Lab tomorrow: Bacterial Diseases

- Quiz for Koch's postulates (p. 17-19), Botrytis predisposition (p. 97), and Fungicide resistance (p.105-107). And, intros for Bacteria (pp 67-69) and Biocontrol of Crown Gall (p. 113).
- Continue Koch's postulates experiment (a several week process)
- Record data from *Botrytis* experiments.
- Bacterial pathogens lab.
- Biocontrol of Crown Gall experiment.
- Finish 'Disease of the Week' unknown.

Bacteria

5,000+ named species of bacteria - many more unnamed

Great majority of bacterial species are saprophytes

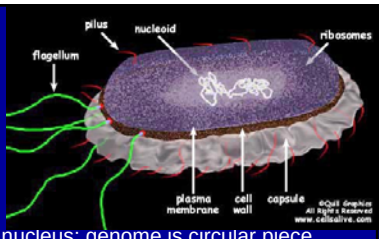
About 80 species are plant pathogens –
These come from five major groups:

Pectobacterium (*Erwinia*) --- soft rots
Pseudomonads --- many diseases – includes Ice+ bacteria
Xanthomonads --- leaf spots, blights – often seed associated
Agrobacterium --- crown gall
Clavibacter and *Xyella* (vascular wilts)

Based on the small number of diseases they cause, bacteria appear to receive more attention than they deserve. Why?

- Bacterial diseases are difficult to control
- Bacteria are excellent model organisms for studying the molecular interactions that occur in disease development
- Saprophytic species are commonly involved with biological control of plant disease
- In biotechnology, the bacterial pathogen, *Agrobacterium tumefaciens*, is used for transformation of plants

Bacterial structure



Single celled organisms:
- No membrane-bound nucleus; genome is circular piece of double stranded DNA
- No membrane-bound organelles such as mitochondria
- Very small size - a typical bacterial plant cell is ~1000 times smaller than a plant cell.

The cell is surrounded by a rigid wall and the wall is coated with a secreted slime layer (capsule) for protection.

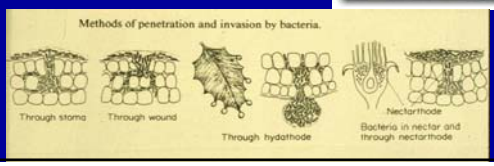
For bacterial plant pathogens, rod- shaped cells are typical, and they are flagellated (whip-like appendages used for swimming)

How bacteria cause disease

The typical life strategy of a bacterial plant pathogen is either facultative parasite or facultative saprophyte

Many survive grow and reproduce saprophytically (no disease) on plant surfaces. This growth is called 'epiphytic' (upon the plant).

Entry into plants is through wounds or natural openings:



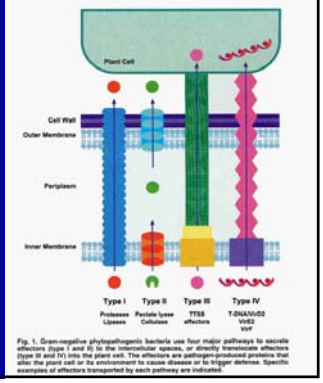
How bacteria cause disease

Can secrete enzymes such as pectinases that degrade the middle lamella - type II secretion system (soft rotters)

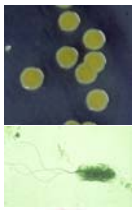
Can secrete virulence factors (effectors) to cause plant cell disruption and lysis – type III secretion system (leaf spots and canker pathogens)

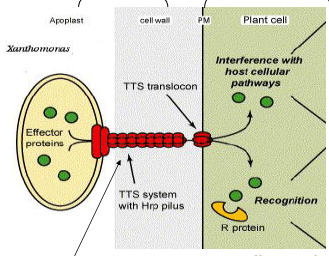
Can secrete genetic material (DNA) that is incorporated into the host genome. The genetic material codes for hormones and for synthesis of specific nutrients that only the bacterial pathogen can utilize. – type IV secretion system (crown gall bacterium)

Systems used by bacteria to secrete molecules involved in pathogenesis



Xanthomonas campestris pv. *campestris*





type III secretion system: secretes virulence factors (effectors) to cause plant cell disruption and lysis

Effectors determine host range

e.g., *X.c.* pv. *carotae* has different effectors than *X.c.* pv. *campestris*

Types of Symptoms

- Soft rotters
  - Pectolytic bacteria – Type II sectretion
- Leaf spots, blights and cankers
  - Bacteria with type III secretion system
- Galls
  - *Agrobacterium tumefacians*
- Vascular wilts
  - *various, tend to be* ***fastidious***

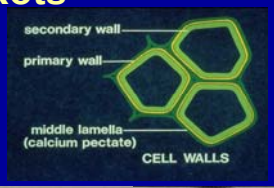
Types of Symptoms

Soft Rots

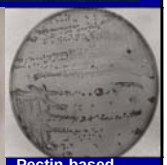
Bacteria reside in lenticels

Under anaerobic conditions, they hydrolyze the pectin in the middle lamella, which leads to necrosis and collapse of tissue.

Cause disease in stems, fruits, and storage organs (tubers).



