Seed borne pathogens
Bacteria

- General characteristics

Prokaryotic vs. Eukaryotic Organisms

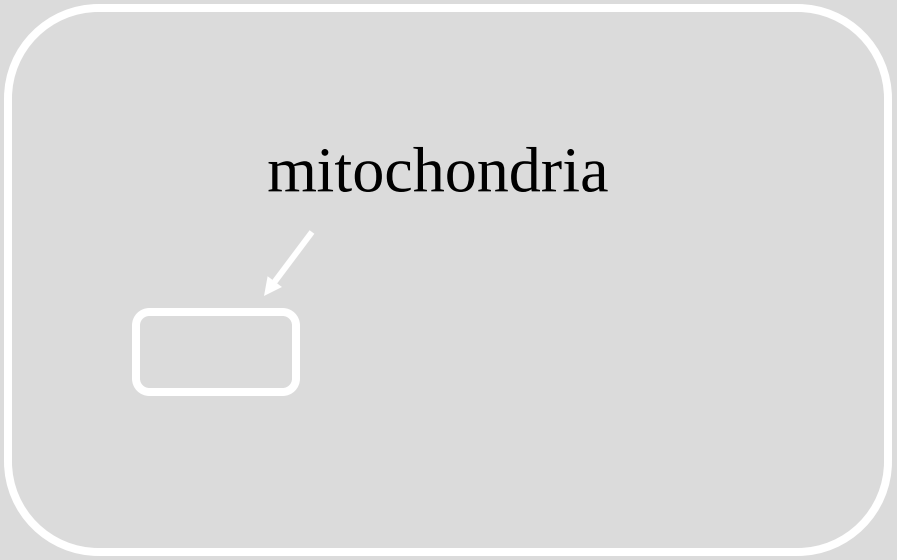
Prokaryotic

- **Single cell organisms**
- **Cell membrane**
- **Cell wall; + or -**
- **Cytoplasm**
  - **Usually 1 chromosome**
  - **Small ribosomes**
  - **plasmids**
- **No organelles**

Eukaryotic

- **One or more cells**
- **Cell membrane**
- **Cell wall**
- **Cytoplasm**
- **Membrane bound organelles**
  - Mitochondria
  - Nucleus
  - (chloroplast)

Size

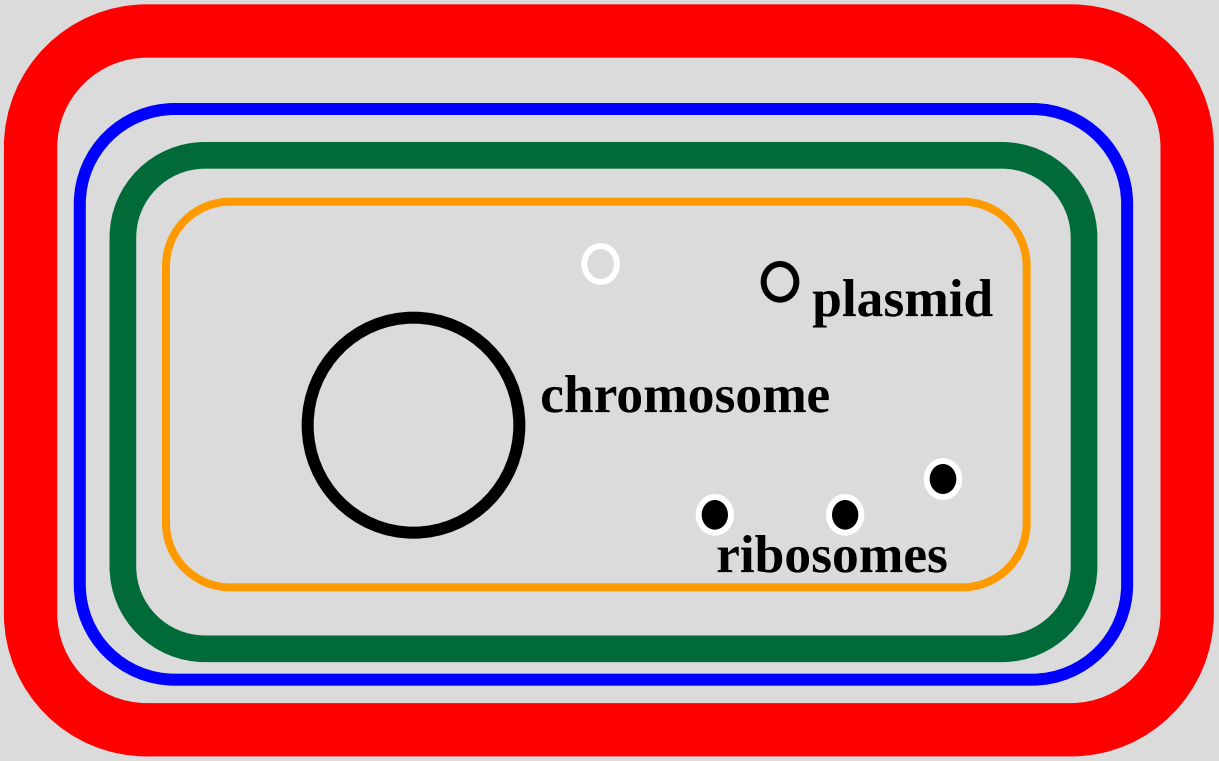


Eukaryotic cell

Prokaryotic cell

Plant Pathogenic Prokaryotes

- **Bacteria**
  - **Gram negative**
    - **12 genera; 6 are common**
  - **Gram positive**
    - **6 genera; 2 are common**
    - **1 genus; filamentous**
- **Mollicutes**
  - **Phytoplasma**
  - **Spiroplasma**

