Diagnosing Plant Diseases Caused by Bacteria-Classical Methods

IPDN Diagnostics Training Workshop

March 5-9, 2007

KARI-NARL,Nairobi

Sally Miller

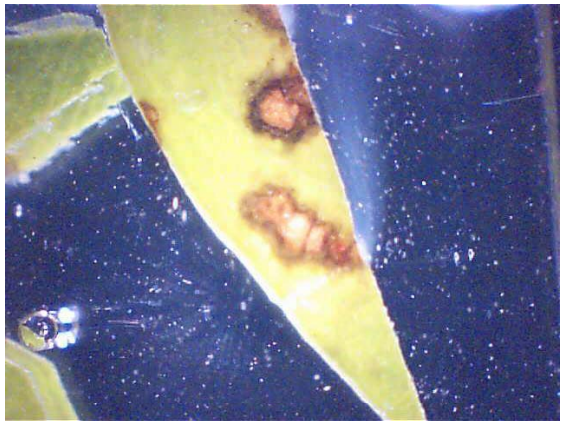
THE OHIO STATE UNIVERSITY

Diagnostic Signs and Symptoms

- Bacterial ooze
- Bacterial streaming
- Odor

- Wilting
- Cankers
- Soft tissue
- Spots
- Watersoaking
- Halos -chlorotic

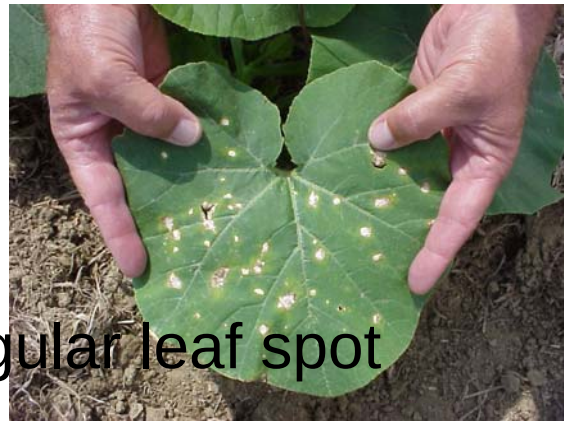
Bacterial Streaming



- First diagnostic test when bacterial disease is suspected
- Diagnostic for bacterial diseases as a group
- Some crops/diseases require experience for accurate use

