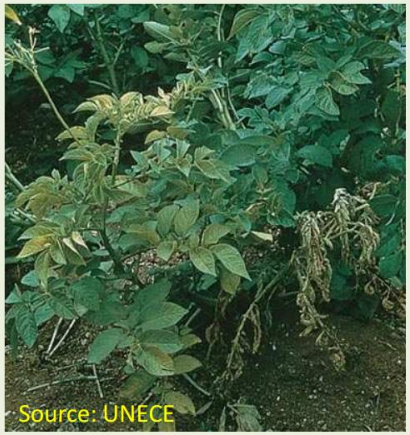
Ralstonia solanacearum

Detection, identification

- Step 1: Symptoms look-alike

Root damage, drought, nutrient deficiency or other plant pathogens, such as the *Verticillium* and *Fusarium* fungi (potato, tomato), or *Xanthomonas campestris* on geranium.

Fusarium wilt of tomato



Verticillium wilt of potato

Bacterial blight of geranium

Ralstonia solanacearum

Detection, identification

- Step 1: IMPORTANT notes on symptoms

Symptoms induced by R3b2 (PII, seq1) CANNOT BE distinguished from those induced by other strains (PII, seq 7 or 38)

LATENT INFECTIONS

- Infected but symptomless plant
- Tolerant cultivars, secondary hosts
- Vegetative propagating material
- Major epidemiological trait

Geranium cuttings ready for shipment to the US

Ralstonia solanacearum

Detection, identification

- Step 1: Signs of the pathogen - later stages

Photo: M. Daughtrey, Cornell U.

Bacterial ooze

P. Champoiseau, UF-IFAS, 2008

Photo: D. B. Langston, UG, Bugwood.org

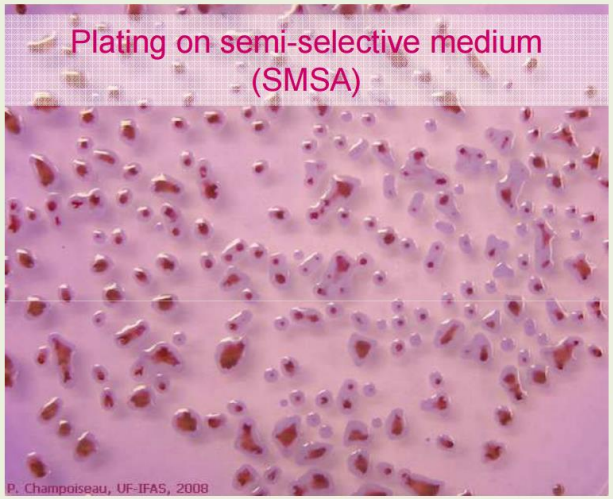
Bacterial streaming test

Ralstonia solanacearum

Detection, identification

- Step 2: Early screening tests

Species level
Fast and easy
Field or lab



- Late stages
- Low sensitivity

Use of immunostrips

Ralstonia solanacearum

Detection, identification

- Step 3: Confirmation of the genus/species

Species level
Approved diagnostic screening lab (PPQ permit)

Immunodiagnostic assays (species-specific Abs)

Enzyme-linked immunosorbent assay (ELISA), immunofluorescence-antibody staining (IFAS), or immunofluorescence-colony staining (IFCS).

DNA-based assays

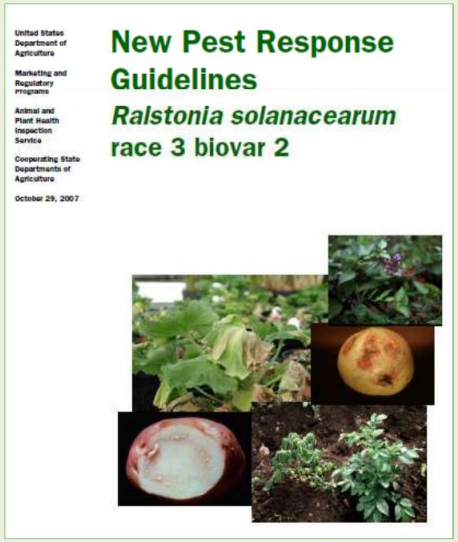
Polymerase chain reaction (PCR)

Universal species-specific primers

Primer set 759/760: 280bp fragment
(Opina et al., 1997)