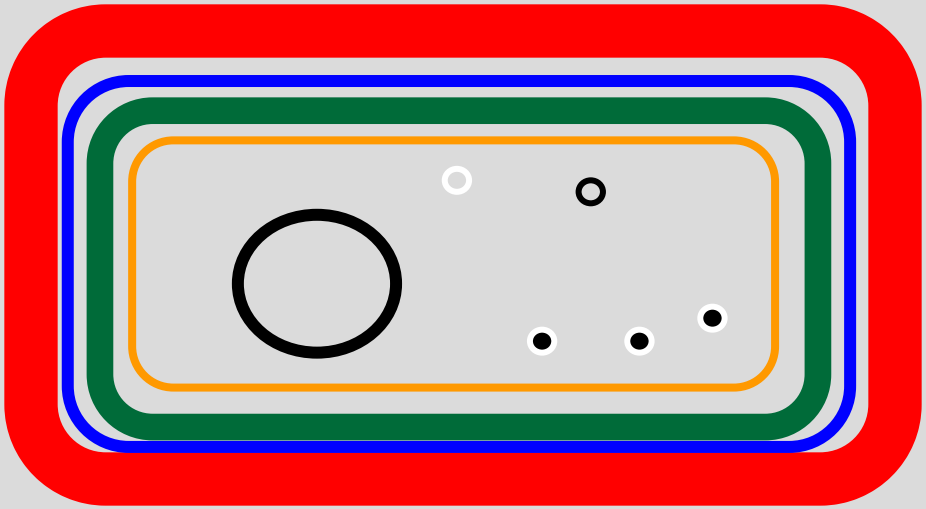


Inner membrane

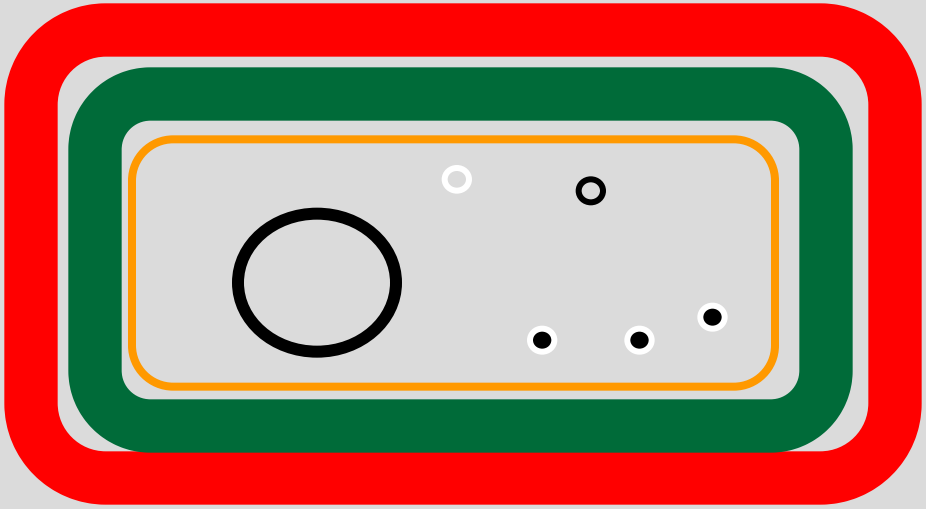
Peptidoglycan layer — Thin gram -, thick gram +

Outer membrane and lipopolysaccharide, LPS — gram -

Capsule and extracellular polysaccharide



gram -

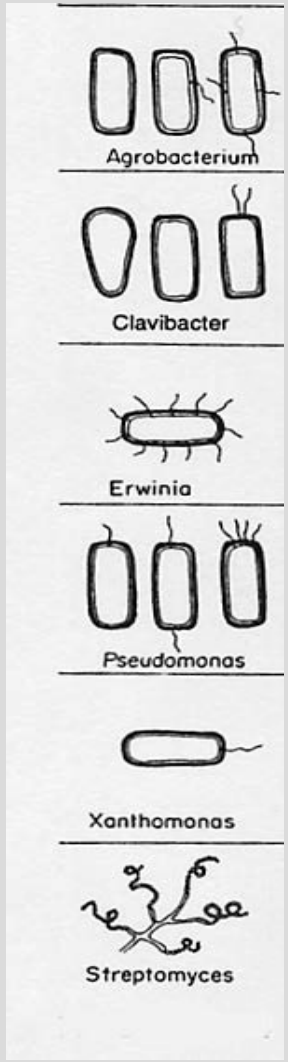


gram +

Plant Pathogenic Bacteria

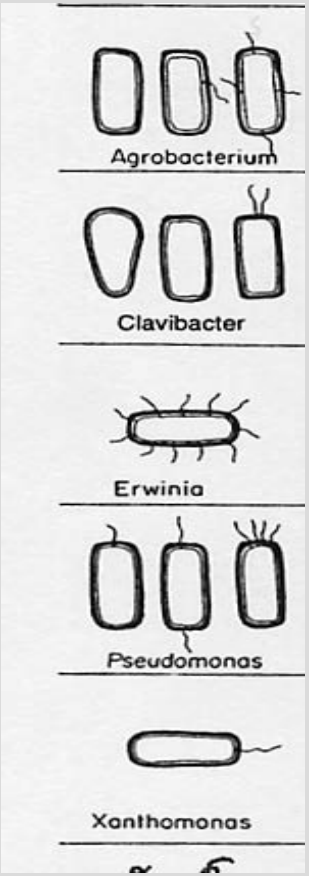
- **Gram negative**
  - ***Pantoea***
  - ***Pectobacterium***
  - ***Erwinia***
  - ***Pseudomonas***
  - ***Ralstonia***
  - ***Xanthomonas***
  - ***Agrobacterium***
  - ***Xylella***
- **Gram positive**
  - ***Clavibacter***
  - ***Streptomyces***

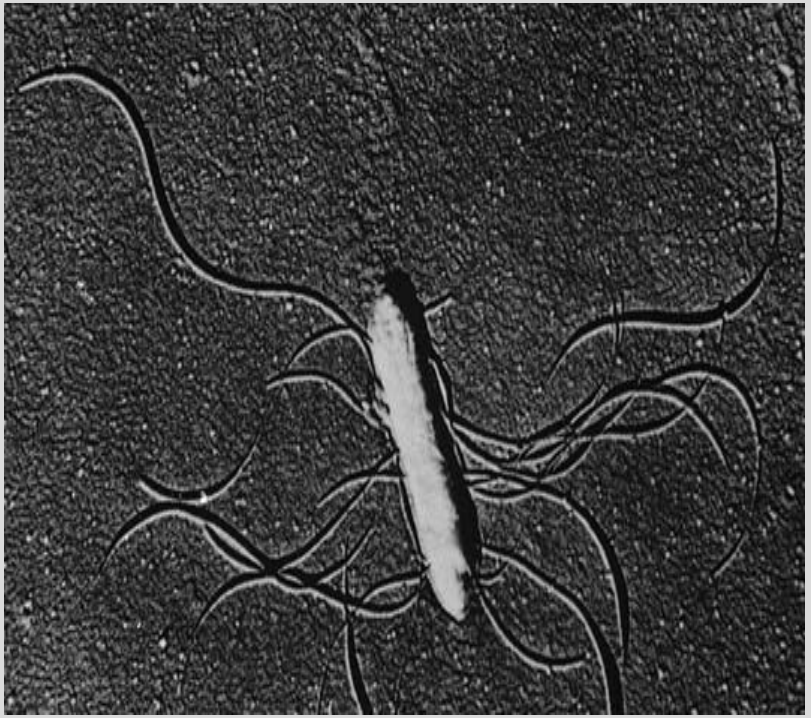
- **Shape**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Rod**
  - **Filamentous**



Flagella on Rod Shaped Bacteria

- **Most have one to several flagella**





Reproduction

- **Rod-shaped bacteria**
  - **Binary fission**
- **Filamentous bacteria**
  - **Cells consist of branched threads**
  - **Produce conidia**

Symptoms

- Blights and Spots
  - *Pseudomonas*, *Xanthomonas*, *Erwinia*
- Cankers
  - *Xanthomonas*
- Wilts, vascular diseases
  - *Ralstonia*, *Pantoea*, *Clavibacter*
- Soft rots
  - *Pectobacterium*
- Gall diseases
  - *Agrobacterium*, *Pseudomonas*, *Clavibacter*
- Scab
  - *Streptomyces*

Leaf Blights: *Pseudomonas* & *Xanthomonas*

- **Chlorosis**
- **Lesions**
- **Necrosis**
- **Halo from excreted toxin if** *Pseudomonas*

Bacterial Spot on Tomato

Xanthomonas campestris pv. vesicatoria

Wilts

- **Vascular diseases**
  - ***Pantoea***
  - ***Clavibacter***
  - ***Ralstonia***

