التعليم العالي و البحث العلمي

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Titre: Course of Characterization and Taxonomy of Plant Pathogen: Party of Bacteria

Polycopié destiné aux étudiants de

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Summary

1. CHARACTERISTICS OF PHYTOPATHOGENIC BACTERIA

1.1. Introduction

The immense diversity of plant pathogens, which include viruses, bacteria, fungi, nematodes, and insects, approximates 7100 species. Among these, roughly 150 are bacterial species that cause diseases to plants. The major ways that bacterial pathogens cause plant diseases are by obtaining nutrients one or more host plants for their own growth. Bacteria are using specific mechanisms to secrete proteins and other molecules to locations on, in, and near their hosts. It can exploit these proteins and other molecules modulate or avoid plant defense circuitry to enable parasitic colonization. Bacterial plant diseases are most frequent and severe in many countries, where warm and humid conditions are ideal for bacterial growth. In Plant pathogenic bacteria cause irritation and pathological changes (disease) in host plants.

1.2. Bacteria structure

Bacteria are single celled microorganism, extremely minute, rigid unicellular and devoid of chlorophyll. Bacteria are microscopic, unicellular prokaryotes. These microorganisms are with a primitive nucleus lacking a clearly defined membrane. Considering ultra-structure of bacteria, its size ranges from 0.2 µm to 250 µm. Small size of bacteria has two important consequences. One is it increases physiological activity of bacteria and second is it limits enzymatic activity. Due to small size of bacteria, it can easily enter through vascular bundles giving a systemic appearance of symptoms. Bacteria cell wall is not made up of cellulose except *Acetobacter*, *Zymosarcina* sp; it is made up of peptidoglycan. Due to high surface to volume ratio, it has high physiological activity and so as high growth rate. 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 animal, plant, and fungi. Some bacteria also have small gene-carrying entities within their cytoplasm called plasmids.

The adaptive nature of enzyme production is one of the greatest virtues of bacteria. It synthesizes organic polymers which have deposited outside the cell wall as loose or more amorphous layer called capsule or slime layer. This protects bacteria from hostile environment.

1.3. Morphology of bacteria

1.3.1. Shapes of bacteria

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.

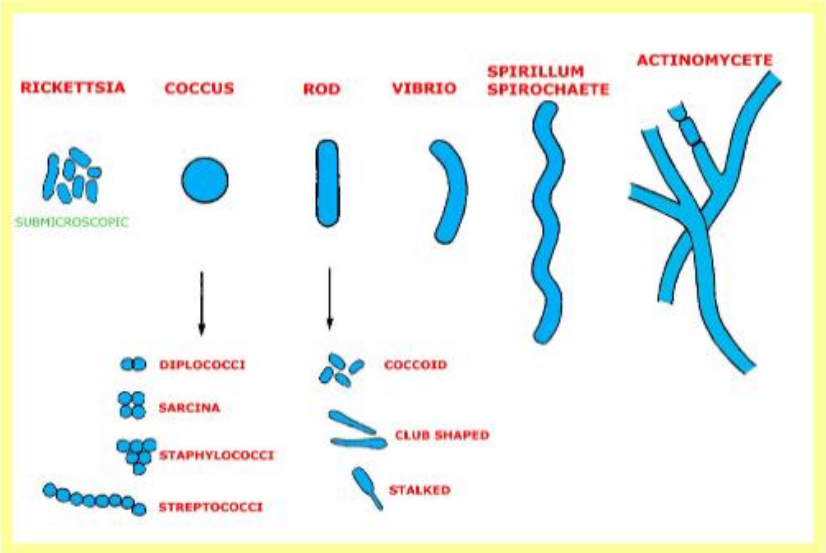


Figure1: Morphology shape of the bacteria cell.

Most of bacteria produce out of the cell delicate flagella, which are filamentous structures build of proteins. Flagella are significantly longer than bacterial cells; in some species only one flagellum occurs (monotrichous flagellation), in others flagella are located on both ends of cell (lophotrichous), there are also bacteria flagellated at various places on cell (peritrichous). When single cell multiplies on artificial agar media, it produces the colony. The shape and size of colonies of various bacterial species vary and constitute an important feature helping their identification. The colonies can be flat, raised or wrinkled, circular or oval, shiny or mat. The colony colour of most species is whitish or greyish, sometimes yellowish.

Staining dyes can divide bacteria into groups. The most well-known example of a differential stain is the Gram stain. It is discriminating between Gram-positive bacteria (which remain blue-purple stained by crystal violet, even after a decoloration action with alcohol) and Gram-negative bacteria (which lose the purple stain upon decoloration and which are subsequently stained red with a counter stain, such as safranin). The discrimination is based on a difference in cell wall structure and composition.

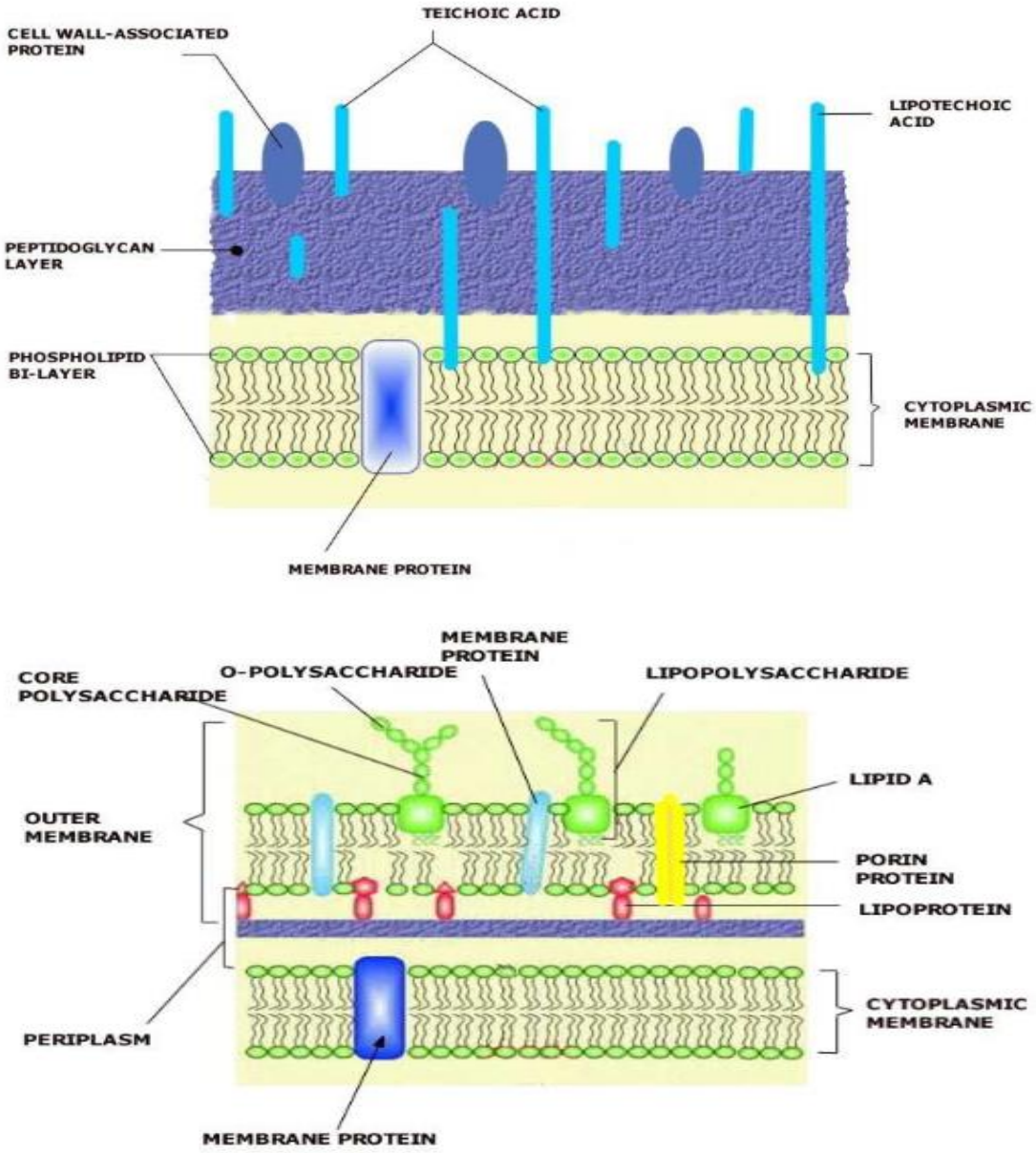


Figure 2: Schematic representation of the structure of the cell wall of a Gram-positive (top) and Gram-negative (bottom) cell wall.

The bacterial proteins play protective, nutritional and transportation functions. In cytoplasm nucleoid (chromosome) built of deoxyribonucleic acid (DNA) is located. Moreover, autonomic DNA structures called plasmids are often present. The plasmid genes are responsible for various traits of bacteria, like synthesis of toxins and other compounds related to their pathogenicity, as well as the resistance to unfavorable environmental conditions. Some of gram positive bacteria produce endospores, which help them survive unfavourable conditions.