Symptoms of Bacterial Diseases

Canker - fire blight



Angular leaf spot

Bacterial wilt

Cabbage soft rot

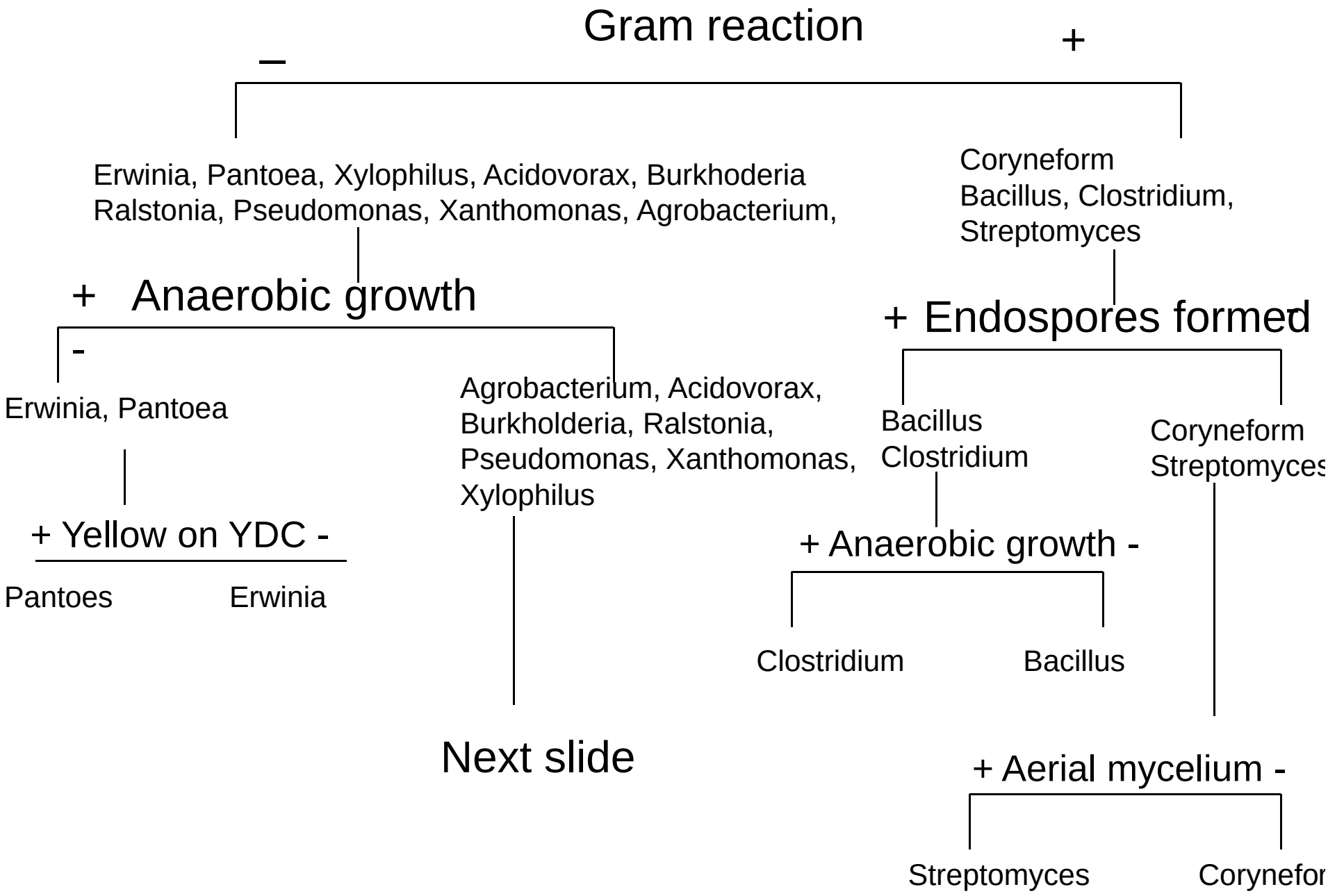
Identification of Common Genera of Bacteria Infecting Plants

