

Week 1

BACTERIAL PLANT PATHOGENS, BIOLOGY AND SYMPTOMOLOGY

Bacteria are microscopic **prokaryotic** (a cell in which the nuclear material is not enclosed by a nuclear membrane) and, for the most part, single-celled microorganisms. A single teaspoon of healthy topsoil contains about a billion bacterial cells, 120,000 fungal cells and 25,000 algal cells. Bacteria are single-celled microorganisms, generally ranging from 1-2 μm in size that cannot be seen with the unaided eye (Figure 1). Plant associated bacteria may be beneficial or detrimental. All plant surfaces have microbes on them (termed epiphytes), and some microbes live inside plants (termed endophytes). Some are residents and some are transient. Bacteria are among the microbes that successively colonize plants as they mature. Individual bacterial cells cannot be seen without the use of a microscope, however, large populations of bacteria become visible as aggregates in liquid, as biofilms on plants, as viscous suspensions plugging plant vessels, or colonies on petri dishes in the laboratory. For beneficial purposes or as pathogens, populations of 10^6 CFU (colony-forming units/milliliter) or higher are normally required for bacteria to function as biological control agents or cause infectious disease.



Figure 1

Basic Biology

Bacteria associated with plants have several morphological shapes as can be seen with conventional microscopes at 400x to 1000x magnification. These shapes initially provided simple ways to differentiate them. There are bacilli (rods), cocci (spherical), pleomorphic rods

(tendency toward irregular shapes) and spiral shapes. The majority of plant-associated bacteria are rods. However, modern science has shown by biochemical, genetic and molecular biological analyses that these bacteria are quite heterogeneous. Some are related to and grouped with animal and human pathogens.

By different types of microscopy (Basic Microscopy), principally fluorescent, confocal, phase-contrast and electron microscopy, one can see different parts of bacterial cells (Figure 8). Stains are often useful in the differentiation of structures.

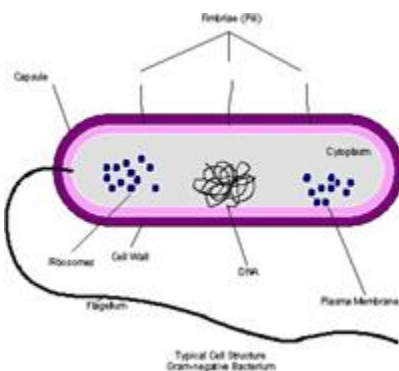


Figure 8

Chromosomes composed of DNA are coiled and there may be more than one per cell. Plasmids, or extra-chromosomal genomic entities may be present, and can code for essential virulence factors or conversely, biological control factors, which are chemicals effective against deleterious bacteria or fungi. Storage granules can be seen in some bacteria. Bacterial cells may or may not have appendages: flagella, usually at the poles of the cells (for movement) and fimbriae or pili, smaller thread-like appendages, usually at multiple locations. There is some evidence that flagellated cells produce larger lesions than non-flagellated mutants. The fimbriae are believed to be helpful in attachment, somewhat like a Velcro® fastener. Flagella and fimbriae, as well as different parts of the cell wall and cell membrane may contain receptor sites for bacterial viruses (bacteriophage) (Figure 9). However, bacteria without detectable appendages can be effective pathogens.

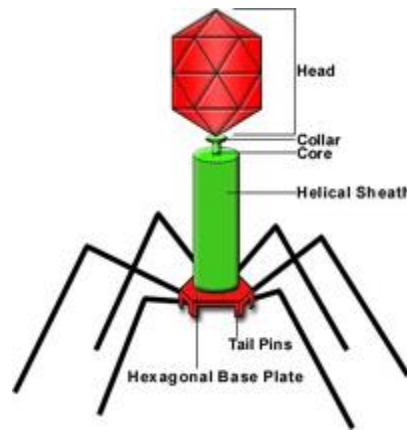


Figure 9

On laboratory media, plant pathogens usually grow more slowly than non-pathogenic bacteria isolated from plants, with optimal temperatures of 20-30°C (68-86°F). This makes isolation sometimes very challenging. A few grow at 37°C (99°F) (or higher), the temperature at which human pathogens, e.g. *Burkholderiacepacia*, (APSnet Feature: *Burkholderiacepacia*: Friend or Foe), are able to grow. Some can grow slowly at 10-12°C (50-54°F). Most are aerobic, some are facultative anaerobes (i.e. they can grow with or without oxygen), and a rare few are anaerobes. At high concentrations accompanying the growth of colonies (each colony is about 10^7 to 10^8 cells) on solid media, characteristic pigments may form within the colony or can be excreted into the growth medium, and may require special lighting (e.g. UV) for detection (Figure 10). Occasionally, bacterial pigments can be detected in seed (Figure 11). Medium requirements for growth may be simple or complex; some bacteria haven't been cultured. (Fastidious Vascular-Colonizing Bacteria). Some bacteria can produce characteristic volatile compounds, often with an unpleasant odor. Think of the smell of rotten potatoes, for example.