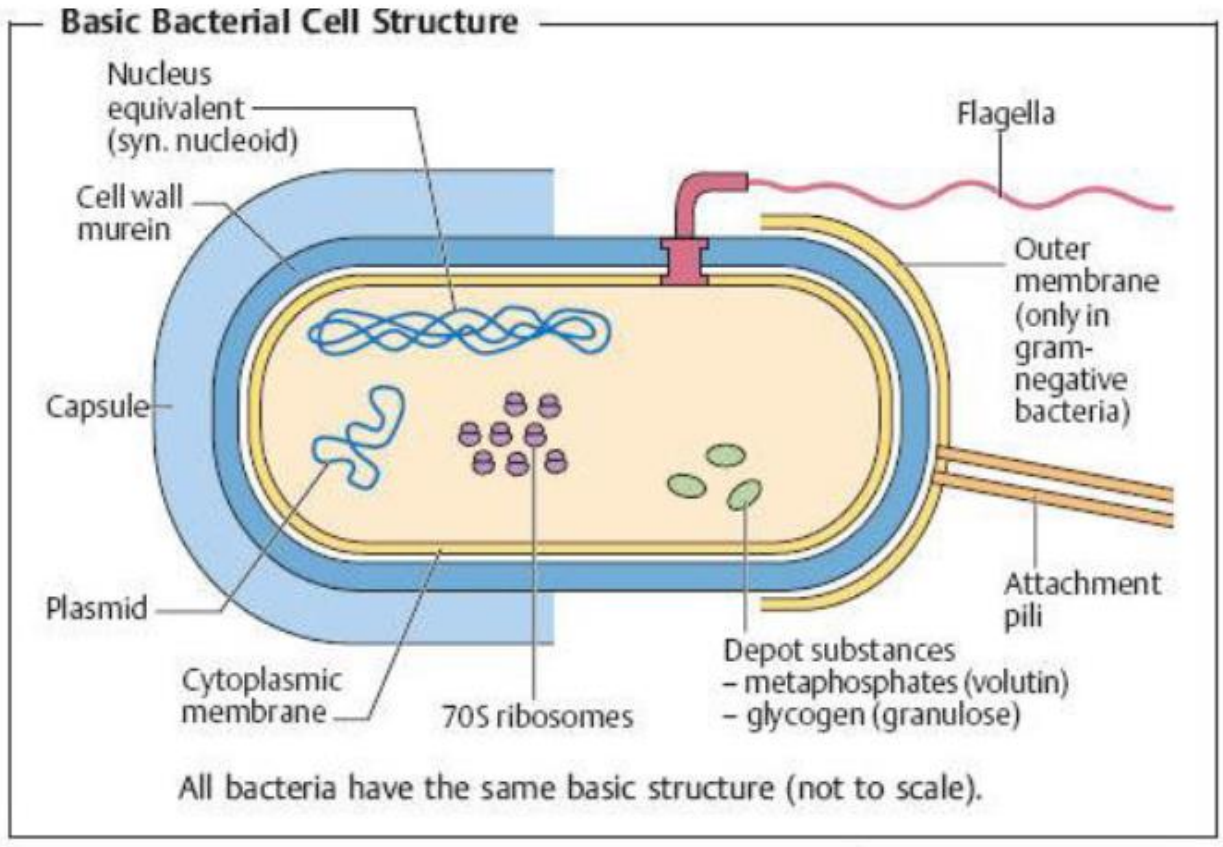


Figure 3: The basic structure of bacteria.

1.4. Biology of bacteria

1.4.1. Endospore

Bacteria are capable of forming a range of sporulation structures but the main is endospore.

Spores are resting bodies produced by some species of bacteria within the cell. However endospore is formed only in the gram positive bacteria. The free endospore is metabolically inactive and may retain viability for several years. This dormancy is known as cryptobiosis. So endospores are highly heat resistant and tolerant to UV radiations, toxic chemicals etc. The second type of spore is the exospore. These exospores are mainly formed by the hyphae-forming Actinomycetes, including *Streptomyces* spp. They are not so thick walled as endospores, are not completely dormant, and mainly function in dissemination of the organism. Exospores are formed on special hyphae, so-called aerial hyphae.

Bacteria requiring molecular oxygen for growth are called aerobic. Those growing only in the absence of oxygen are called anaerobic, those growing in the presence or absence of oxygen facultative anaerobic and those growing at low oxygen tensions. Bacteria multiply by division. In addition to cell enlargement that takes place between two divisions, division (asexual reproduction) itself is also called growth. Growth rate of bacteria is dependent on the bacterial species, physical factors (temperature, osmotic value, pH), and nutritional factors.

Most of the plant pathogenic bacteria grow well between 25-30° C except *Pseudomonas* which has optimal growth at 35-38° C and *Clavibacter* 20-25° C. Thermal death point of bacteria is 50-55° C. The UV light, ionizing temperature atwhich the organism is killed in10 minute of exposure. The growth of bacteria if drawn in a graph shows lag phase followed by log/exponential phase, stationary phase and finally death phase. Gene transfer in case of bacteria occurs through process of transformation, transduction, and conjugation.

1.5. Conservation, Propagation and penetration of phytopathogenic bacteria (epidemiology)

Plant pathogenic bacterial disease epidemics can cause such huge losses in crop yields that species extinction can occur. Triangulation of epidemic elements includes susceptible host, pathogen, and favorable environment; each of these is vital in the occurrence of a disease and the duration of an epidemic. Epidemics can be monocyclic (typically, soil-borne) or polycyclic (typically, airborne). Polyetic epidemics can be caused by both monocyclic and polycyclic pathogens. In such cases, an initial infestation by inocula continues over several growing seasons, causing the same disease each time, because bacteria are able to travel to previously uninfected areas.

Bacterial plant pathogens can survive quite well in the environment even though they do not form spores. Active cells may be present as epiphytes on plants or in their root zones, or as less active cells within the plant (endophytes, latent forms). They survive in plant debris on the soil surface, in soil, in and on seed, in or on insects, and as epiphytic populations on other host plants.

Bacteria reproduce very rapidly by dividing in half (binary fission). The process may be repeated every twenty minutes producing millions cells per day. Infectious doses are in the order of millions of cells. Bacteria are spread by wind, dust, rain splash, surface water, irrigation water, insects, mites, animals, soil, nursery staff and their equipment. In the field spread and infection are favoured by windblown soil and sand particles during storms that also cause plant damage. In the nursery bacteria are splashed from the soil to leaves and from leaf to leaf by overhead irrigation. Bacteria are also spread in plant parts especially seed. Seed transmission is especially important for vascular bacterial pathogens which can infect seed externally and internally. Non-vascular pathogens can contaminate and infect the seed coat. Bacteria are also spread in tubers, bulbs, cuttings, and transplants.

Most bacteria require a wound or natural opening (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

1.6. Symptoms

Symptoms caused by different genera of plant pathogenic are mentioned by the following classes of symptoms: a) Leaf spots, b) Excrescences and galls, c) tumours, d) vascular diseases and wilting, e) necroses and cankers, f) rotting, g) bacteria embedded in slime.

a. Leaf spots

Bacterial leaf spots can often be distinguished from those caused by other organisms. It characterized by a chlorotic halo which is formed under the influence of toxins, followed by a

water-soaked zone formed by EPS, a brown to black necrotic part and a grayish to brown papery dry centre. When leaf spots are bordered by larger, lignified veins they are angular in the case of dicotyledonous plants and longitudinal in the case of monocotyledonous plants; for the rest they are circular or irregular. When leaf spots coalesce larger areas of the leaf lamina are killed. Development of leaf spots often stops when the weather becomes dry. Bacterial slime is pressed out of the plant under humid conditions via stomata and ruptures the slime can be observed as a thin silvery film under dry conditions.

Figure 4: Bacterial leaf spots, caused by *Pseudomonas syringae pv. pisi*.

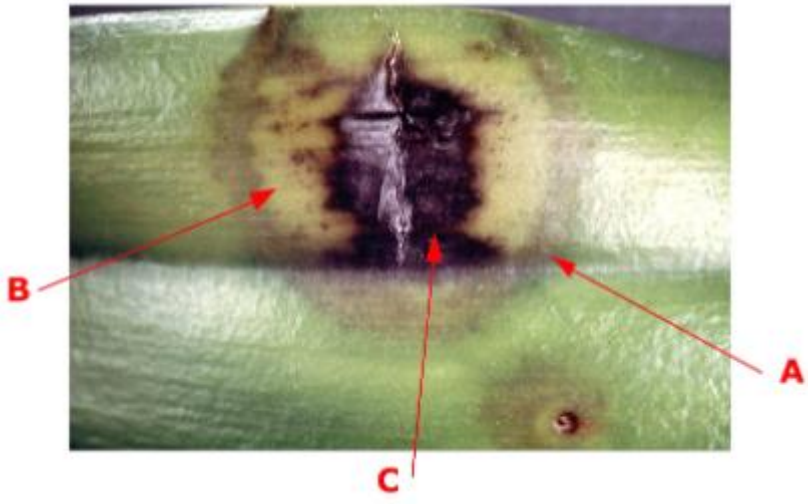


Figure5: Water-soaked spot, typical of bacterial infections, on leaf of Cattleya orchid, caused by *Acidovorax avenae subsp. Cattleyae* **A** water-soaked margin, where cells are leaking, but bacteria are not yet present; **B** yellow, chlorotic ring due to action of bacterial toxin; **C** necrotic area, where the pathogen, but also secondary pathogens and saprophytic organisms can be found.

b. Excrescences and galls

The filamentous bacterium *Streptomyces scabiei* causes excrescences, called scab, on potato, (sugar) beet, radish and carrot. The bacterium often penetrates the plant via young, not yet suberized lenticels or wounds. *S. scabiei* produces pectinases, enabling the hyphae to grow between cells. After plant cells have been killed, intracellular growth also takes place. Because *S. scabiei* does not produce toxins that rapidly kill plant cells, cork layer formation can take place in tissue at a distance from the lesions. The plant tries to localize the infection. The cork layer meristem (phellogen) sometimes forms many other parenchymal cells apart from cork cells, in that case so-called raised scab will develop. When the processes as described for potato scab persist for a number of years, excrescences are formed as are found with the bacterial knot disease caused by *Pseudomonas savastanoi pv. fraxini***.**