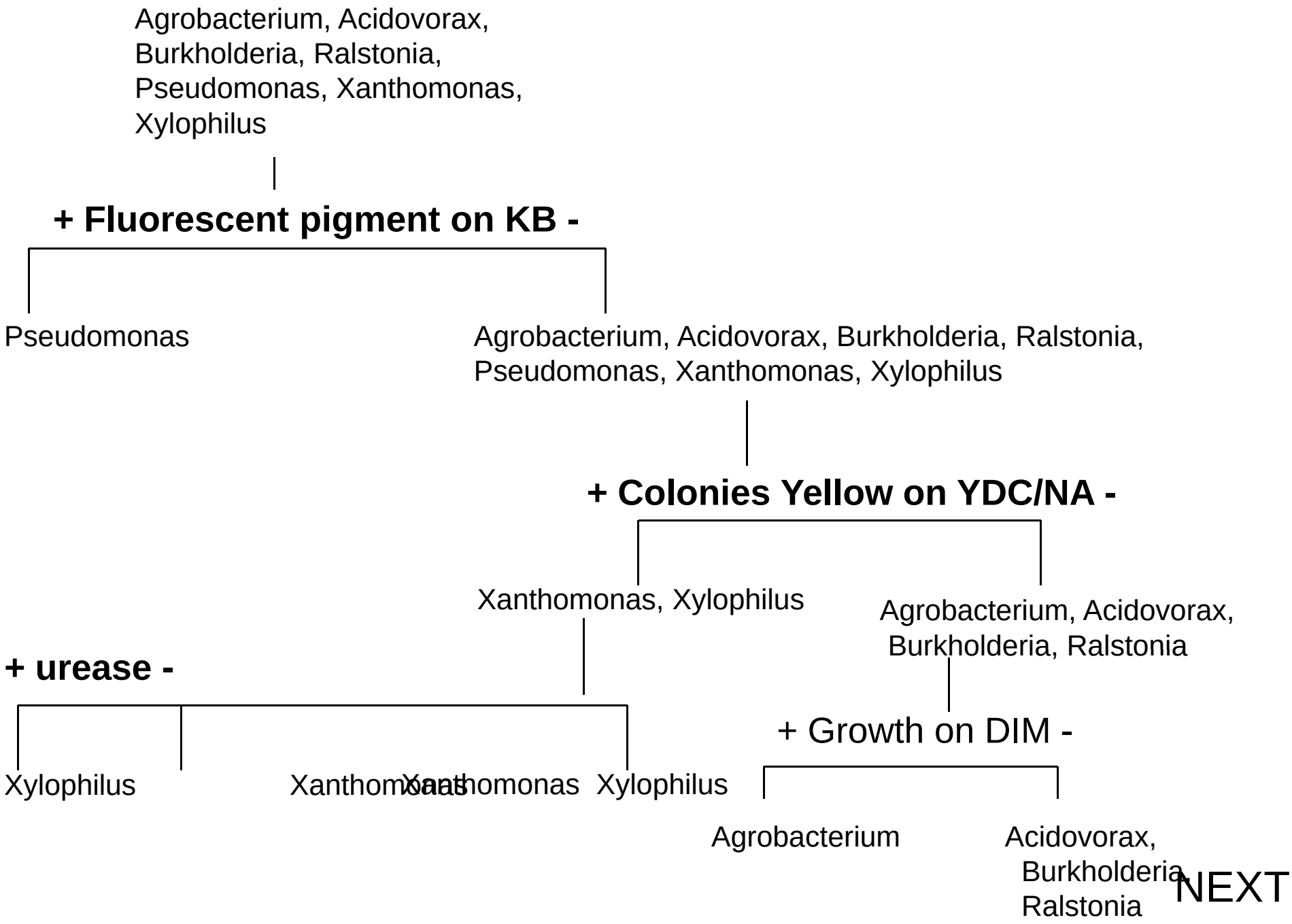
Sample

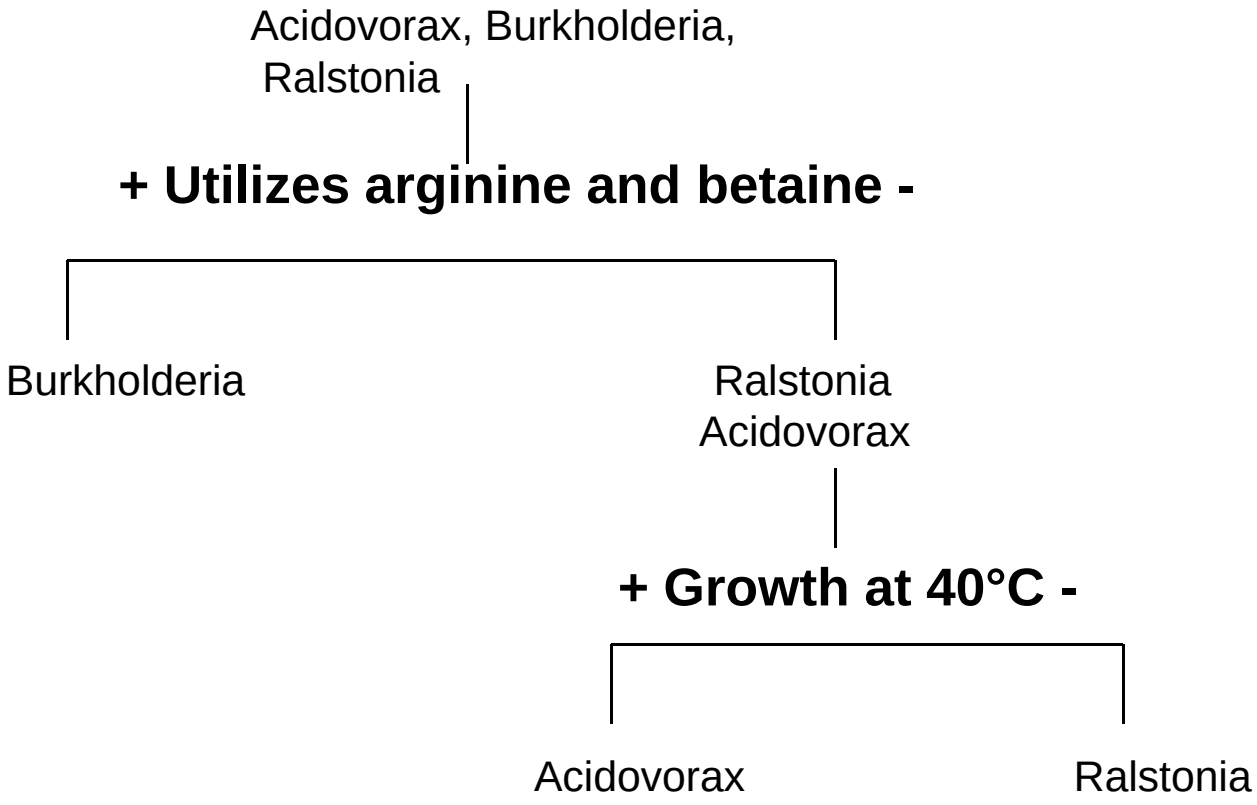
Easily Culturable		**Non-culturable or w/ difficulty**
Erwinia	*Xylophilus*	*Rhizomonas*
Pantoea	*Agrobacterium*	*Xylella*
Acidovorax	*Clavibacter*	*Spiroplasma*
Pseudomonas	*Clostridium*	*Phytoplasma*
Ralstonia	*Bacillus*	*Phloem-limited*
Burkholderia	*Streptomyces*	
Xanthomonas		

Characters differentiating fastidious and non-culturable plant pathogenic bacteria

Character	Rhizo-monas	Xylella	Spiro-plasma	Phyto-plasma	Phloem-limited
Growth on S-medium	+	-	-	-	-
Growth on PW agar	-	+	-	-	-
Possess cell wall	+	+	-	-	+
Growth on serum agar	-	-	+	-	-
Helical morphology	-	-	+	-	-


Gram reaction
–
+
Erwinia, Pantoea, Xylophilus, Acidovorax, Burkhoderia
Ralstonia, Pseudomonas, Xanthomonas, Agrobacterium,
Coryneform
Bacillus, Clostridium,
Streptomyces
+ Anaerobic growth
-
+ Endospores formed
-
Erwinia, Pantoea
Agrobacterium, Acidovorax,
Burkholderia, Ralstonia,
Pseudomonas, Xanthomonas,
Xylophilus
Bacillus
Clostridium
Coryneform
Streptomyces
+ Yellow on YDC -
Pantoes
Erwinia
+ Anaerobic growth -
Clostridium
Bacillus
Next slide
+ Aerial mycelium -
Streptomyces
Corynefor