Figure 10

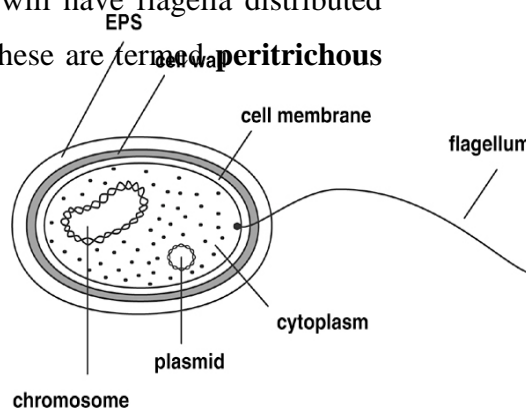


Figure 11

The genetic material of bacteria consists of a single DNA

molecule suspended in the cells cytoplasm. Bacteria do not have a true nucleus as do animals, plants and fungi. Some bacteria also have small gene-carrying entities within their cytoplasm called **plasmids**. Plasmids are extra-chromosomal, self-replicating genes that are responsible for such characteristics as resistance to streptomycin, copper and other antibiotics. Bacteria come in four shapes, there are **coccus** (spherical), **bacillus** (rod shaped) and **spirochetes** (spiral). Most phytopathogenic bacteria are rod shaped bacillus the only exception being *Streptomyces* (family Actinomycetes) which is a **filamentous** (thread-like, filiform) bacteria. Also, most of these bacteria have flagella which are whip-like structures projecting from a bacterium that functions as an organ of locomotion.

Some species of bacteria have only one flagellum (**monotrichous**) or a tuft of two or more flagella at one end of the cell. These are called **polar** flagella. Other species will have flagella distributed over the entire surface of the cell. These are termed **peritrichous** flagella.



WEEK 2

HISTORY

Individual bacteria were first seen by humans about 325 years ago when they were magnified by the first microscope. It's only been a little over 100 years since a bacterium was first implicated as a causal agent in a plant disease. Bacteria were shown in 1878 to be associated with fireblight of apples and pears in Illinois and New York, USA (Burrill 1878). (fire blight disease lesson). The disease caused by *Erwinia amylovora*, now widespread throughout much of the temperate world, remains a limiting factor in growth of healthy apple and pear trees (Figure 4). In 1885, J.C. Arthur was able to isolate a bacterium from diseased plants, culture it, and then inoculate the same host to reproduce a naturally occurring disease. He recovered it subsequently from diseased tissue, fulfilling what is known as Koch's postulates (Arthur 1885). And it's only been about 120 years since the development of sterilized semi-solid media, first gelatin and then agar with various nutrients added, that enabled the isolation of purified cultures, a technique taken for granted today (Koch 1881).

Of the over 15,000 identified species of bacteria most are saprophytic and are of great benefit in decomposing dead and rotting organisms thereby releasing their nutrients back into the environment. This is the most important roll that bacteria play in nature.

Plants rely on nitrogen from the soil but cannot directly

acquire it from the gaseous nitrogen in the atmosphere. The primary way nitrogen is supplied to plants is through the mineralization of organic material in the soil. However, **nitrogen fixation** by bacteria such as *Rhizobium spp.* and *Cyanobacteria spp.* is almost as important as mineralization, and is a primary source of nitrogen. As these bacteria metabolize they convert gaseous nitrogen into nitrates or nitrites that become available to plants.

Most phytopathogenic bacteria are **aerobic** (live in the presence of oxygen) and some are **facultative anaerobes** which can grow with or without oxygen. Some bacteria have thick, rigid cell walls which will retain dye from a cell staining method developed by Christian Gram, while other bacteria will not accept this stain. This method of staining results in the bacteria being classed as **Gram-positive** or **Gram-negative** and is an important factor in identification and classification. Gram-positive bacteria appear purple and Gram-negative bacteria appear pink under magnification. Bacteria are also distinguished by the different kinds of enzymes they either can or cannot use for nourishment and the nutrient media on which they can grow.