Cankers: Citrus canker *Xanthomonas*

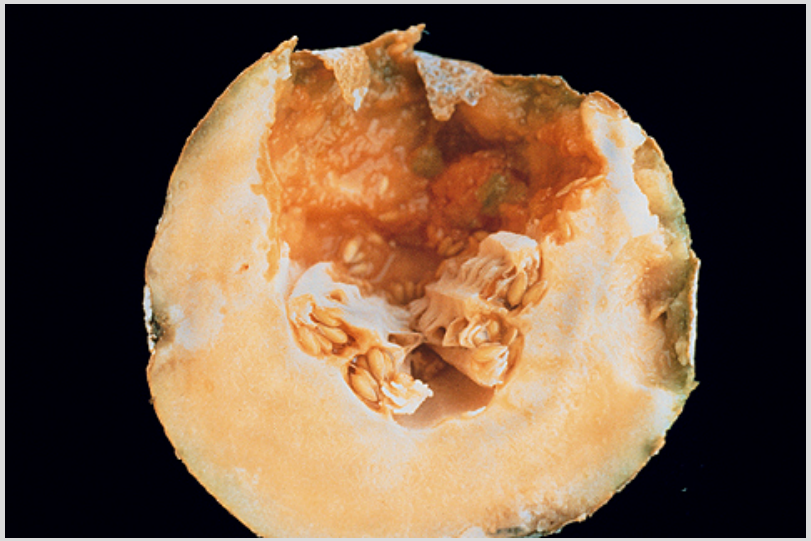
Potato Scab Caused by an Actinomycete: *Streptomyces*

Pectobacterium diseases

- *Pectobacterium carotovorum*,
- *P. chrysanthemi*
  - Soft rots, stem rots

Soft Rot: *Pectobacterium*

- **Major problem in pre- and post harvest vegetables and fruits**
- ***P** carotovorum, P. chrysanthemi*
- **Active pectolytic enzyme system**
- **Degrade pectin between cells**



Important seed borne bacteria

Pseudomonas savastanoi pv. *phaseolicola*

- **Incidence**
- The halo blight pathogen is extensively seed-borne in beans. Of eight test samples of bean (Phaseolus vulgaris) seeds from Germany, six contained seed infected with P. savastanoi pv. phaseolicola. Routine laboratory testing for the presence of P. savastanoi pv. phaseolicola, using immunofluorescence, indicated that 27.5% of 710 bean seed lots of various origins, were infected with the pathogen (Vuurde et al., 1991).

Halo Blight: *Pseudomonas savastanoi* pv *phaseolicola*

- Very similar to Common Blight by X.c. pv phaseoli
- Halolike zone of greenish yellow forms around lesion
- Exudate yellow for *Xanthomonas*, light cream for *Pseudomonas*

Xanthomonas axonoponis pv. *phaseoli*

- **Incidence**

- X. axonopodis pv. phaseoli is extensively seedborne on Phaseolus spp. A survey of navy bean (P. vulgaris) seeds showed that approximately 35% had internal contamination with X. axonopodis pv. phaseoli (Saettler and Perry, 1972). Incidences of seed infection by X. axonopodis pv. phaseoli as high as 16.1%, with populations of the bacterium averaging between 100,000 and 1,000,000,000 c.f.u./100 seeds, have recently been recorded in farmers commercial and research seeds in Africa (Opio et al., 1993), when approximately 1 in 10,000 seeds is capable of causing an outbreak of blight (Sutton and Wallen, 1970)

Common Blight Bean *Xanthomonas axonoponis* pv. *phaseoli*

- Host range - bean
- Source of inoculum
  - Seed
  - Host residue
- Primary inoculum
- Dissemination
  - Wind & water
- Environment
  - Hot & humid

Xanthomonas campestris pv. *campestris*

- **Incidence**
- Seeds are considered to be the most important source of inoculum of Xcc on cabbage and other cruciferous crops (Russel, 1898; Walker, 1952; Randhawa and Schaad, 1984; Schultz and Gabrielson, 1986). Seed infection usually varies from 0.25 to 80% (Shrestha et al., 1977; Schaad, 1982; Mariano et al., 1985; Shakya and Malla, 1988; Shen and Chen, 1990; Sharma et al., 1992; Shiomi, 1992; Kobayashi et al., 1994; Gaetan et al., 1995).

pv = pathovar, designates host specificitiy of a strain of bacterium.

Xanthomonas campestris* pv. *phaseoli

Pathogenesis

- **Dissemination**
  - **Water**
  - **Wind**
  - **Insect vectors**
  - **Seed borne**
  - **Mechanical**
  -

Survival

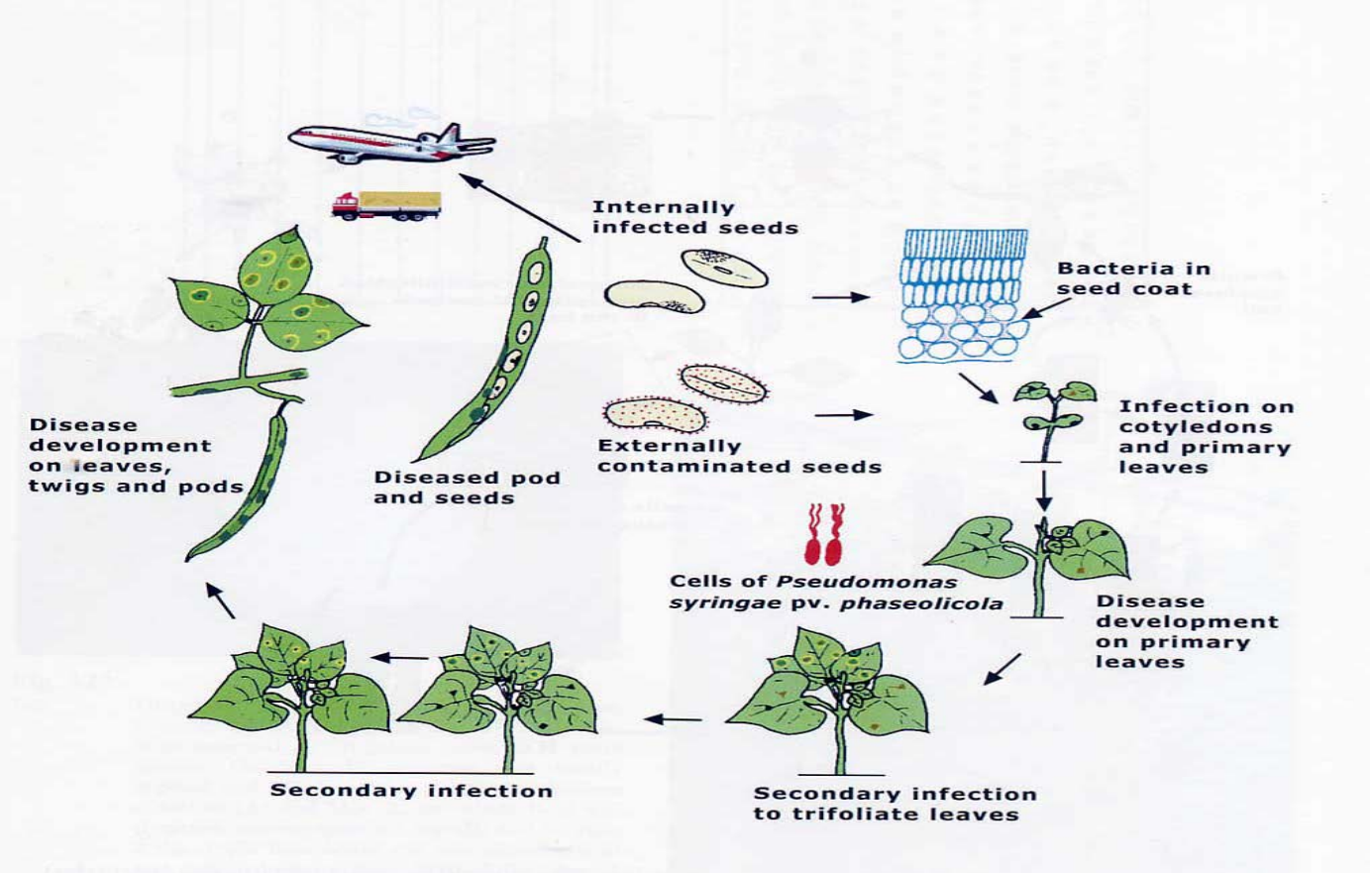
- **in plant debris**
- **in rhizosphere**
- **epiphytes**
- **in insects**
- **alternate hosts**
- **in seed**