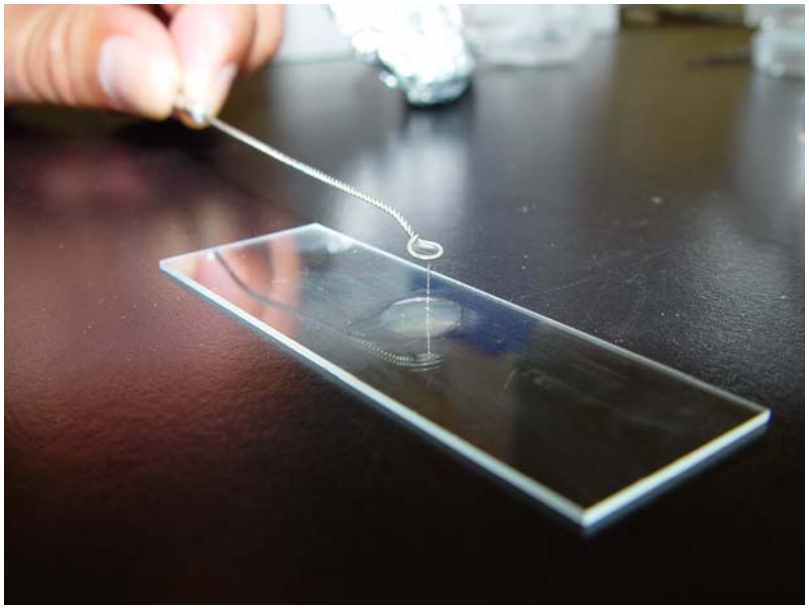

Agrobacterium, Acidovorax, Burkholderia, Ralstonia, Pseudomonas, Xanthomonas, Xylophilus
+ Fluorescent pigment on KB -
Pseudomonas
Agrobacterium, Acidovorax, Burkholderia, Ralstonia, Pseudomonas, Xanthomonas, Xylophilus
+ Colonies Yellow on YDC/NA -
Xanthomonas, Xylophilus
Agrobacterium, Acidovorax, Burkholderia, Ralstonia
+ urease -
+ Growth on DIM -
Xylophilus
Xanthomonas
Xanthomonas
Xylophilus
Agrobacterium
Acidovorax, Burkholderia, Ralstonia
NEXT


Acidovorax, Burkholderia, Ralstonia
+ Utilizes arginine and betaine -
Burkholderia
Ralstonia
Acidovorax
+ Growth at 40°C -
Acidovorax
Ralstonia

Key Tests in Identification of Easily Culturable Bacteria

- Gram reaction (stain or KOH)
  - Use rapidly-growing bacteria
- Anaerobic test
  - For identification of *Erwinia*, *Pantoea* and *Clostridium*
- Presence of spores
  - For *Bacillus*, *Clostridium*

Gram reaction -KOH test



Gram negative reaction

- A few drops of 3% KOH onto slide
- Pick 24-48 hr colony with loop; stir into KOH 5-10 sec
- Pull up loop
- Gram - : viscous slime
  - Gram rxn:red
- Gram + : no slime
  - Gram rxn: purple-black

Key Tests in Identification of Easily Culturable Bacteria

- For *Acidovorax*, *Burkholderia*, *Ralstonia*, *Pseudomonas:*
  - Colony morphology
  - Pigmentation
  - Growth at 40C
  - Oxidase reaction

Critical Points

- Include known cultures as positive and negative controls
- Use purified cultures - clone at least twice by streaking from well separated colony onto a non-selective agar medium
  - Allow culture to grow several days to rule out contamination

Isolation of Bacterial Pathogens

- Use several different media for unknowns
  - YDC, NBY, NA, NDA, King's B
  - Bacteria vary in robustness of growth on these media
  - Water agar if *Streptomyces* is expected
- Streak known cultures at the same time
- Wash tissue containing lesions in sterile water or surface sterilize in 10% Clorox for 3 min., followed by sterile water rinse.
- Chop tissue in a drop of sterile water; incubate several minutes, then streak plates