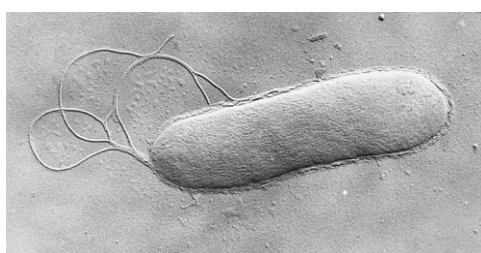
Rod shaped bacteria reproduce asexually by the process of **binary fission** (the transverse splitting in two of a bacterial cell). This process takes place when the **cytoplasmic membrane** grows inward dividing the cytoplasm into two approximately equal parts.

When the cell walls are completely formed the cell splits into two cells. During this process the nuclear material duplicates itself and becomes distributed equally between the two cells. Bacteria can reproduce at a very rapid rate; some species can divide every 20 minutes under ideal conditions. It is conceivable that a single bacterium could produce one million progeny in less than 24 hours. However, with limited food supply, environmental conditions and other factors the optimum conditions rarely occur in nature.



There are around 200 species of phytopathogenic bacteria and almost all of them are **parasites** within the plant, on its surface, in plant debris or in the soil as **saprophytes**. Dissemination of bacteria can be accomplished by several means. Some bacteria

can survive on inanimate objects, in water or inside insects. It is important to know the survival characteristics of bacteria for effective management strategy and intervention in dissemination. Some species have the ability to move short distances in water on their own power by use of their **flagella**. Most bacteria, however, are disseminated by passive agents such as air and insects, water and soil movement, and to a lesser degree by humans, water and other animals. Infected seeds and transplants can also be a source of



inoculums. Most bacteria require a wound or natural opening (e.g. stomata, lenticels or hydathodes) to gain entry into the host tissue and also require warm, moist conditions to establish a **colony**. Windblown soil and sand will commonly cause wounds which can facilitate bacterial infections.

Bacteria colonize a host by growing between the cells and absorbing the cells nutrients that leak into intercellular space or grow within the vascular tissue of the plant.

Depending on the species of bacteria and the tissue infected they produce and release **enzymes** that degrade cell walls, **growth regulators** that alter the plants normal growth, **toxins** that degrade cell membranes and **complex sugars** that plug water conducting tissue.

WEEK 3

SYSTEMATICS OF BACTERIA

Most of the plant pathogenic bacteria are either Gram-positive, classified within the Phylum *Actinobacteria*, or Gram-negative, in the Phylum *Proteobacteria*. Gram-positive and Gram-negative cells appear purple or red, respectively, with specific stains when viewed at 1000x magnification with a light microscope (Figure 12). The different colors largely reflect differences in stain retention by the respective cell walls of the bacteria during the staining process. Further differentiation is based on chemical or physiological characteristics, e.g. cell wall composition, enzyme production, substrate utilization, etc. Molecular characterization of 16S ribosomal RNA also may distinguish bacteria from one another. Ribosomes are coded by a highly conserved part of the bacterial chromosome and represent only a small part of the genome. But, the 'gold standard' for determining phylogenetic relationships is DNA:DNA homology by hybridization or genomic sequencing. Such analyses are sometimes at variance with ribosomal analyses.

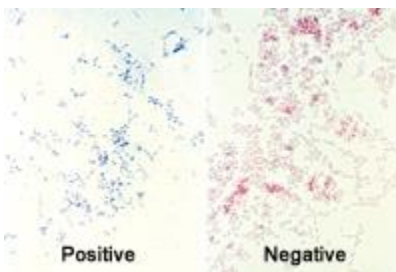


Figure 12

Interpretations of relationships vary with time, new techniques and more data. Thus, bacterial names can change from one era to another. It is sometimes challenging to read scientific literature and make legislative decisions without knowledge of all the synonyms for a particular bacterium. Updated nomenclature of bacterial plant pathogens can be found in Young et al. (1996) or accessed electronically in web-based bacterial nomenclature databases (Table 1), which are frequently updated. These listings include the historical and all published names for a particular bacterium. In addition, due to the inability of humans to otherwise differentiate some host specific plant pathogenic bacteria, the concept of pathovars or pathogenic variants and races, differentiated by host range, can be found in the literature. This system is at variance with naming of pathogens of animals and humans, where host range differences may be recognized

but are not part of an organism's name. Also, complicating the systematics of plant pathogenic bacteria is the presence of essential plasmids. Pathogenic *Agrobacterium tumefaciens* causes crown gall on a large number of hosts (crown gall disease lesson). Without its specific tumor-inducing plasmid (termed tumor-inducing or Ti plasmid) the strains are equivalent to nonpathogenic *A. radiobacter*.