Xylem (vessel) tissue and bacterial cavities surrounded by cork layers. The galls develop under the influence of hormones indole acetic acid (IAA) and cytokinins, excreted by the bacteria. The plant cells are not transformed as in the case of tumours (see below). The other type of gall is called organoid, such as those caused by *Rhodococcus fascians*. This bacterium stimulates resting meristematic tissue in buds and (stem) bulbs, by excreting cytokinins and IAA, to abnormal growth and sprout formation. No bacterial cavities are found in this case. Witches' broom symptoms, dwarfing, and deformation occur in diseases caused by phloem-inhabiting fastidious bacteria (PLFB) and phytoplasmas

Figure6: Histoid galls of *Pseudomonas savastanoi pv. savastanoi* on olive (*Olea europaea).*

Figure 7: Top left: Organoïde galls (distorted stembubls) on lily (*Lilium spec*.), caused by *Rhodococcus fascians*. **Top right**: Large tumour, formed on a wound, which was caused by grafting, on Ficus species, caused by *Agrobacterium tumefaciens*. **Centre**: Histoid galls (common scab of potato), caused by the bacterium *Streptomyces scabiei*.

c. Bacterial Vascular Wilts

Vascular wilts caused by bacteria primarily affect herbaceous plants such as vegetables, field crops, ornamentals 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. Vascular pathogens can excrete toxins (glycoproteins), which diffuse more rapidly than the bacteria, causing wilting, yellowing, and sometimes large glassy, later necrotic spots. In these spots no bacteria are

found. Because the bacteria degrade non-lignified parts of vessels and walls of neighbouring parenchymal cells, vascular tissue is damaged, transport is disturbed, and bacterial cavities are formed. 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. Vascular diseases may be caused by infection through roots or stolons by (soil) pathogens such as *Ralstonia solanacearum*, causative organism of brown rot in potato, tomato, tobacco, etc., *Clavibacter michiganensis* subsp. *sepedonicus*, causing ring rot of potato. Other possibilities are infection through infected seed or hydathodes at the leaf margin, such as in infection caused by *Xanthomonas campestris pv. campestris*.

Figure 8: Wilting of tomato and onion due to blocking of vascular tissue, caused by *Ralstonia solanacearum*.

d. Soft rot

Soft rot, caused by several types of bacteria, but primarily subspecies and pathovars of *Erwinia carotovora* and *E. chrysanthemi*, is a widespread and destructive disease of fleshy fruits, vegetables, and ornamentals throughout the world. Other bacterial species that cause soft rot include *Pseudomonas cichorii*, *P. marginalis*, and *P. viridiflava*. Soft rot losses may occur in the field, garden, greenhouse, or after harvest during transit, storage, or marketing.

The symptoms of soft rot are similar on most plants. The disease starts on leaves, stems, and underground parts as small, water soaked, translucent spots (lesions). These rapidly enlarge in both diameter and depth. Bacterial soft-rot symptoms are caused primarily by secreted pectinases that degrade pectin in the middle lamella and primary plant cell walls, macerating the plant tissues, and causing a wet, often foul-smelling rot of the plant organ. The bacteria tend not to degrade cell walls completely; thus, outlines of the cells can still be seen in macerated tissues. The bacteria reach high concentrations in the xylem and can cause necrosis in the vascular tissue. Soft rot is typically seen on plant storage organs, such as tubers, rhizomes, and bulbs. The host tissue softens and becomes mushy or watery. Slimy masses of bacteria and cellular debris frequently ooze out from cracks in the tissues. Within 20 to 72 hours, entire fleshy fruits, roots, tubers, stems and rhizomes, bulbs, corms, buds, leafstalks, and leaves may rot and collapse, sometimes leaving only the outer "skin" intact. Decaying tissue, which may be opaque, white, cream-colored, gray, brown, or black frequently gives off a characteristically putrid odor. The odor is caused by secondary invading bacteria that are growing in the decomposing tissues.

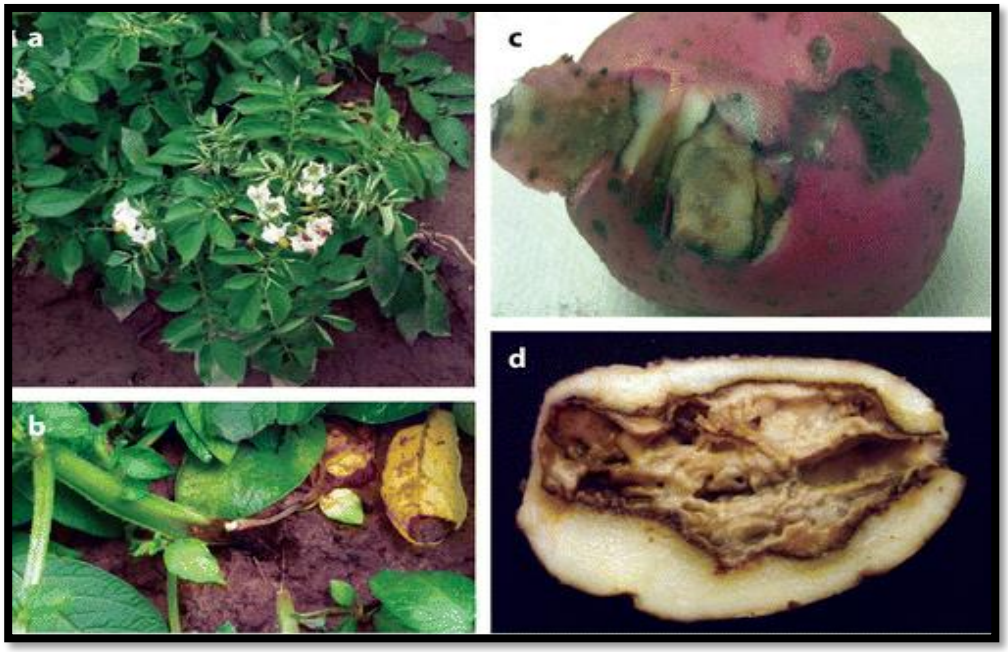


Figure09: Typical soft-rot bacteria symptoms on potato. (a) Potato plant leaf curl and wilting due to *Dickeya dianthicola*. (b) *D. dianthicola blackleg* on potato. (c) Potato tuber infected with *Pectobacterium*. (d) Potato tuber infected with *Pectobacterium* that entered the

tuber through the stolon. The bacteria are unable to decay the periderm; thus, the tuber becomes a protected anaerobic environment.

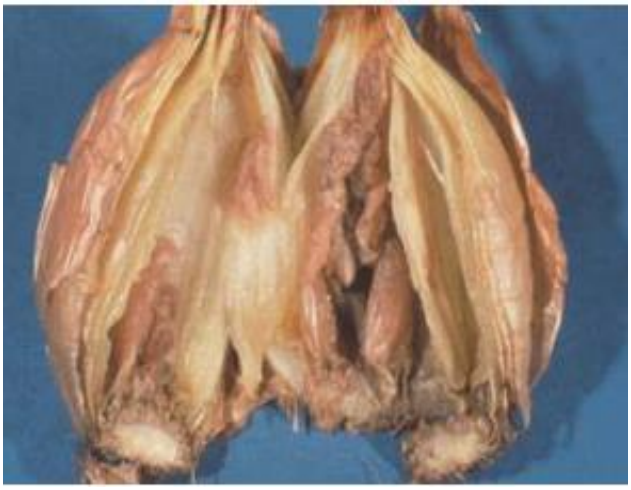


Figure 10: Bacterial soft rot of green onions.

Figure 11: Bacterial soft rot affecting cabbage plant.

c. Bacterial Canker and necrosis

Bacterial canker is a potentially serious disease of tomato that can occur in commercial plantings and residential gardens. This infectious disease is caused by the bacterium *Clavibacter michiganensis subsp. michiganensis* it's capable of spreading rapidly, resulting in devastating losses. Infected seedlings may not show symptoms until after transplanting. Wilting is often the first symptom observed on mature plants. Later, infected stems split, resulting in open cankers that give this disease its name. When cut lengthwise, the vascular system of diseased stems has a reddish- brown discoloration. Stem centers (pith) may be discolored and grainy or pitted. As symptoms progress, marginal browning, or necrosis becomes evident on older leaves. Secondary foliar infections from rain splashed bacteria develop circular, somewhat raised lesions with brown centers and white-tan margins. The primary fruit symptom appears as raised lesions with white margins. These "bird's eye" spots, which can reach 1/4 inch in diameter, reduce fruit quality. A yellow to brown internal breakdown of fruit can occur when the bacterium invades the fleshy tissue.

Figure 12: Wilting of tomato plants.

Figure 13: Stem canker and pith necrosis.

Figure14: Marginal necrosis of older leaves (often called "firing") occurs as a consequence of foliar infections and the infected fruit develop "birds-eye" spots that reduce fruit quality

d. Bacteria embedded in slime

Bacteria do not form fructifications on or in the host as fungi do. They are only visible as masses embedded in slime, which protrude from the tissue, either spontaneously or after cutting or wounding of the tissue. For some diseases, like fire blight or brown rot of potato, this slime may have diagnostic value. Bacterial slime may be present as very thin threads, so-called strands. The colour of the slime is often grey, but may be also yellowish or orange.