Sampling protocols

Plant tissues, water, soil

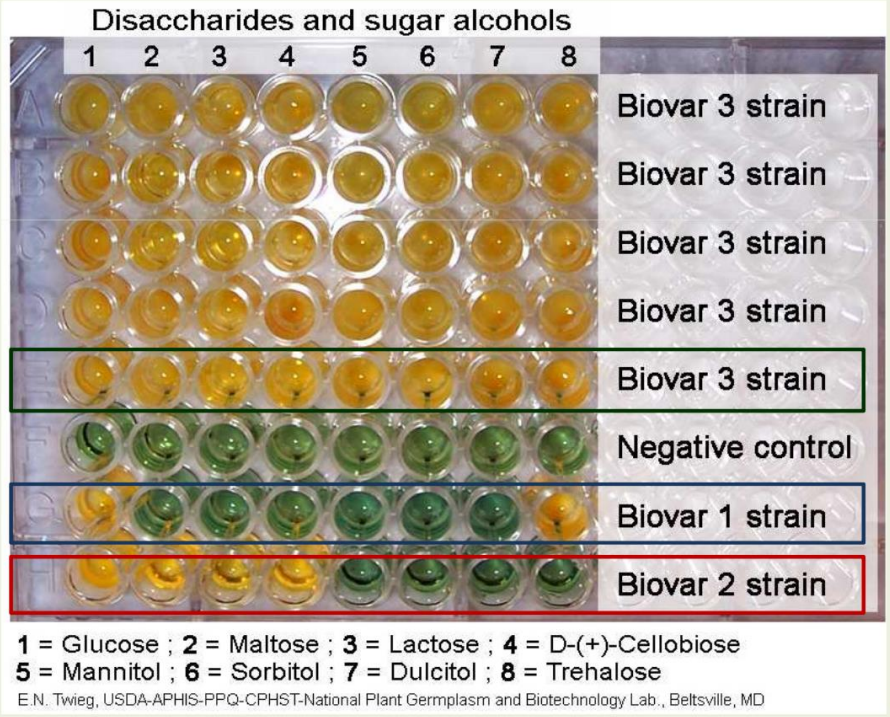


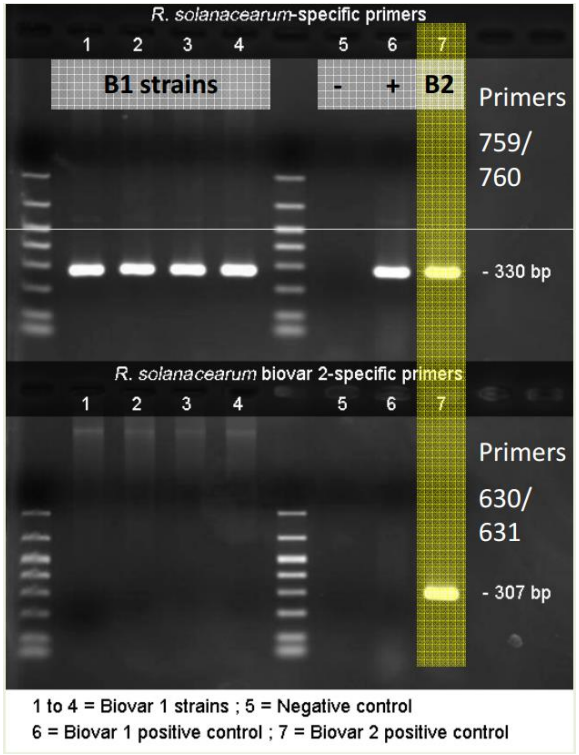
Ralstonia solanacearum

Detection, identification

- Step 4: Identification of the biovar

Biovar test





Ralstonia solanacearum – sub species

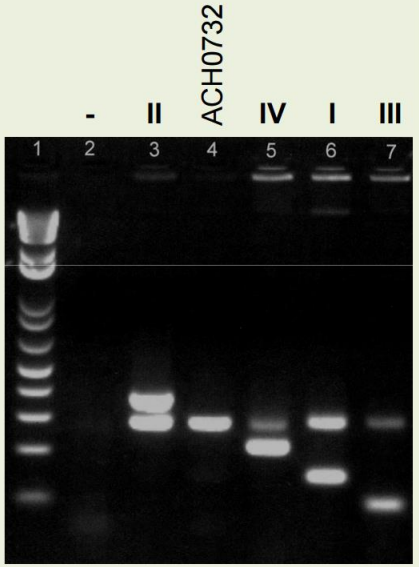
Detection, identification

- Step 4: Identification of phylotype/sequevar

Sub-species level

Multiplex PCR (Fegan and Prior, 2005)

PCR-Phylotype		
Nmult:21:1F	CGTTGATGAGGCGCGCAATTT	Phylotype I (Asiaticum)
Nmult:21:2F	AAGTTATGGACGGTGGAAGTC	Phylotype II (Americanum)
Nmult:22:InF	ATTGCCAAGACGAGAGAAGTA	Phylotype IV (tropical)
Nmult:23:AF	ATTACGAGAGCAATCGAAAGATT	Phylotype III (Africain)
Nmult:22:RR	TCGCTTGACCCTATAACGAGTA	Amorce reverse unique
759R	GTCGCCGTCAACTCACTTTCC	Marqueur interne
760F	GTCGCCGTCAGCAATGCGGAATCG	*R. solanacearum*