Survival

Survival of plant pathogenic bacteria in nature occurs most commonly in plant debris left on the soil surface, in and on seeds, in soil, and in association with perennial hosts. But some bacteria can also survive in water and some do well on inanimate objects or on or inside insects. *Clavibacter michiganensis* subsp. *sepedonicus*, causative agent of potato ring rot, is notoriously known for surviving on machinery and packaging material. Knowledge of survival is usually essential to intervene in dissemination and for disease management.

Dissemination

Dissemination of plant pathogenic bacteria is easy, but fortunately does not always result in disease. Dissemination commonly occurs by windblown soil and sand particles that cause plant wounding, particularly during or after rains or storms (Figure 13). Wounding is essential for entry by many plant pathogens. Aerosols generated by diurnal temperature fluctuations enable dissemination, if temperature and humidity are aligned (Hirano and Upper 1989). Some plant diseases require certain temperature conditions e.g. *Pseudomonas syringae* (synonym: *P. savastanoi*) pv. *phaseolicola* causes disease below 22°C (72 °F) and *Xanthomonas campestris* (syn: *X. axonopodis*) pv. *phaseoli*, above 22°C on dry bean (*Phaseolus vulgaris*). Both diseases can occur simultaneously under growth conditions in which day and night temperature differentials enable disease progression in susceptible plants. Infested (surface contamination) or infected seed or any plant part can be sources of bacterial inoculum. Machinery, clothing, packing material and water can also disseminate pathogens, as can insects and birds. Continual monoculture in an area will usually enable increases in inoculum, making it easier for pathogens to be disseminated.

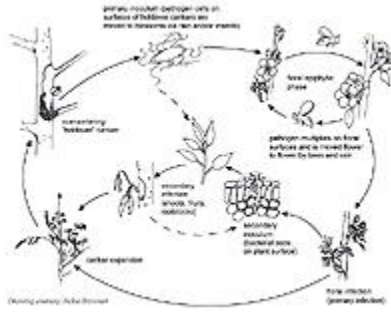


Figure 13

WEEK 4
REPRODUCTION IN BACTERIA

WEEK 5

CLASSIFICATION OF BACTERIA

The following is a general classification (Agrios, 5th Ed, 2005) of phytopathogenic prokaryotes with the exception of the Division Tenericutes, Class Mollicutes, which will be addressed in a later section. Genera in **bold** type are common plant pathogens

Kingdom: Procaryotae

Bacteria – Have cell membrane and cell wall and no nuclear membrane.

Division: Bacteria – **Gram-positive: Gracillicutes**

Class: Proteobacteria – Mostly single celled bacteria.

Family: Enterobacteriaceae

Genus: ***Erwinia***, causing fire blight of pear and apple,
Stewart's wilt in corn, and soft rot of
fleshy vegetables.

Pantoea, causing wilt of corn.

Serratia, ***S. marcescens***, a phloem-inhabiting bacterium
causing yellow vine disease of cucurbits.

Sphingomonas, causing brown spot of yellow Spanish
melon fruit.

Family: Pseudomonadaceae

Genus: ***Acidovorax***, causing leaf spots in corn,
orchids and watermelon.

Pseudomonas, causing numerous leaf
spots, blights, vascular wilts,
soft rots, cankers, and galls.

Ralstonia, causing wilts of solanaceous crops.

Rhizobacter, causing the bacterial gall of carrots.

Rhizomonas, causing the corky root rot of lettuce.

Xanthomonas, causing numerous leaf spots, fruit spots, blights of annual and
perennial plants, vascular wilts and citrus canker.

Xylophilus, causing the bacterial necrosis and canker of grapevines.

Family: Rhizobiaceae

Genus: ***Agrobacterium***, the cause of crown gall disease.

Rhizobium, the cause of nitrogen-fixing root nodules in legumes.

Family: still unnamed

Genus: ***Xylella***, xylem-inhabiting, causing leaf scorch and dieback disease on trees and vines.

Candidatus liberobacter, Phloem inhabiting, causing citrus greening disease.

Unnamed, laticifer-inhabiting, causing bunchy top disease of papaya.

Division: Firmicutes - Gram-positive bacteria.

Class: Firmibacteria – Mostly single celled bacteria.