Soft rot of potato



Pectin-based Selective media for isolation

Types of Symptoms

Leaf and Fruit Spots

Bacteria colonize stomates or hydathodes

Or enter by wounds (such as those caused by hail)

Bacterial leaf spots tend to be more angular than fungal spots, and bacterial spots often have a chlorotic, or water-soaked halo

Types of Symptoms

Blights

Initially, the bacteria reside on surface of foliage as epiphytes; enter plant via a natural opening (nectarthodes, hydathodes) or wounds

Fire blight of pear and apple

Black rot of cabbage

Guttation drops on hydathodes

Types of Symptoms

Cankers

Necrosis of woody stem tissue, usually involves phloem tissue

Bark of stem appears sunken with sharp borders

Often initiated in early spring by freezing/ thawing injury

Cutaway trunk showing canker advance to ground line. Courtesy George Philley, TAES - 1996.

Types of Symptoms

Galls

Bacterium colonizes wounds on roots and crowns of plants

Tumor formation in response to *host* production of hormones. Gene for hormone production came from bacterium

Crown gall

Types of Symptoms

Vascular Wilts

Potato ring rot

Pierce's disease of grape

Bacterial wilt of cucurbits

Colonize vascular tissue; disrupt transpiration stream and nutrient transport in vascular system.

Identification of bacteria

Gram strain

positive — negative

Polar flagella — Peritrichous flagella

Peritrichous flagella: Aerobic — Facultative Anaerobe

Erwinia carotovora

Polar flagella: fluorescent — Non-fluorescent

fluorescent: induce HR in tobacco — no HR

Pseudomonas syringae

Identification of bacteria is commonly conducted by performing a series of tests and or observations on the bacterial strain

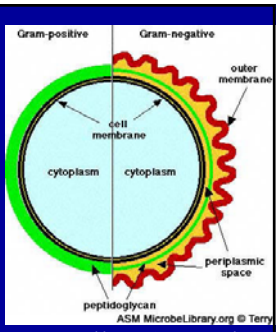
Gram stain

First test in identification of a bacterium

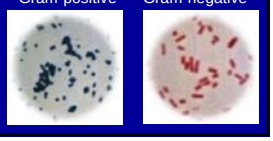
Consists of a staining procedure that detects 'peptidoglycan', which is exposed on wall of gram positive bacteria

The test was developed by Dr. Hans Gram, a Danish physician (1853-1938)

Most species of plant pathogen bacteria are Gram-negative



Gram-positive Gram-negative



Arrangement of flagella

whip-like threads types used to propel cell

Polar (lophotrichous)
Pseudomonas

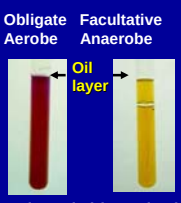
Peritrichous
Erwinia

Other tests for Identification of bacteria

Oxidation-fermentation test: this test is used to determine if bacterium can multiply in absence of oxygen

Colony color, morphology, and fluorescence:
Colors range from white to pink, and can depend on growth medium

Fluorescence is checked with UV light



Medium is sealed from air with oil, color change indicates growth

Hrp test in tobacco

Inject bacterial suspension into tobacco leaf. If hypersensitive reaction occurs, then the bacterium possess a 'Type III secretion 'system.

Type III secretion systems are used by bacteria to inject virulence factors into host cells.

Many bacterial pathogens of both plants and animal possess a Type III system.

'Hrp' means hypersensitive response, pathogenesis-related

Ice nucleation phenomenon

Catalysis of ice crystal formation in supercooled water has been demonstrated for some plant pathogenic bacteria e.g. *Pseudomonas syringae*

These epiphytic bacteria initiate ice formation at temperatures higher than normally required.

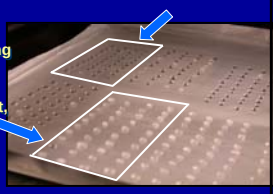
A gene in the bacterium codes for protein in cell wall that confers ice nucleating ability.

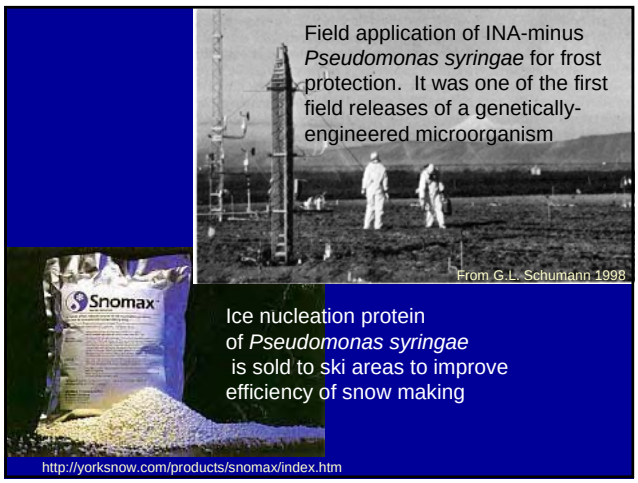
Ice damage creates wounds

Droplet freezing assay:

In this sample, ice-nucleating bacteria were not present. The droplets supercool but do not freeze.

In this sample, ice-nucleating bacteria were abundant, and ice was formed




Field application of INA-minus *Pseudomonas syringae* for frost protection. It was one of the first field releases of a genetically-engineered microorganism
From G.L. Schumann 1998
Snomax
Ice nucleation protein of *Pseudomonas syringae* is sold to ski areas to improve efficiency of snow making
http://yorksnow.com/products/snomax/index.htm