Cannot invade directly

- **Natural openings – stomates, hydathodes and lenticels**
- **Wounds - mechanical, insect, nematodes**

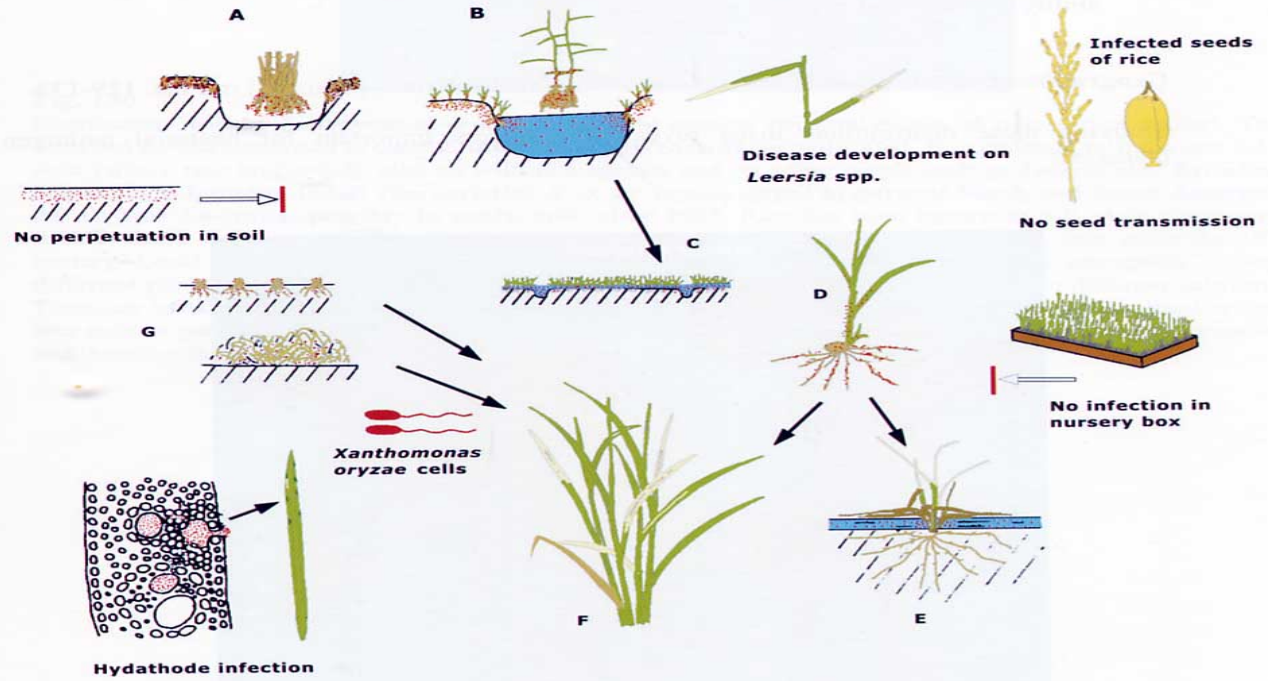
Detection of seed-borne bacteria

- Extraction
- Purification
- Identification
  - Pathogenicity test
  - Biochemical test
  - Serological test



Pseudomonas savastanoi pv. *phaseolicola*

DISEASE CYCLE OF BACTERIAL LEAF BLIGHT (*XANTHOMONAS ORYZAE* PV. *ORYZAE*) IN RICE (*ORYZA SATIVA*) IN JAPAN



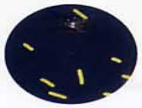
KOCH'S POSTULATES IN BROWN ROT DIAGNOSIS

Diseased plant

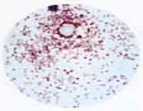
Healthy plant

1 Screening test

Positive: suspected bacterial cells



IF test



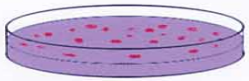
Sudan Black stain

PCR

Negative: no bacterial cells

No PCR band

2 Isolation on selective medium SMSA



Typical colonies

No (typical) colonies

Pure culture

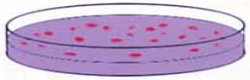
Identification by fatty acid analysis
and/or other biochemical/molecular tests