Genus: *Bacillus*, causing rot of tubers, seeds, and seedlings and white stripe of wheat.

Clostridium, causing rot of stored tubers and leaves and wetwood of elm and poplar.

Class: Thallobacteria – Branching bacteria.

Genus: ***Arthrobacter***, causing bacterial blight of holly, thought to be the cause of Douglas-fir bacterial gall.

Clavibacter, causing bacterial wilts in alfalfa, potato, and tomato.

Curtobacterium, causing wilt in beans and other plants.

Leifsonia, causing ratoon stunting of sugarcane.

Rhodococcus, causing fasciation of sweet pea.

Streptomyces, causing common potato scab.

WEEK 6-8

DIAGNOSTICS SYMPTOMS OF BACTERIAL INFECTIONS

Symptoms of bacterial infection in plants are much like the symptoms in fungal plant disease. They include leaf spots, blights, wilts, scabs, cankers and soft rots of roots, storage organs and fruit, and overgrowth.

Bacterial spots:

the most common symptom of bacterial disease is leaf spots. Spots appear on leaves, blossoms, fruits and stems. If the spots appear and advance rapidly the disease is considered a **blight**. Spots on leaves of dicotyledonous plants often have a rotten or fishy odor, are water soaked and are initially confined between the leaf veins and will appear **angular**. In some cases **bacterial ooze** will be present; this is diagnostic for bacterial infections. Sometimes a **chlorotic halo** will surround the



bacterial lesion of an infected leaf. Spots may coalesce causing large areas of necrotic tissue. Bacterial spots will appear as streaks or stripes on monocotyledonous plants. Almost all bacterial leaf spots and blights are caused by the genera *Pseudomonas* and *Xanthomonas*.

Cankers: primarily *Pseudomonas* and *Xanthomonas* cause canker disease of stone fruit and pome fruit trees, and canker disease of citrus respectively. Canker symptoms can appear on Trunks, stems, twigs and branches. The most conspicuous symptom of a bacterial canker disease in stone and pome fruit trees is the development of **cankers** and **gum** exudation (gummosis). Cankers can be slightly sunken, dark brown and



much longer than broad. The cortical tissue of the canker can be orange-brown to dark brown. Gum is produced in most cankers and some branches and twigs. Cankers that do not produce gum may have a sour odor and be soft, sunken and moist. Cankers that girdle trunks and branches can result in leaf stress and eventual dieback of the portion of the tree distal to the canker.



Bacterial Galls: bacterial galls can be produced by the genus *Agrobacterium* and certain species of *Arthrobacter*, *Pseudomonas*, *Rhizobacter* and *Rhodococcus*. *Agrobacterium tumefaciens*, *A. rubi* and *A. vitis* alone are responsible for galls in over 390 plant genera worldwide. Galls of these genera have been referred to as crown gall, crown knot, root knot and root gall. Species of

these bacteria are thought to be present in most agriculture soil. A wound in the host is required for the pathogen to gain entry into the host tissue. Gall tissue is composed of disorganized, randomly proliferating cells that multiply in the intercellular (between the cells) spaces in the vicinity of the wound. In the presence of the pathogen rapid and continuous cell division (hyperplasia and hypertrophy) of the plant tissue persists.

Gall damage can be benign to deadly. Crown gall first appears as **small, whitish, soft round overgrowths** typically on the plants **crown** or at the main root. The color of galls (tumors) caused by *A. tumefaciens* can be orange-brown and

as it enlarges the surface can become **convoluted** and dark brown. This is most often found in commercial nurseries.

Bacterial Vascular

Wilts: Vascular wilts caused by bacteria primarily affect herbaceous plants such as vegetables, field crops, ornamentals



and some tropical plants. The causal pathogen enters, multiplies in, and moves through the **xylem vessels** of the host plant and interferes with the translocation of nutrients and water by producing gum. The pathogen will often destroy parts of the cell wall of the xylem vessels resulting in pockets of bacteria, gums and cellular debris. The symptoms of bacterial wilt disease include **wilting** and **death** of the

aboveground parts of the plant. In some cases **bacterial ooze** seeps out through stomata or cracks onto the surface of infected leaves. Usually this ooze does not occur until the infected plant tissue is dead.