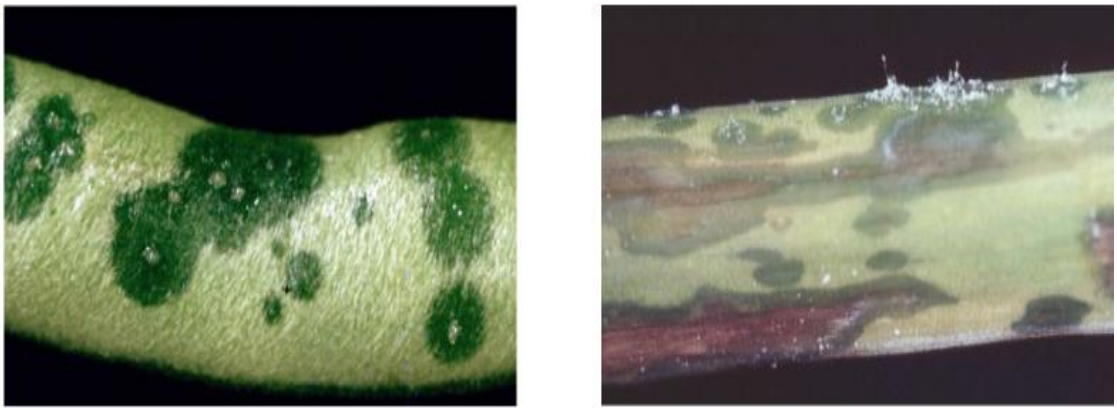


Figure 15: Left: Droplets of slime on water-soaked spots, in an infection of *Pseudomonas syringae pv. phaseolicola*, causing halo blight of bean (*Phaseolus vulgaris*). **Right:** Strands of bacterial slime (that can be spread by wind) on water-soaked spots, caused by Pseudomonas *syringae pv. porri* on leek (*Allium porrum*).

2. BASICS OF BACTERIA CLASSIFICATION

2.1. Taxonomy

Taxonomy is a scientific activity trying to create order in a complex diversity of organisms. In bacteriology taxonomy comprises:

1) Classification, orderly arrangement of organisms in entities, sub-entities, etc.

2) Nomenclature, giving names or labels to entities defined in 1), in agreement with accepted rules laid down in the International Code of Nomenclature of Bacteria of 1975.

3) Identification of unknown organisms with entities defined and named in 1) and 2).

In the taxonomy of bacteria morphological, serological, and metabolic (nutritional) characteristics (so-called phenotypic characteristics) are used traditionally. More recently, characteristics of the genetic material itself are also used (genotypic characteristics), such as base composition of DNA, usually G (uanidine): C (ytosine) ratios and degree of homology of total DNA and/or RNA of different bacteria. DNA and RNA hybridization show how much similarity there is in DNA or RNA sequences of the organisms that are compared.

2.2. Morphological identification

Generally plant pathogenic bacteria produce round, raised, glistering non pigmented or pigmented colonies on nutrient agar medium or specialized medium. The colony type with colony pigment is an important characteristic of the bacterial genus and species of plant

pathogenic bacteria. Colonies with a markedly different appearance can be assumed to be either a mixed culture or a result of the influence of the environment on a bacterial culture which normally produces known colony characteristics or a newly discovered species. The features of the colonies on solid agar media include: their shape (circular, irregular or rhizoid), size (the diameter of the colony: small, medium, large), elevation (the side view of a colony: elevated, convex, concave, umbonate/umbilicate), surface (how the surface of the colony appears: smooth, wavy, rough, granular, papillate or glistening), margin/border (the edge of a colony: entire, undulate, crenated, fimbriate or curled), colour (pigmentation: yellow, green among others), structure/opacity (opaque, translucent or transparent), degree of growth (scanty, moderate or profuse) and nature (discrete or confluent, filiform, spreading or rhizoid). Cell shape has also been used in the description and classification of bacterial species.

The morphological characters for identifying bacteria are few and limiting. This not only provided a challenge, but also an opportunity for creativity. Gram staining those bacteria could be differentially classified as either Gram positive or Gram negative, a convenient identification and classification tool that remains useful today. Although there are few morphological traits, and little variation in those traits, identification based on morphology still has significant taxonomic value.

2.3. Physiological Characterization of Bacteria (Effect of Environmental Conditions on Growth of Bacteria)

Temperature is one of the most important physical factors affecting microorganisms. Termal death point (for plant pathogenic bacteria usually 50-55ºC, when kept for 10 minutes at this temperature in liquid medium); growth at different levels of NaCl, 2.5 and 7%. The pattern of antibiotic resistance may also be determined. Testing sensitivity to an antibiotic is important when this antibiotic is used to control a particular bacterium in the field (streptomycin against *Erwinia amylovora*). Furthermore screening for toxin production and ice-nucleation activity will yield additional characteristics useful in the identification of plant pathogenic bacteria, especially those belonging to the *Pseudomonas syringae* group and *Clavibacter* spp.

2.3.1. Effect of pH on the bacterial growth

Bacteria infecting the plant system multiplies in the plant tissues having plant sap that has specific pH. The plant sap of different plants may have some variation which either promote

or inhibit the bacterial growth. Therefore, the requirement of pH is an important aspect in the studies of bacterial plant pathogen. Although specific pH range for bacteria is between 4 and 9, the optimum growth usually occurs between 6.5 and 7.5.

2.4. Biochemical Tests

Metabolic reactions that release energy (ATP) from the breakdown or degradation of a substrate (complex organic molecules) are called catabolic reactions, while the ones that use energy to assemble smaller molecules and produce biosynthetic building blocks are called anabolic reactions and when there is involvement of both the reactions, catabolism and anabolism in biochemical pathways, it is called as amphibolic reactions.

All these biochemical reactions that occur both outside and inside the cell are precisely controlled by some governing factors the enzymes. An enzyme is a biological catalyst, a substance that accelerates the rate of a specific chemical reaction. The enzymes are either exoenzymes (extracellular) or endoenzymes (intracellular). Exoenzymes, which are a few in number, are released from the cell and act on the substrates. These are mainly hydrolytic enzymes that degrade, by the addition of water, high-molecular weight substrates (like polysaccharide, lipids, and proteins) into smaller components (glucose) that can enter into the cell and are later assimilated. Enzymes required for the hydrolysis of cellulose, starch, pectin, lipid, casein, and gelatin belong to the category of exoenzymes. Endoenzymes are utilized by the cell for further metabolic degradation of carbohydrates and are mainly responsible for synthesis of new protoplasmic requirements and production of cellular energy from assimilated materials.

Some commercial applications of testing for acid formation from C and N sources use miniaturized test tubes and the results can be obtained in 24-48 h (API system strips). Again other systems use oxidation-reduction reactions to determine C and N utilization in a miniaturized (ELISA-plate) format (BIOLOG system). Other biochemical tests determine the formation of certain end-products by the bacteria, formation of H_2S from cysteine, indole from tryptophane, NO_2 or NH_4 from nitrate (NO_3). Effects of enzymes can also be visualized directly or after staining on certain (agar) media containing the substrates for the enzymes, hydrolysis of pectin, gelatin, starch and casein by pectinases, gelatinase, amylases and casease, respectively, or hydrolysis of 41 cellulose by cellulases or fats/fatty acids by lipases (esterases), respectively. When all the tests mentioned in 1-4 have given a certain pattern, this pattern is compared with those of plant pathogens described earlier.

Furthermore it may be necessary or fruitful to compare the isolated bacteria with a living reference culture of the pathogen in the tests performed.

2.5. Identification of Bacteria by Using the Immunodiagnostic Technique

Modern biological sciences have developed efficient and precise biochemical and immunological techniques for the diagnosis of various important diseases, thus replacing more conventional methods. Immunodiagonostic techniques are used in the identification of plant pathogenic bacteria in the laboratory or directly under field condition to identify the pathogen. These are specific in the identification of plant pathogen where specialized antibodies or antiserum are used.

2.5.1. Serological techniques

Serological techniques are often used for detection, mainly immuno-fluorescence (IF) and the enzyme-linked immune-sorbent assay (ELISA); for a description of these techniques, and also the immunofluorescence-colony (IFC) staining. Advantages of these techniques are that they 1) are less time-consuming; 2) are simple and robust; 3) have a fairly high detection level (e.g. the IF test has a detection level of 10^3-10^4 cells. ml^{-1}); and 4) have possibilities for screening many samples and automation. A disadvantage of ELISA is its higher detection level (10^5-10^6 cells. ml^{-1}). Both IF and ELISA have the disadvantage of showing disturbing cross-reactions of non-target bacteria with antisera used. Some newer reagents have been developed to solve this problem, such as monoclonal antibodies or polyclonal antibodies against specific antigenic determinants. Cross-reactions, however, cannot be ruled out completely with these new approaches. In some detection methods antibodies are used in combination with nucleic acid methods mentioned below and/or with magnetic capture. In the latter case magnetic beads, which are coated with antibodies, are used to trap the bacteria present in complex extracts of soil, plant tissue. The following methods and techniques are mostly used:

2.5.2. Agglutination test

On a microscopic slide if antibodies (in antiserum) and a suspension of intact bacteria are mixed in a certain concentration they will clump together and an agglutination reaction takes place. In the so-called latex agglutination test antibodies are coated to sensitized latex beads. In this way the antibodies are enlarged so to say, and the reaction is more readily visible, making the test more sensitive than slide agglutination. Unfortunately the agglutination test is often liable to disturbing cross-reactions. New is the development of so-called lateral flow kits (by CSL, York, UK) where an agglutination test can be performed with a kit in the field on symptomatic material.

2.5.3. Immuno-fluorescence (IF).

This is a very sensitive and robust serological test (detection level of c. 10^3-10^4 cells.ml^{-1} of plant extract) because the primary reaction of antigen and antibody is made visible. Binding reactions can be observed at very high titres (titre = highest dilution of the antiserum where a clear reaction is still visible) of antiserum. In the IF test antibodies are marked with a chemical dye that fluoresces in blue light, mostly fluoresce in isothiocyanate (FITC). For IF a light microscope fitted for epi-fluorescent light is necessary with the suitable excitation and barrier filters for FITC. In so-called direct IF antiserum against a certain plant pathogen is already labelled with FITC. In indirect IF the bacteria are first treated with a pathogen-specific rabbit or mouse antiserum (against the target bacterium). After incubation and washing, a second, labelled anti-rabbit or anti-mouse serum, prepared in another animal (e.g. goat), is applied. This anti-rabbit or anti-mouse serum is called the conjugate. Only the antibodies bound to the bacteria will fluoresce, while the others are removed by washing. Indirect IF is slightly more sensitive and less specific than direct IF.