Pure culture, similar to
that used for inoculation

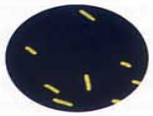
4 Reisolation

3 Pathogenicity test on tomato

Diseased plant



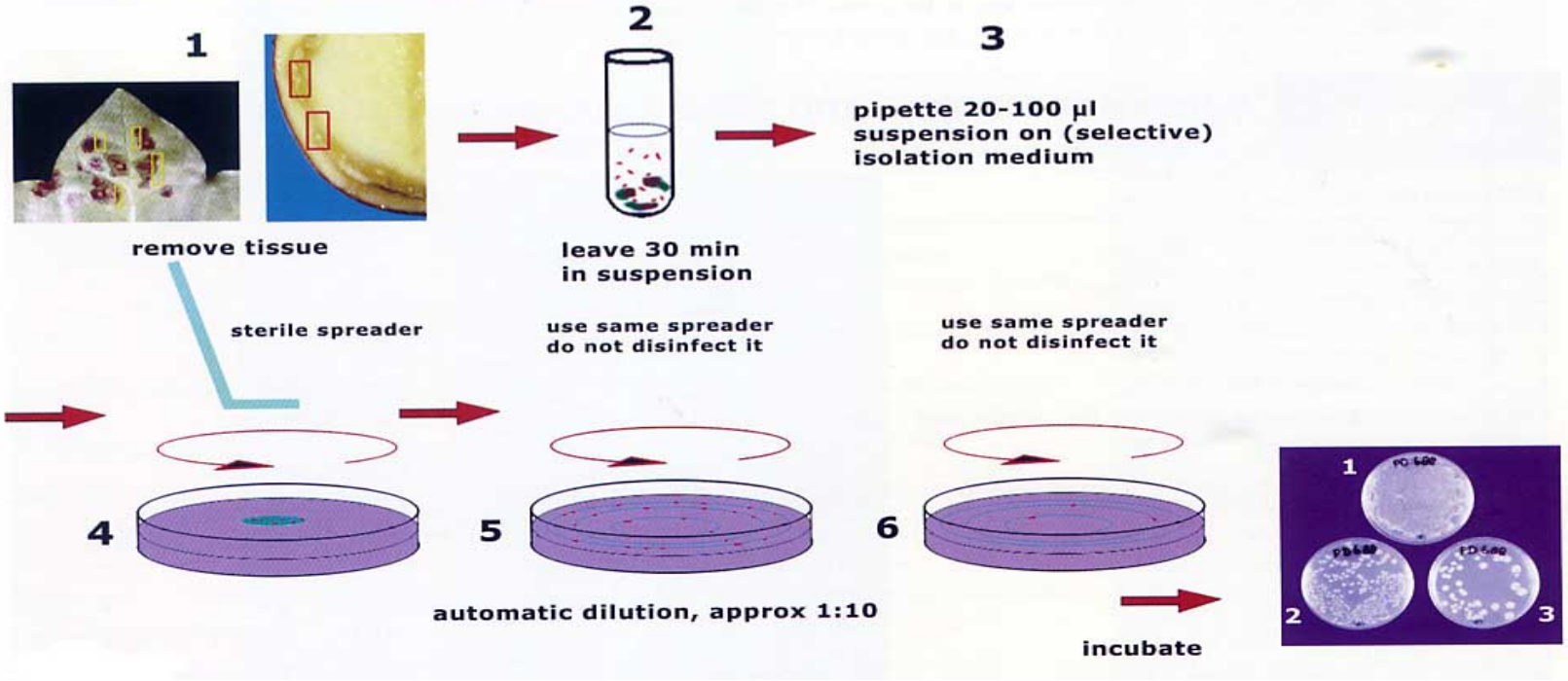
Typical colonies

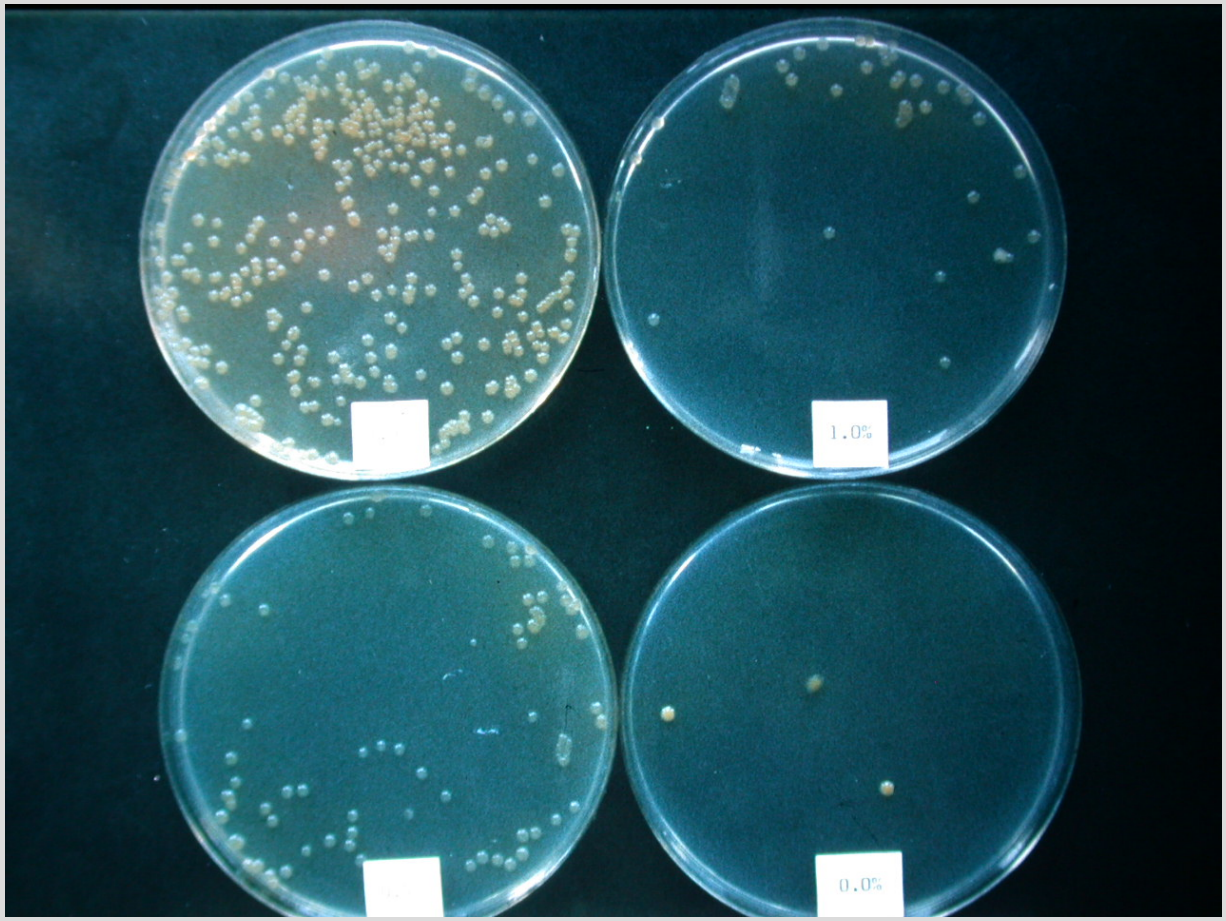


Screening of inoculated host plant

Final IF or PCR
test of reisolated
culture

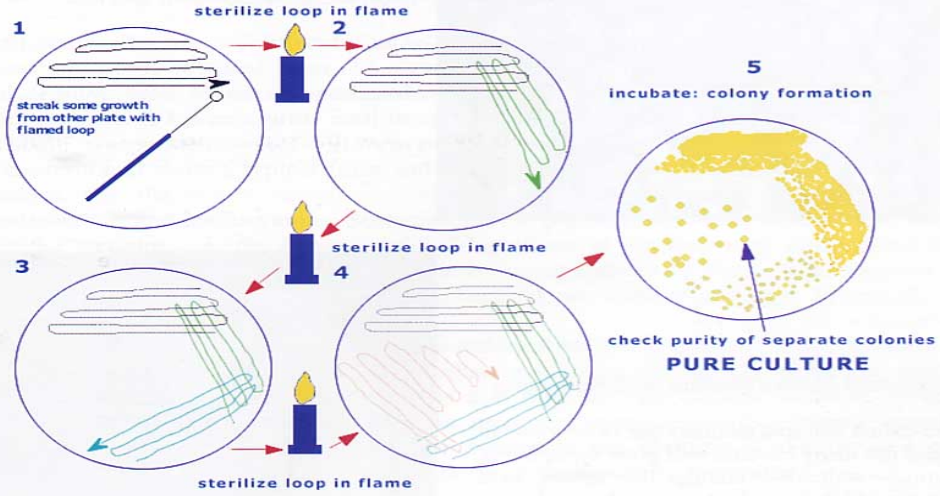
Isolation of bacteria from borderline of healthy and diseased tissue after short disinfection of external tissue