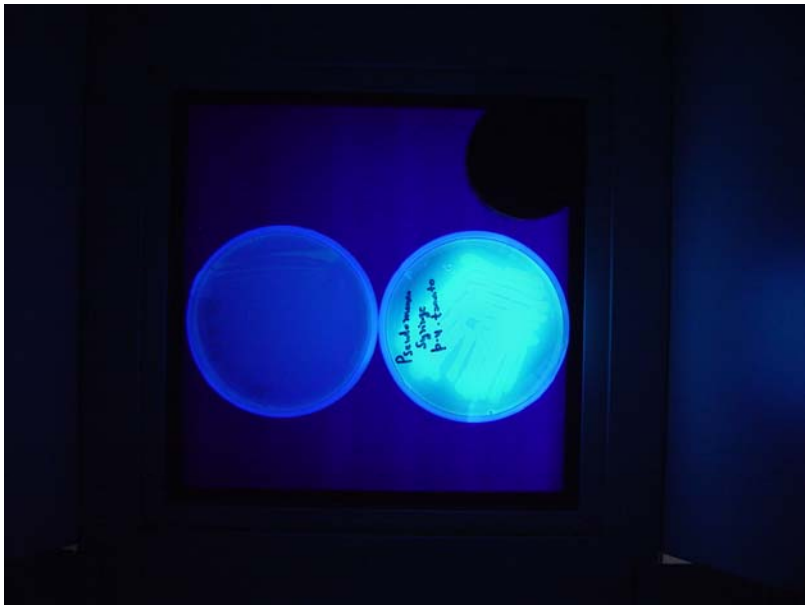
Non-culturable or Not-easily-culturable Bacteria

- Determine if anaerobic (*Clostridium*)

- Rely on other techniques
  - Dienes' stain (phloem limited bacteria e.g. phytoplasmas)
  - Microscopy
  - PCR

What are Selective Media?

- Media that
  - Selectively enhance growth of certain bacteria
    - Nutrient sources
  - Selectively inhibit growth of bacteria, fungi
    - Additives
  - Distinguish bacteria based on specific morphology, color, reactions in media, etc.
    - Fluorescent pigments
    - Pectin, tween, citrate utilization, etc.



- Media are only semi-selective
- Use known controls
- Use general media at the same time

Plant tests

- Hypersensitivity test
  - Tobacco
  - Four O-Clock
- Bacteria with Hrp genes cause HR; saprophytes do not
- 10^{8-9} CFU/ml
- Gently force bacteria into intercellular spaces using syringe

- Soft rot (potato slice)

Erwinia and *Pantoea*

- Straight rods, G-, peritrichous flagella; facultative anaerobes, oxidase negative, catalase positive
- Soft rot “carotovora” group
  - *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, *E*. *chrysanthemi*, etc.
- Yellow-pigmented “herbicola” group
  - *Pantoea herbicola*, *P. agglomerans, P, ananas, P. stewartii, etc.*
- “amylovora” group
  - *E. amylovora, E. tracheiphila,* etc.

Pseudomonas

- G-, straight or curved rods, motile by 1-several polar flagella, strict aerobes, catalase positive
- **Produce fluorescent pigments** (except *P. corrugata*)
- Diverse symptoms:
  - *P. syringae* pvs:cankers, diebacks, blossom, leaf, twig or kernel blight, leaf spots
  - *P. viridiflava, P. marginalis*: soft or brown rots
  - *P. savastanoi*: tumors or galls

Xanthomonas

- Yellow pigmented (xanthomonadin)
- G-, aerobic, rods, oxidase negative, catalase positive, 1 polar flagellum
- Over 100 pathovars identified
- Taxonomy in flux

Agrobacterium

- Soil-borne bacteria that infect a wide range of dicots
- Some strains incite gall or tumor formation
- All pathogenic strains contain a tumor-inducing (Ti) or root-inducing (Ri) plasmid
- G-, aerobic, rod-shaped, motile by 1-6 peritrichous flagella, slimy on nutrient-rich media

Coryneform Plant Pathogens

- G+, pigmentation of colonies yellow to orange on rich media, motile, coryneform
- Limited range of pathogenicity
- *Clavibacter*
- *Curtobacterium*
- *Rhodococcus*
- *Arthrobacter*

Recommended Reference

- Schaad, N. W., Jones, J. B and Chun, W. 2001. Laboratory Guide for the Identification of Plant Pathogenic Bacteria (3rd ed.). St. Paul, MN USA:APS Press