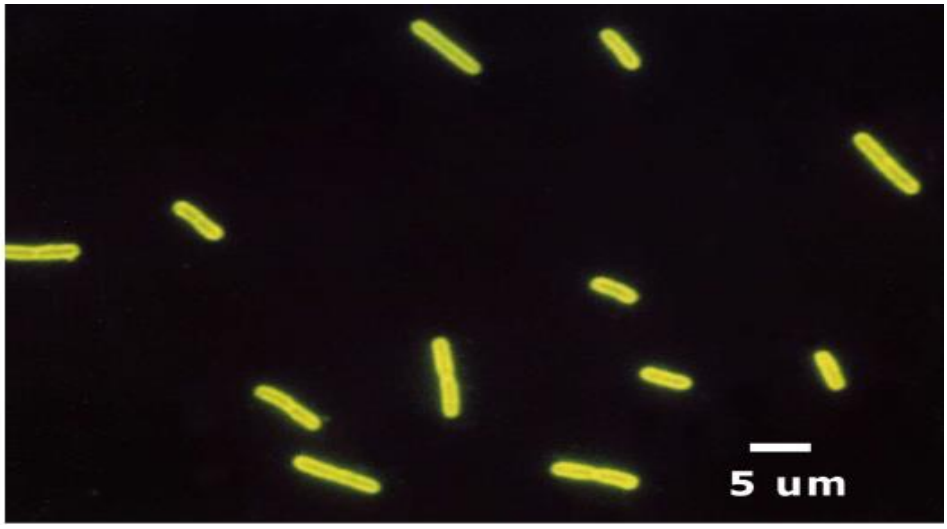


Figure16: Principle of the immunofluorescence (IF) test, often used for detection and identification of plant pathogenic bacteria.

2.5.4. Enzyme-linked immuno sorbent assay (ELISA)

Specific antibodies can be estimated quantitatively by ELISA. After incubating the test serum in an antigen-coated polystyrene tube or plate. Basically antibodies are first adsorbed (coated to) in the wells of a plastic ELISA plate. Bacterial antigens in buffered sample solution are subsequently pipetted in the wells and trapped by the coated antibodies. After incubation and washing an enzyme-labelled antiserum (conjugate) is added.

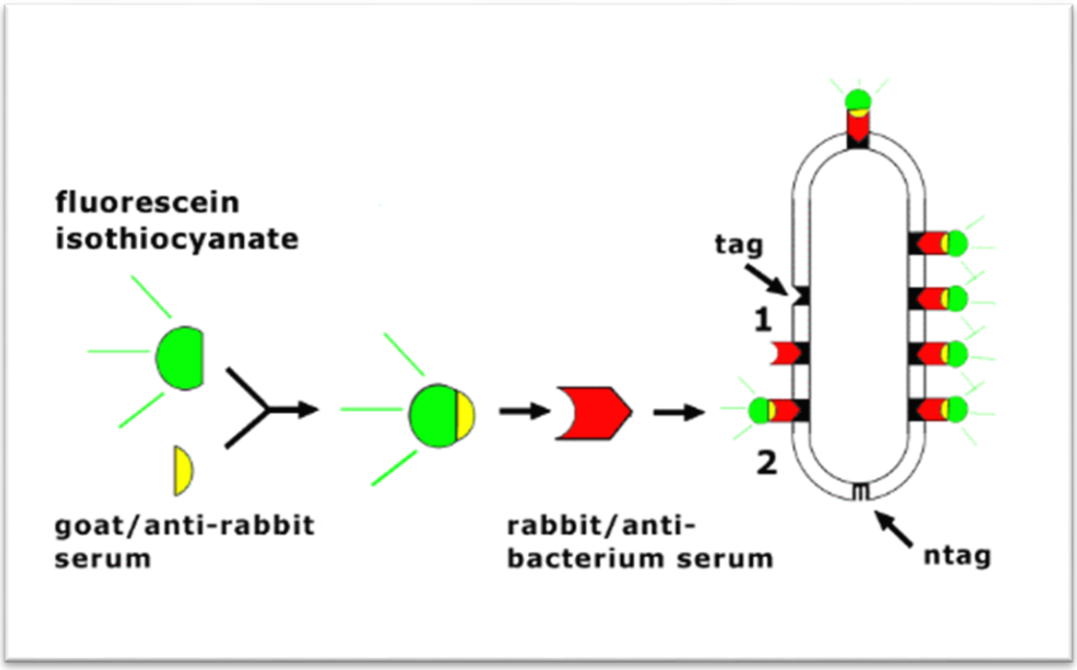


Figure 17: Principle of serology and cross-reacivity of polyclonal antisera. Antigens present in the cell wall of the bacterium (represented here as small sphere, square, etc. on the cell wall), provoke the production of antibodies in an animal (here: rabbit), when this animal is injected with a suspension of the bacterium. The antibodies are specific against the antigens and therefore against the bacterium.

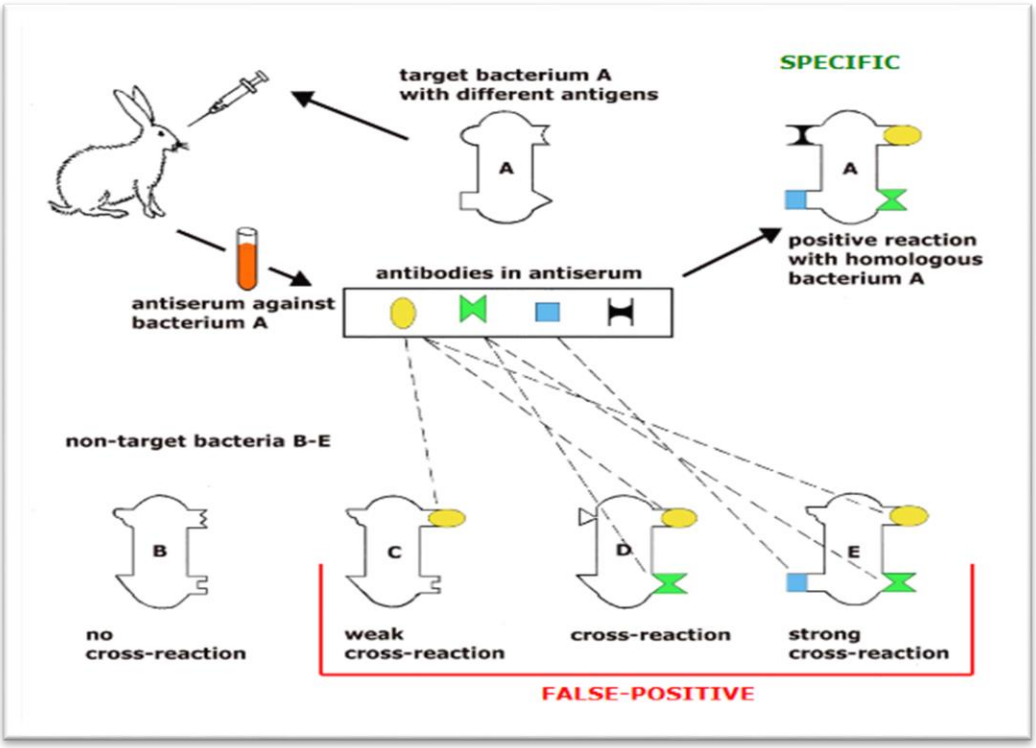


Figure 18: Principal of serology and cross reactivity of polyclonal antiserum.

2.6. Matrix-assisted laser desorption/ ionisation time-offlight mass spectrometry (maldi-tof ms)

A rapid, high-throughput identification method, MALDITOF MS, has been introduced in bacterial taxonomy. MALDI-TOF is the only polypeptide fingerprinting-based methods even to be used for bacterial identification. The MALDI-TOF MS technique offers easily determinable peptide/protein fingerprints for the identification of bacterial species. This technique has the ability to measure peptides and other compounds in the presence of salts and to analyse complex peptide mixtures, making it an ideal method for measuring non-purified extracts and intact bacterial cells. Bacterial cultures to be queried are spotted on the MALDI-TOF plate which is placed in the time-of-flight (TOF) chamber. Each sample is spotted at least in duplicate, to verify reproducibility. A control specimen of known identity is included to ensure correct identity. The samples are allowed to air-dry at room temperature, inserted into the mass spectrometer and subjected to MALDI-TOF MS analysis.

2.7. Biological tests

To confirm a diagnosis and prove that the isolated pathogenic agent is really responsible for the symptoms observed, artificial inoculations of the bacteria are done on a host plant in conditions favourable to the infection

2.7.1. Koch's postulate

Koch's postulate specifies the successive steps that have to be validated to establish a causal relationship between a disease and a micro-organism. The steps of Koch's postulate applied to phytopathology are as follows:

1. The micro-organism must be present in affected plants, and absent in unaffected ones;

2. It must be possible to isolate the micro-organism from the diseased plants and grow it in an axenic culture;

3. When the micro-organism in pure culture is inoculated to a normal plant, it must induce symptoms characteristic of the disease;

4. It must be possible to re-isolate the initial micro-organism as from plants infected experimentally.

In practice, the diagnosis of several known diseases do not necessitate all the stages of the Koch's postulate. In each case, it is possible to stop at the observation or isolation stage.