Dilution plate

BASIC TECHNIQUE OF OBTAINING PURE CULTURES IN BACTERIOLOGY

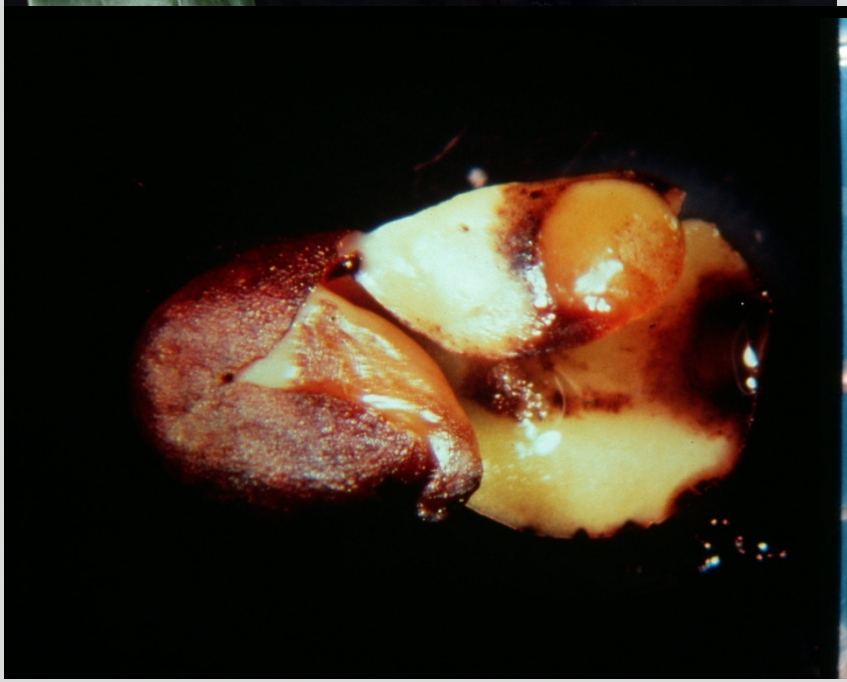


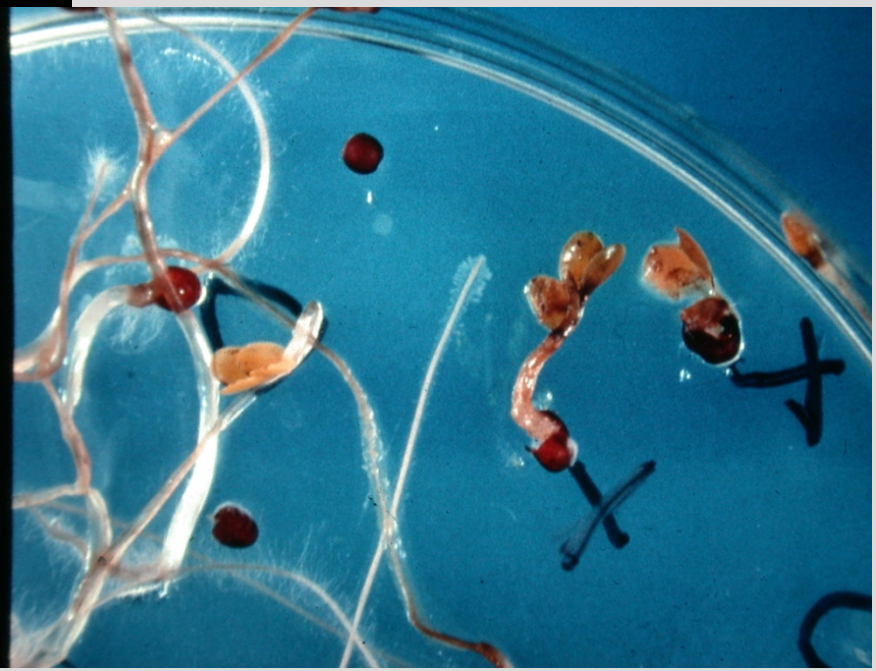
Top: Basic technique to obtain pure cultures.
Bottom: **Pure culture** of *Pseudomonas savastanoi* subsp. *fraxini*, after restreaking of a colony from an isolation plate and 3 days' growth at 27°C on nutrient agar.