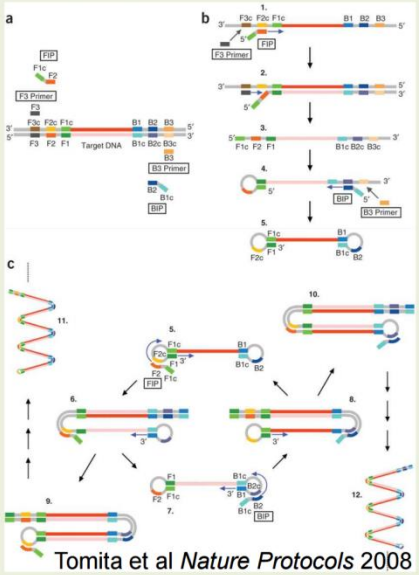


Sequevar: sequencing and analysis of partial endoglucanase (*egl*) gene

Ralstonia solanacearum – new methods

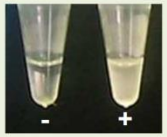
Coming diagnostic tools

- LAMP: Loop-mediated isothermal AMPlification



Tomita et al *Nature Protocols* 2008

- **A simple rapid PCR-like way to generate large quantities of DNA**
- Highly specific (3 primer sets)
- Only need 2 temperatures
- No thermocycler required



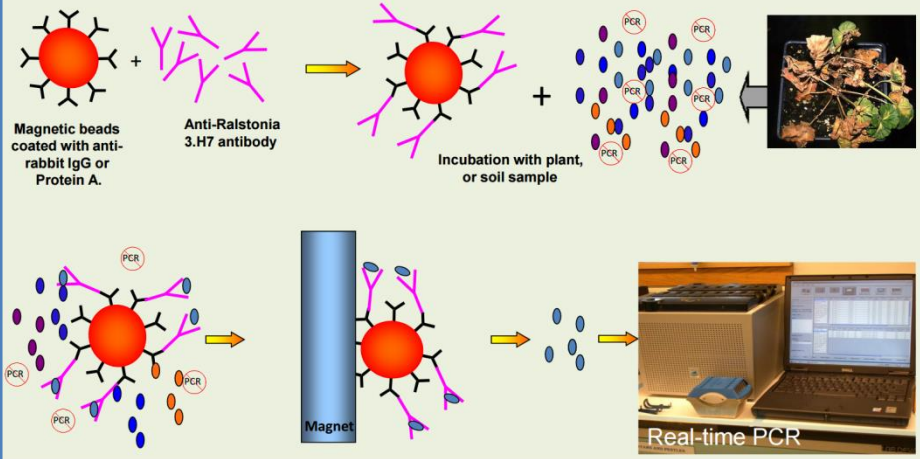
Rapid Visual Assessment

- Sensitive to PCR inhibitors

Coming diagnostic tools

- ImmunoMagnetic Separation (IMS):

Use magnetic beads coated with Ab 3.H7 to simply and easily purify plant or soil samples from other bacteria and PCR inhibitors.



http://plantpath.ifas.ufl.edu/rsol/

Web Links for Further Information

APHIS-PPQ Fact Sheet on *Ralstonia solanacerarum Race 3 Biovar 2*
http://www.aphis.usda.gov/publications/plant_health/content/printable_version/faq_phraston.pdf
Article: Southern Bacterial Wilt caused by *Ralstonia solanacearum*, Margery Daughtry
http://www.greenhouse.cornell.edu/pestsdis/MD.SAFtalk.pdf
EPPO Data Sheets on Quarantine Pests: *Ralstonia solanacearum* Race 3 Biovar 2
http://www.eppo.org/QUARANTINE/bacteria/Ralstonia_solanacearum/PSDMSO_ds.pdf
Bacterial Wilt, Massachusetts Introduced Pests Outreach Project
http://massnrc.org/pests/pestFAQsheets/ralstonia.html
Pictorial Guide to Geranium Wilt Disorders, NCSU
http://www.ces.ncsu.edu/depts/hort/floriculture/GeraniumWilt.pdf
Article: It Pays to Know the Difference, Dr. S.G.P. Nameth, Ohio State University
http://floriculture.osu.edu/archive/apr02/Bacterial.html
NCIPM *Ralstonia solanacearum* Race 3 Biovar 2 Pest Alert
http://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/ralstoniapstalert.pdf