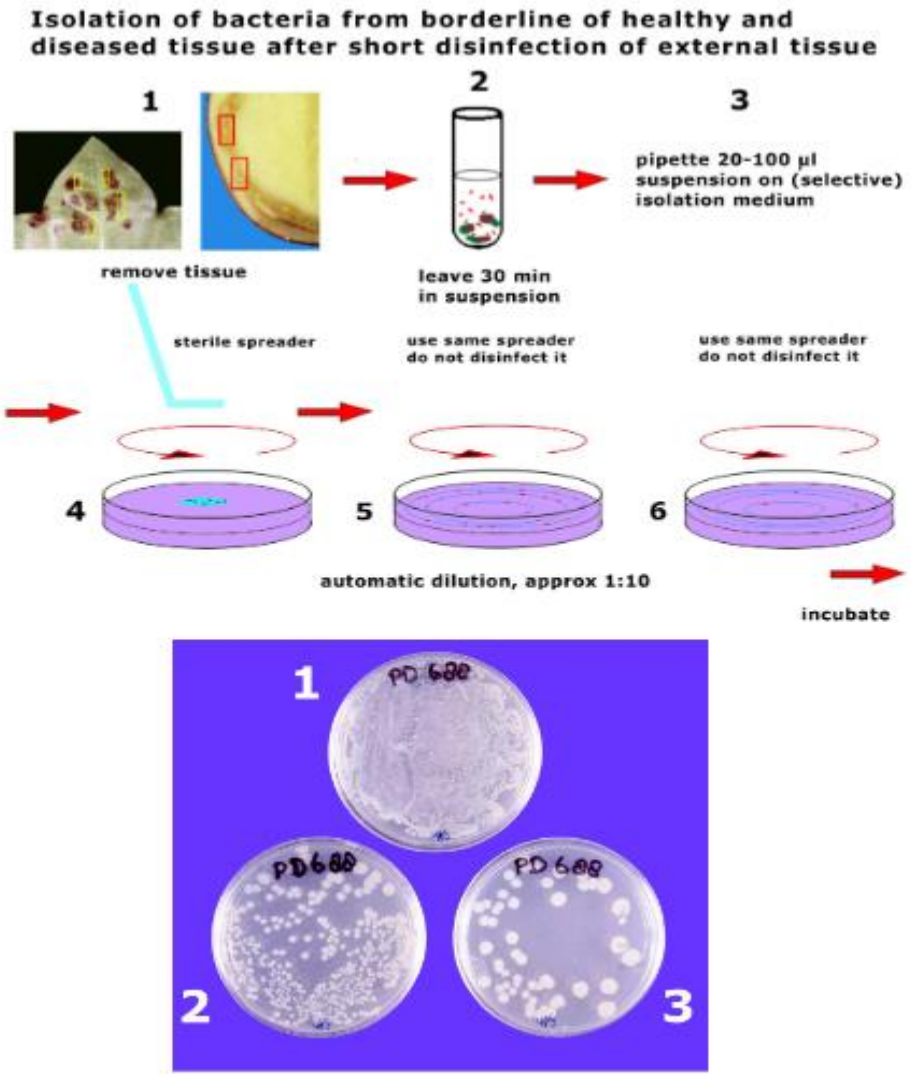


Figure 19: Isolation procedure from symptomatic material and the result.

2.8. Identification of Bacteria by using Molecular Techniques

Identification of various strains of plant pathogens has traditionally been based upon microscopic examination of morphological and biochemical characters and growth characteristics of the pathogen on specific media. Variations in the morphological characters exist and lead to difficulties in accurate identification by traditional methods. Accurate and rapid characterization is important for precise diagnosis of related plant pathogens and understanding of the population structure and genetics of the pathogen for several reasons.

Technological advances in PCR-based methods enable fast, accurate detection, quantification and characterization of plant pathogens and are now being applied to solve practical problems. In plant pathogenic bacteria the variation exits at molecular or genetic level in a given pathovar itself and therefore the races exit in the given pathovar of the bacterium, which cannot be identified by cultural, physiological, or biochemical characteristics. For example, the use of molecular techniques in bacterial taxonomy allows different taxa of etiologically significant bacteria to be separated. Therefore, molecular diagnostics can provide the degree of discrimination needed to detect and monitor plant diseases, which is not always obtained by other types of analysis. To study the molecular variability the molecular techniques like hybridization-based techniques, PCR-based techniques, and guanine-plus-cytosine-ratio–based techniques are generally used.

2.8.1. Sequence analysis of the 16s RNA gene

Analysis of ribosomal RNA genes is a suitable tool for bacterial species identification and taxonomic categorisation. Moreover, ribosomal RNA genes are conserved but have sufficient variation to distinguish between taxa. In prokaryotes, ribosomal RNA genes occur in copies of three or four in a single genome. The 16S rRNA gene has become a reliable tool for identifying and classifying bacteria. Analysis of the 16S rRNA gene requires that this gene be amplified by polymerase chain reaction (PCR) and the resultant PCR product sequenced. The gene sequence can then be matched with previously obtained sequences obtainable from various DNA databases. This method has been so widely adopted that DNA sequence database databases are flooded with sequences of the 16S rRNA gene. Almost all new sequences deposited for query have matches and any 16S rRNA gene copy which does not match any known bacterial species is believed to be new.

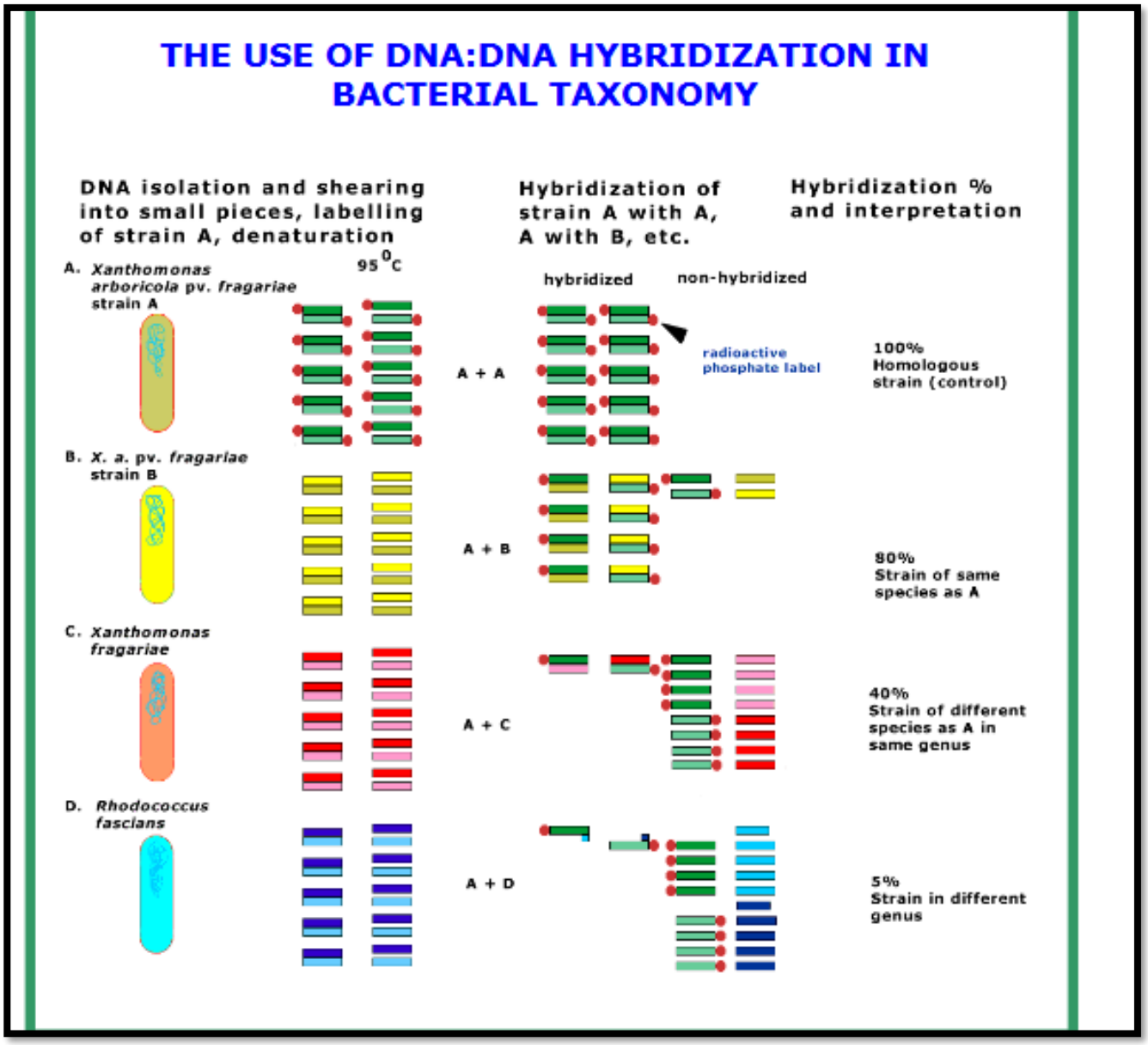


Figure 20: The use of genomic DNA: DNA hybridization in bacterial taxonomy.

2.9. Phage typing

forgiveness to systematic phage typing, epidemiologists can trace the lineage of cases, go back to the very source of the contamination or to the different sources, suspect the responsibility of a germ carrier in the outbreak of bacilli are all of the same phage type, or on the contrary demonstrate his non-responsibility if they are of a different phage type. They can thus take, with full knowledge of the facts, the most appropriate measures for the outbreak or epidemic they are studying to stop its spread and sometimes even achieve its total eradication in a given region.