Hypersensitivity test on tobacco

Black rot of crucifer

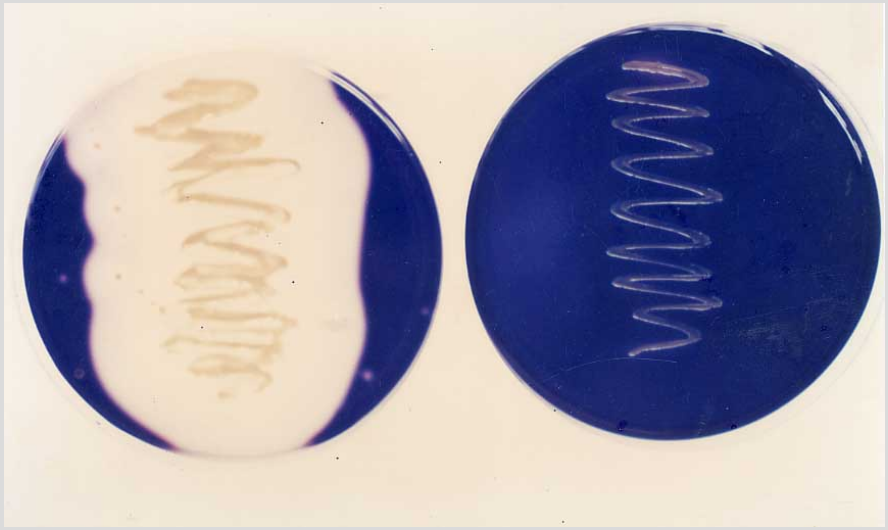
Xanthomonas campestris pv. *campestris*





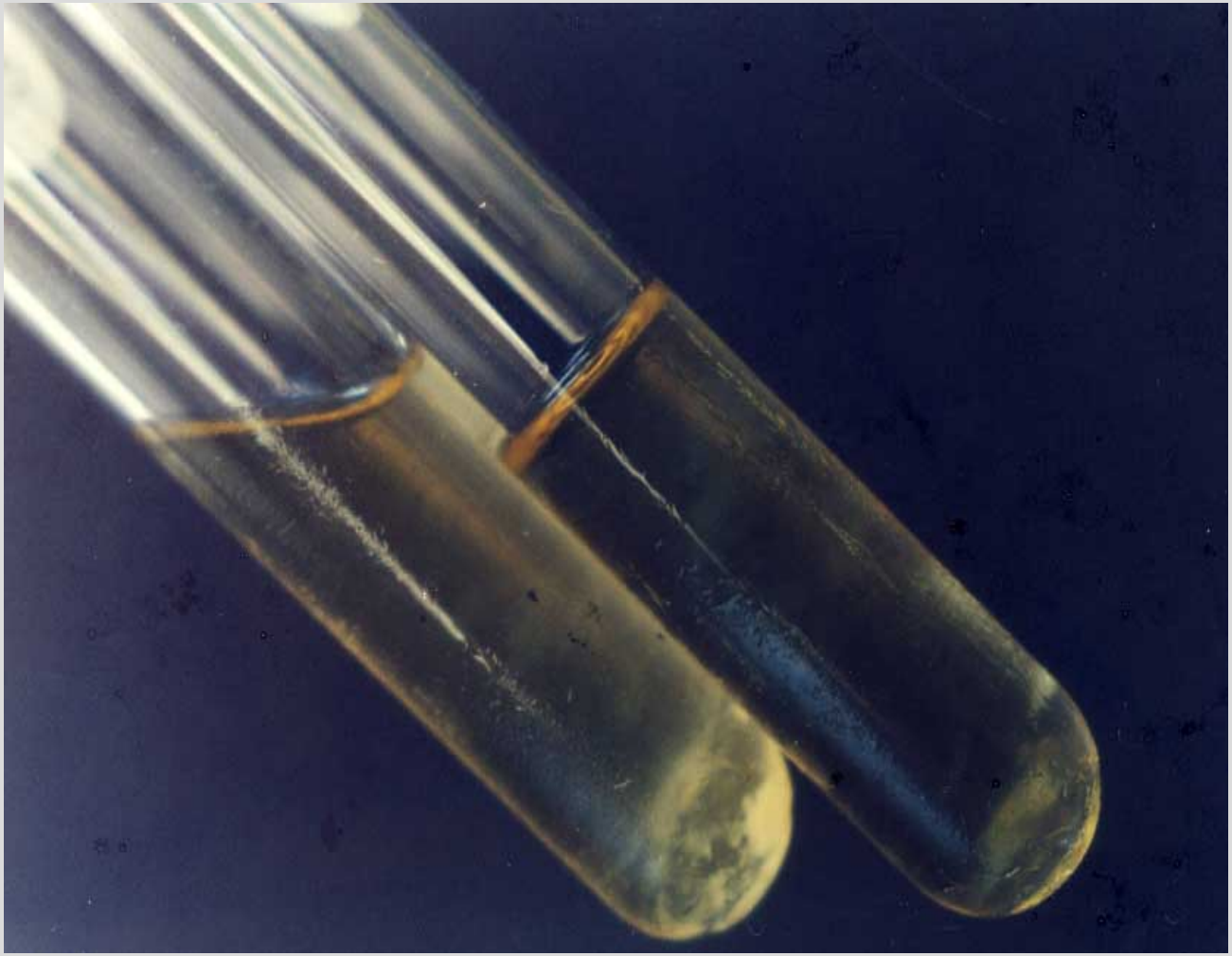
Levan formation

MXP medium

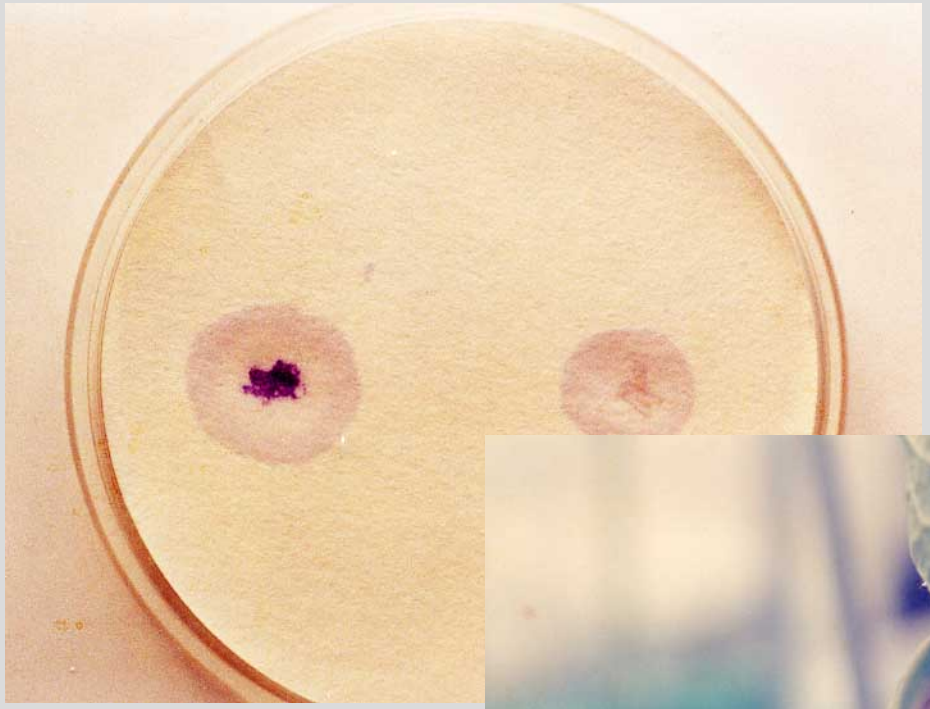


Starch hydrolysis

O/F test



Gelatin liquefaction



Oxidase test

Pathogenicity test

Cultural, physiological and biochemical characteristics of the species of the genus *Erwinia*

	E. amylovora	*E. tracheiphila*	*E. mallotivora*	*E. rubrifaciens*	*E. quercina*	*E. salicis*	*E. herbicola* (*P.l. agglomerans*)	*E. rhapontici*	*E. carotovora*	*E. chrysanthemi*	*E. cypripedii*	*E. nigrifluens*	*E. stewartii* (*P.l. stewartii*)	*E. uredovora* (*P.l. ananas*)
Motility	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Anaerobic growth	w	w	+	+	+	w	+	+	+	+	+	+	+	+
Growth factors required	+	+	+	-	+	-	-	-	-	-	-	-	-	-
Pink diffusible pigment	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Blue pigment	-	-	-	-	-	-	-	-	-	d	-	-	-	-
Yellow pigment	-	-	-	-	-	-	+	-	-	-	-	-	+	+
Mucoid growth	+	-	+	+	+	+	d	+	d	d	d	-	+	-
Symplasmata (cell aggregates)							d						-	d
Growth at 36°C	-	-	-	+	+	-	+	d	d	+	+	+	d	+
H_2S from cysteine	-	+	-	+	+	+	+	+	+	+	+	+	-	-
Reducing substances from sucrose	+	d	+	-	+	+	d	d	d	-	-	-	d	+
Acetoin	+	d	+	-	+	+	+	+	+	+	-	+	-	+
Urease	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Pectate degradation	-	-	+	-	+	-	-	+	+	-	-	-	-	-
Gluconate oxidation	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Gas from D-glucose	-	-	-	-	-	-	-	-	d	+	+	-	-	-
Casein hydrolysis	-	-	-	-	-	-	-	-	d	d	-	-	-	-
Growth in KCN broth	-	-	-	-	-	-	-	+	d	d	+	-	-	-
Cotton seed oil hydrolysis	-	-	-	-	-	-	-	d	d	d	+	-	-	-
Gelatine liquefaction	+	-	-	-	-	-	+	-	+	+	-	-	-	+
Phenylalanine deaminase	-	-	-	-	-	-	+	-	-	-	+	-	-	-
Indole test	-	-	-	-	-	-	-	-	-	+	-	-	-	+
Nitrate reduction	-	-	-	-	-	-	+	+	+	+	+	-	-	+
Growth in 5% NaCl		-	-				+	+	+	d	+		+	+
Deoxyribonuclease	-	-		-	-	-	-	-	-	-	-	-	-	+
Phosphatase			-					d	-	+	d			
Lecithinase								-	-	+	-			
Sensitivity to erythromycin (15 µg/disk)								+	-	+	+			

+ = positive; - = negative; d = doubtful; w = weak; 1 = *Pantoea*

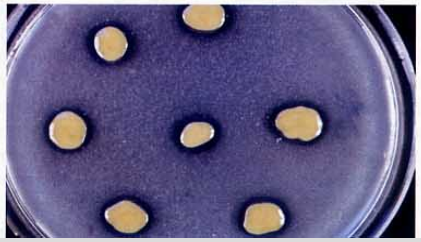


Fig. 39
Hydrolysis of fat, demonstrated on a medium containing tributyrin, seen as clear zones around colonies of *Xanthomonas axonopodis* pv. *begoniae* (7 days after inoculation).