Bact





Primarily the bacteria that cause soft rots in living plant tissue include *Erwinia spp.*, *Pseudomonas spp.*, *Bacillus spp.* and *Clostridium spp.* Many soft rots are caused by non-phytopathogenic bacteria which are saprophytes that grow in tissue that has been killed by pathogenic or environmental causes. Soft rots attack a large number of hosts and are best known for causing disease in fleshy plant structures both above and below ground. These bacteria are almost always

present where susceptible plants under stress are in the field or in storage. Soft rot pathogens enter the host through wounds. After entering the host tissue these bacteria produce enzymes that break down the middle lamella causing separation of the cells at the site of the infection. The cells die and disintegrate. Rotting tissue becomes **watery** and **soft** and bacteria will form a **slimy**

foul smelling ooze that will ooze out of infected tissue. Bacterial ooze is diagnostic of soft rot diseases.

Bacterial scabs:

bacterial scabs primarily infect belowground parts of plants such as potatoes. Common scab of potato is caused by *Streptomyces scabies* which cause localized **scabby lesions** on the outer surface of the tuber. Typically **corky tissue** will form below and around the lesion. Rot pathogens can gain entrance into the host tissue through these lesions and further degrade the host.



Diagnosis of non-fastidious bacterial diseases depends on characteristic symptomatology,

isolation of the presumed infectious agent, and physiological and/or molecular tests (Plant Disease Diagnosis). In heavily infected plants, bacterial populations in leaves or lesions may reach 10^8 or 10^9 CFU/gram of plant tissue, and actually visibly ooze from leaves or stems (Figure 18). A simple way to determine if a disease is caused by a bacterium is to cut a typical lesion or discolored area near its boundary with healthy tissue and suspend it in a droplet of water on a microscope slide. If a mass of moving small rods or 'dots' is seen at 400-1000x magnification flowing from the cut tissue under a microscope, you are observing bacterial streaming (Figure 19) which is an indicator of a bacterial disease. However, not all bacterial infections show streaming, or it may not be visualized without special microscope attachments. Serological tests, usually enzyme-linked, and physiological assays are available commercially for a few common and economically important bacteria. Molecular tests such as the polymerase chain reaction (PCR), based on specific genomic sequences, are becoming more readily available and used. Diagnostic tests are still evolving (Schaad et al. 2001), so that few are standardized and validated by multiple users, including governments.

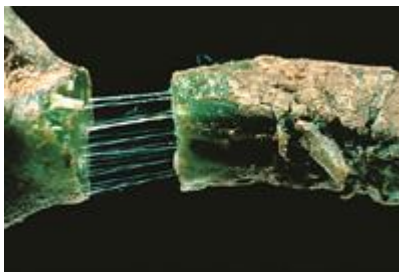


Figure 18

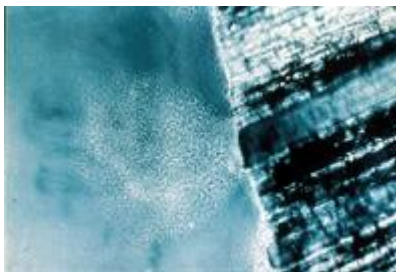


Figure 19

Most plant pathogens are capable of inducing a hypersensitive reaction (HR) in plant species that are non-hosts or indicator plants (Klement et al. 1964). The HR is a plant defense mechanism elicited by the presence of a pathogen in non-host tissue. The tissue becomes sensitized to the pathogen, resulting in a rapid death of local plant cells (Figure 20), and entrapment of the pathogen. This, in effect, limits the spread of infection. One may use an HR test to determine if a colony isolated from infected plant tissue is a pathogen by introducing it, in a pure culture water suspension at 10^8 CFU/ml, into a non-host leaf panel. Tobacco (*Nicotianatabacum*) is frequently used in HR tests because its large leaf panels are easily infiltrated, but Four O'clock (*Mirabilis jalapa*) may be used for some Gram-positive bacteria. Collapse of host tissue in the infiltration area within 48 hours indicates the bacterium is likely a pathogen for another host.



Figure 20

Confirmation that a pathogen causes disease symptoms requires a host and performance of a pathogenicity test. This strategy can be time-consuming (days, weeks, or months). A pure culture of bacteria recovered from diseased tissue is artificially inoculated into the same or related cultivar or another susceptible host species, in an effort to reproduce the same disease symptoms. The bacteria should then be reisolated and compared with the inoculant culture.

With some practice, most bacterial diseases can be easily diagnosed. However, the variation that can occur with different strains may require more sophisticated testing.

Selected References:

Agrios, 5th ed. 2005, Plant Pathology

WSU, OSU U of I, 2005, Pacific Northwest Plant Disease Handbook

Jim Cooper, Master Gardener WSU County Extension, SJI

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