2.9.1. Bacteriophages

Phages are specific viruses of bacteria that subvert the metabolism of their bacterial hosts in order to replicate. Of the phages that have been identified, the majority belong to the tailed phages; and these form the Taxonomic Order: Caudovirales. These phages possess icosahedral heads containing genomes comprised of double stranded DNA. The order Caudovirales is made up of three phage families; Myoviridae which have rigid contractile tails, Podoviridae with short, non-contractile tails and Siphoviridae with long flexible tails. Phages belonging to other families have highly variable morphologies with genomes of varying nucleic acid composition. Bacteriophages may have different life cycles in natural environments. This includes a lytic life cycle, where a bacteriophage infects its bacterial host cell and rapidly induces its breakdown and a lysogenic cycle, where they are able to integrate their injected DNA into the bacterial genome. Bacteriophages have been used as tools to identify and characterize phytopathogenic bacteria. The infection cycle of bacteriophages is begun by the adsorption of bacteriophages with the special receptors located in the cell surface of susceptible bacteria. Upon irreversible attachment, they inject their genomic DNA (gDNA) into the host cell cytoplasm. In terms of lytic replication cycle, after gDNA injection, bacteriophages utilize ribosomes of the host bacterium to manufacture phage proteins. The bacterium also provides resources for bacteriophage genome replication and production of virion-related protein components. At the late stage of the lytic replication cycle, bacteriophages encode holins and lysins, known as endolysins, to lyse the bacterium for release of the phage progenies. In contrast to the lytic cycle, the lysogenic replication cycle has been known as the integration of bacteriophage genome into the bacterial cell chromosome-termed as prophage-or existence as an episomal element, and then they replicate and transfer to daughter bacterial cell. Prophages can spontaneously alter to a lytic cycle and kill their host spontaneously due to certain environmental stresses, metabolic condition of host bacteria, or antibiotic treatment.

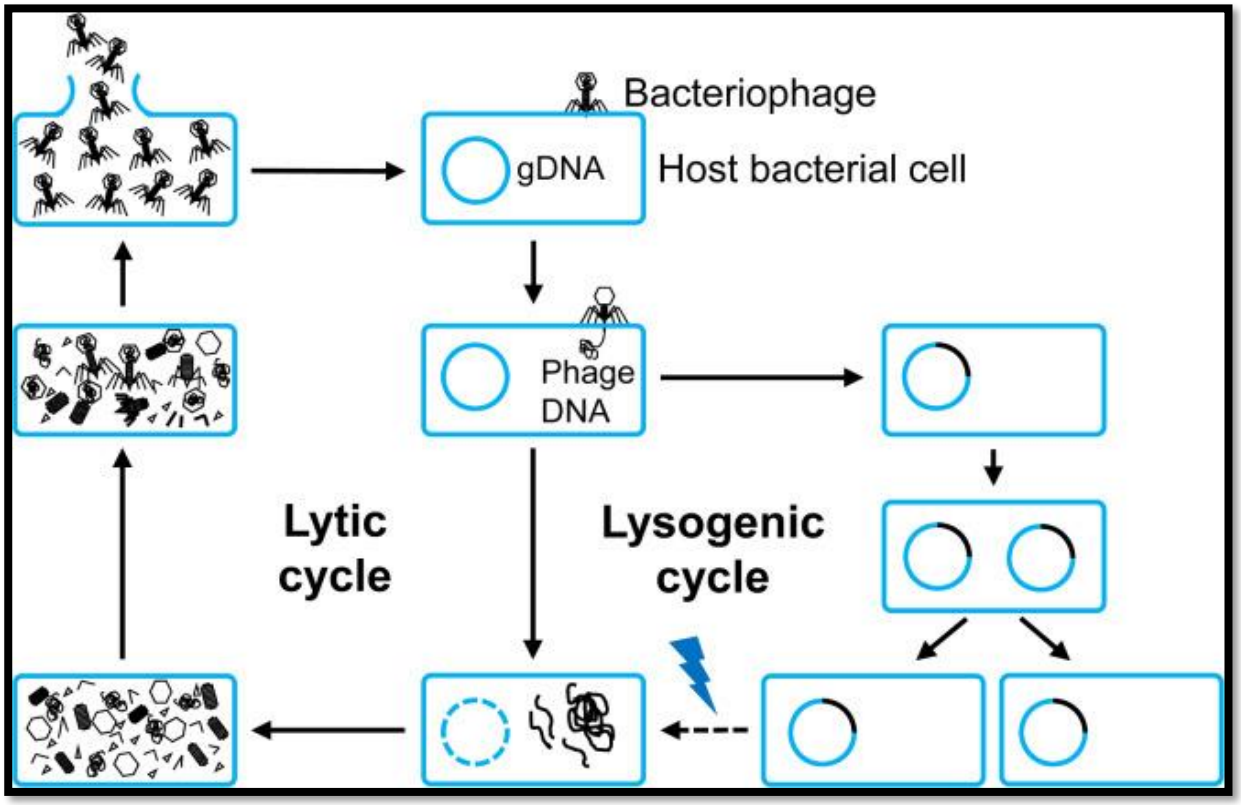


Figure 21: The general life cycles of bacteriophages. They begin with the adherence of a bacteriophage into the host bacterial cell, then the translocation of its genetic material (phage DNA). Based on the destination of the genetic material, a bacteriophage comes to either lytic or lysogenic cycles. In the case of a lytic cycle, a bacteriophage multiplies and lyses the host cell, while in the case of a lysogenic cycle, its genetic material is integrated into bacterial genomic DNA (gDNA). Under certain conditions (indicated by the lightning), a lysogenic cycle can be converted to a lytic cycle.

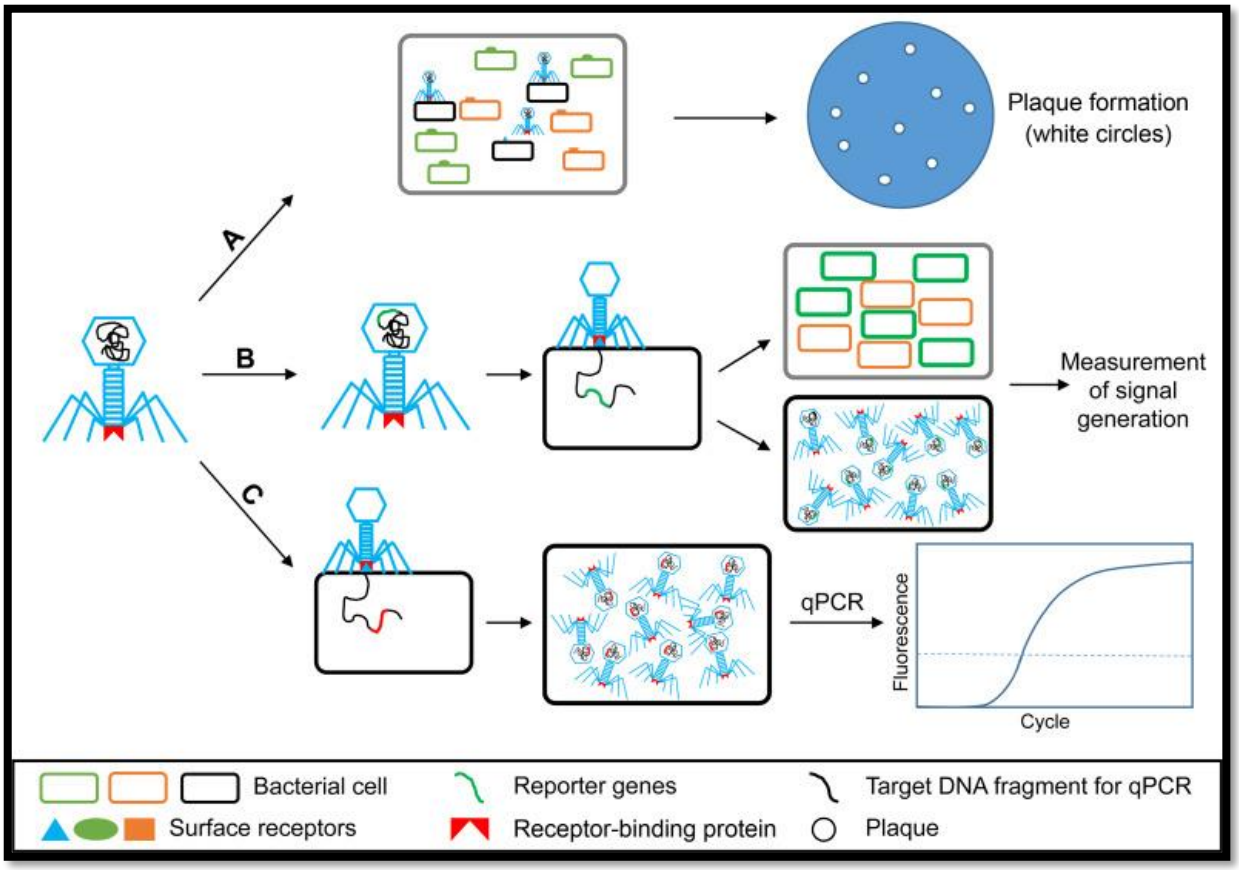


Figure 22: The working models of bacteriophages to detect plant-pathogenic bacteria. A, phage typing: traditionally employed a specific phage for identification and characterization of different pathogenic bacteria based on its lysis activity (Singh et al., 2012); B, reporter phages: engineered phages are used as an importer of marker gene that makes target bacterial cells detectable; C, phage progeny-based detection: using specific bacteriophage to generate rapid amplification of progenies before detection by quantitative PCR (qPCR).

2.10. The binomial system

The bacteriological code uses the binomial system. The first name beginning with a capital indicates the genus, Xanthomonas, the second name the species epithet, citri (no capital). When the exact name has to be mentioned, the author(s) giving the first description of the bacterium with the prefix 'ex', and those who made the last taxonomic (re-) classification are also mentioned, together with the year of publication, i.e. *Xanthomonas axonopodis pv. citri* (ex Hasse 1915) Vauterin, Hoste, Kersters and Swings 1995. When only the genus is known, this is indicated as *Xanthomonas* sp. (= species). The most commonly used classification and

nomenclature of bacteria can be found in Bergey's Manual of Determinative Bacteriology, now named Bergey's Manual of Systematic Bacteriology. Also see Young et al., 1992 and http://www.isppweb.org/names_bacterial.asp. Up-to-date nomenclature can also be found at http://www.dsmz.de/bactnom/bactname.htm of the German Culture Collection of Microorganisms. (DSMZ) in Braunschweig, Germany and the site maintained by J.P. Euzéby, http://www.bacterio.cict.fr/

Kingdom: Procaryotae

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

Division: Bacteria – Gram-positive

Class: Proteabacteria – 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.