Biochemical test

Test	Differential phenotypic characteristics of *Ralstonia solanacearum* and some related (plant pathogenic) bacteria						
	Ralstonia solanacearum	*Ralstonia pickettii*	*Ralstonia syzygii*	Blood Disease Bacterium	*Burkholderia cepacia*	*B. gladioli*	*B. caryophylli*
Colonies on TZC agar	fluidal, >5 mm		minute	viscid, <5 mm			
Diffusible pigment	+	-			+	+	+
Motility	+	-	-	-	+	+	+
Growth at 37°C	+	+	+	-			
Growth at 41°C	-	-			+	V	-
NaCl tolerance	<2%		<1%	<1.5%			
Oxidase	-	-	-	-	+	V	+
Arginine dihydrolase	-	-			-	-	+
Gelatin hydrolysis	-	-/W			+	+	-
Pectin hydrolysis	+	-			+	+	
Nitrate reduction	+/V	+	V	-	-/W	-	+
Oxidation of:							
Glucose	+	+	(+)	-	+	+	+
Sucrose	+	-	(+)	-	+	+	+
Galactose	+/V	+	+	-	+	+	-
Glycerol	+W	+	-	-	-	-	+W
Mannose	+/V	-	-	-	-	+	-
Ribose	+	-	-	-			
Utilization of:							
Cellobiose	V	-			+	+	V
Trehalose	+	-			+	+	+
D-tartrate	-	-/V			-/W	+	-
Mannitol	V	-			+	+	+
Sorbitol	V	-			+	+	+
N-propanol	+	-			+	-	+W
β-Alanine	V	+			-	-	-
Inositol	+	-			+	+	+
Betaine	-	-			+	+	+
L-arginine	-	-			+	+	+
L-lysine	-	-			+	+	V
Heptanoate	-	+			+	+	-
D-arabinose	-	-			+	+	+
D-fucose	-	+			+	+	+
D-raffinose	-	-			V	-	+
% C sources utilized	97		51	36			
Tobacco HR	+/inf.	-	V	+			
Plant pathogenicity	+	- Human	+Clove tree	+Banana	+ Also human	+	+ *Dianthus*

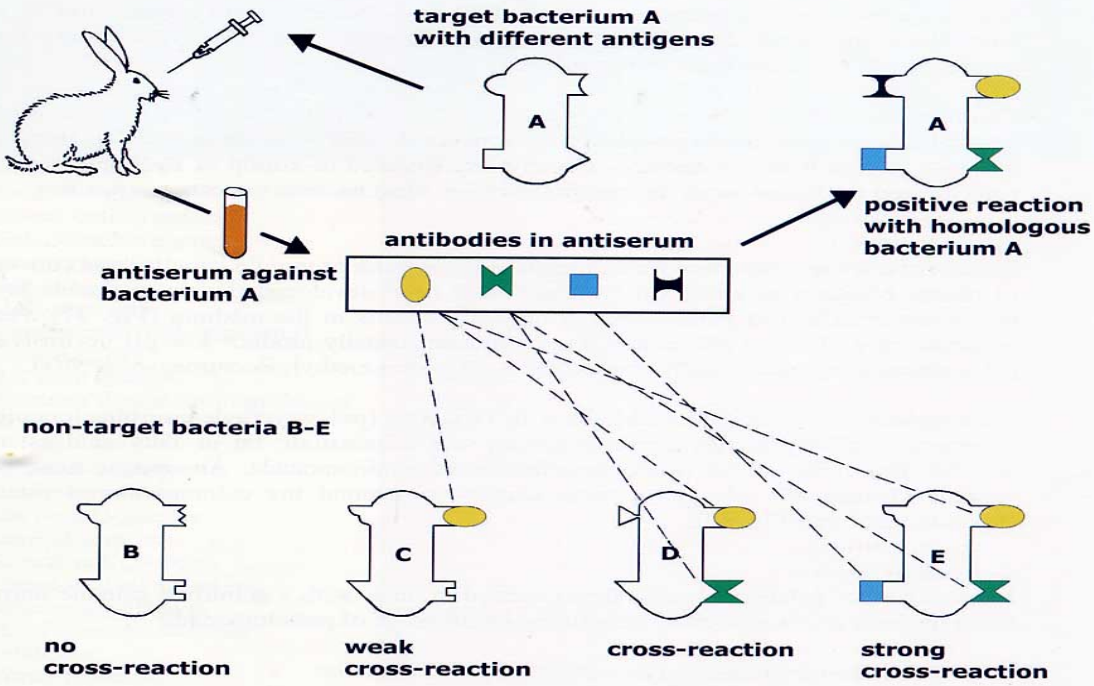
+ = positive; - = negative; V= variable; W = weak; inf. = infection; HR = hypersensitive reaction

Table 3

Some seed-borne and seed-transmitted plant pathogenic bacteria

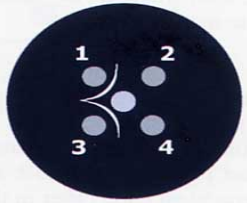
Bacterium	Main host(s)	Disease
Acidovorax avenae pv. *avenae*	oat, rice	bacterial blight and brown stripe
Acidovorax avenae subsp. *citrulli*	watermelon (*Citrullus lanatus*)	bacterial fruit blotch
Burkholderia glumae	rice	bacterial grain rot of rice
Clavibacter michiganensis subsp. *michiganensis*	tomato	bacterial canker
C. m. subsp. *insidiosus*	alfalfa	bacterial wilt
C. m. subsp. *nebraskensis*	corn	bacterial wilt and blight
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	bean (*Phaseolus, Vigna*)	bacterial wilt
Pantoea stewartii subsp. *stewartii*	maize	stewart's disease, bacterial wilt
Pantoea ananatis	onion	centre rot
Pseudomonas syringae pv. *atrofaciens*	cereals	leaf spot, basal glume rot
P. s. pv. *glycinea*	soybean	bacterial blight of soybean
P. s. pv. *lachrymans*	cucumber, gherkin	angular leaf spot
P. s. pv. *phaseolicola*	bean	halo blight of bean
P. s. pv. *pisi*	pea	bacterial blight of pea
P. s. pv. *tomato*	tomato	bacterial speck
Xanthomonas axonopodis pv. *phaseoli*	bean (*Phaseolus, Vigna*)	common blight of bean
X. a. pv. *phaseoli* var. *fuscans*	bean (*Phaseolus, Vigna*)	common blight of bean
X. a. pv. *malvacearum*	cotton	bacterial blight of cotton
X. a. pv. *vesicatoria*	pepper	bacterial spot
X. a. pv. *vitians*	lettuce	bacterial leaf spot
Xanthomonas campestris pv. *campestris*	cabbage	black rot of crucifers
X. c. pv. *carotae*	carrot	bacterial blight
X. translucens	cereals	bacterial leaf streak, black chaff
X. oryzae pv. *oryzae*	rice	bacterial leaf blight
X. oryzae pv. *oryzicola*	rice	bacterial leaf streak
X. vesicatoria	tomato	bacterial spot
Xylella fastidiosa	orange (*Citrus sinensis*)	citrus variegated chlorosis

PRINCIPLE OF SEROLOGY AND CROSS-REACTIVITY OF POLYCLONAL ANTISERA

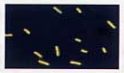


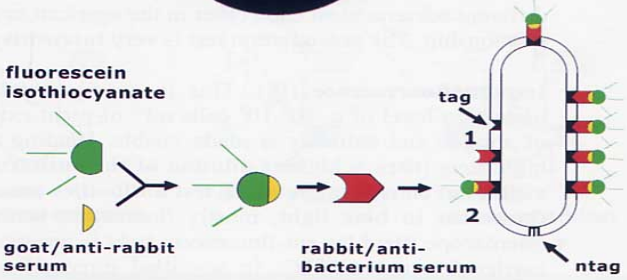
Agglutination
5 minutes

Agar-gel diffusion
1-2 days



Indirect
immuno-fluorescence
2-3 hours





CONJUGATE + SPECIFIC SERUM + BACTERIUM

Serology