2.11. Pathogenesis of bacteria

Microbial pathogenicity has often been defined as the biochemical mechanisms where by pathogenic microorganisms cause disease in a host organism. Microbial virulence is defined as the degree or measure of pathogenicity shown by one or more plants. Pathogenicity and/or virulence of Gram-negative plant pathogenic bacteria are strictly dependent on the presence of secretion apparatuses in host cells, through which they secrete proteins or nucleoproteins involved in their virulence within the apoplast or inject these substances into host cells.

Bacterial pathogens contain several classes of genes, called virulence genes, that are essential for causing disease or for increasing virulence in one or more hosts. Pathogenicity factors that are encoded by pathogenicity genes (pat) and disease-specific genes (dsp) are crucially involved in the establishment of diseases. Some of these genes are essential for the recognition of a host by a pathogen, attachment of a pathogen to a plant's surface, formation of infection structures on or within the host, penetration of the host, and colonization of host tissue. The pathogenicity genes that are involved in the synthesis and modification of the lipopolysaccharide cell wall of Gram-negative bacteria may help condition the host range of a bacterium. Plant pathogenic bacterial virulence factors are associated with the bacterial surface or secreted into the surrounding environment. Proteins secreted by bacteria are

transported via molecular systems out of bacterial cells; unrelated virulence factors often share the same secretion mechanism. Properties that determine phytopathogenicity are:

a) Toxigenicity: This is the ability to produce toxic substances, such as exotoxins, excreted by living bacteria in the tissue (glycoproteins, lipoproteins, and polysaccharides) and endotoxins. The latter are mostly parts of the bacterial cell wall, which are only liberated after death of the bacteria. Toxins of plant pathogenic bacteria are generally non-host- specific and usually there is a direct relation between the toxin produced and a particular symptom. Examples of toxins produced by plant pathogenic bacteria are: Chlorosis inducing dipeptides, tabtoxin produced by *Pseudomonas syringae* pv. *tabaci*, *P.* pv. *coronofaciens* and some other pathovars; Cyclic lipodepsipeptide compounds, syringomycins and syringostatins causing necroses, produced by *Pseudomonas syringae* pv. *syringae*. The glycoprotein toxin causing white necrotic spots on leaves, produced by *Clavibacter michiganensis* subsp. *michiganensis*

b) Extracellular polysaccharide (EPS): Amylovorin of *Erwinia amylovora*. Furthermore production by plant pathogenic bacteria of growth-influencing hormones, such as indole acetic acid (IAA) and cytokinins may play a role in symptom formation, as has been determined for e.g. *Pseudomonas savastanoi*, *Rhodococcus fasciens* en *Agrobacterium tumefaciens*.

2.12. Resistance

Resistance of a plant against a bacterial pathogen can be based on a gene-for-gene relationship, for example between a resistant cultivar and a virulent strain or variety of the pathogen. In this case there is interaction between avirulence genes (so-called avr genes) of the bacterium and resistance gens of the plant, which cause a hypersensitivity reaction (HR). The time lapse of a HR reaction is very short (several hours), the tissue disintegrates, becomes necrotic and the bacteria are encapsulated and killed. The HR can be divided into three stages, namely: induction, latent period and disintegration of tissue and necrosis.

Hypersensitivity reactions are also involved in the interaction between non-hosts and plant pathogenic bacteria and between host plant and avirulent strains or varieties of the bacterial pathogens. Vertical resistance (or gene-for-gene resistance) mostly occurring between cultivars and pathogenic varieties or races of the bacterium. Horizontal resistance is based on many genes. The latter form of resistance is usually longer lasting, but is not complete and the genetic basis often not determined. Vertical resistance is found in cultivars of soybean against

pathogenic races of *Pseudomonas syringae* pv. *glycinea*; in bean against *P. s. pv. phaseolicola*, in tomato against *X. vesicatoria*, and in pea against *P. s.* pv. *pisi*

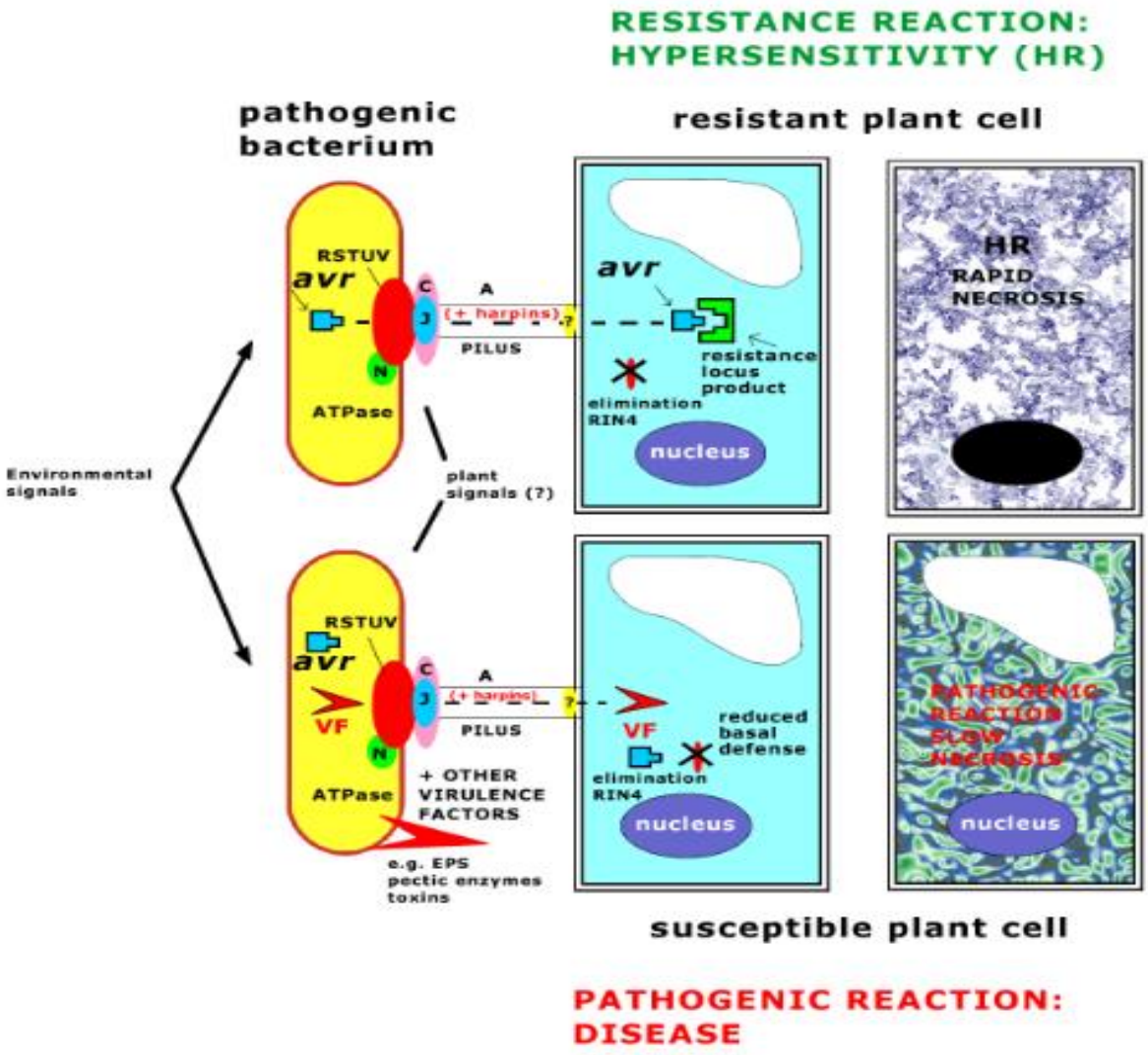


Figure23: Model for the function of so-called hrp genes and of their products.

Table 1: Interactions and reactions between bacteria and plants (after Kleinhempel et al., 1989, changed)

Bacterium	Plant	Reaction of plant	
		HR	Symptoms
Saprophyte	any plant	-	-
Potential pathogen	non-host plant	+	-
Pathogen (virulent)	susceptible host	-	+
	resistant host	+	-
Pathogen (avirulent)	resistant host	+	-
	susceptible host	+	-
Non-pathogenic mutant	susceptible host	+ (-)*	-
Soft rot bacterium	host and non-host	- (+)*	+ (-)*

HR: hypersensitivity reaction; **- :** no reaction; **+:** positive reaction; ***** both reactions occur, the more uncommon between brackets**.**

3. DESCRIPTIVE STUDY OF THE MAIN GENERA OF BACTERIA PHYTOPATHOGENS

Most plant pathogenic bacteria are intercellular (living between plant cells) and/or necrotrophic (living on killed cells) pathogens. Several plant pathogenic bacteria can survive or even multiply saprophytically in the natural environment. Bacteria can enter the plant through small wounds (caused by insects, hail or wind-blown sand), stomata, lenticels, hydathodes, nectarines. A number of them, especially vascular pathogens, are seed transmitted. The pathogenicity (based on virulence or aggressiveness of the bacterium) is determined by the ability of the bacterium to maintain itself in plant tissue and to multiply and often by the ability to excrete substances influencing the growth of plant cells (hormones) or that damage or kill cells. Plant pathogenic bacteria can be divided into xylem, phloem or parenchymal parasites according to their preference for vascular or parenchymatous tissue. Symptoms caused by different genera of plant pathogenic bacteria are: a) Leaf spots; b) Excrescences and galls; c) tumours; d) vascular diseases and wilting; e) necroses and cankers; f) rotting; g) bacteria embedded in slime.

3.1. The characteristics of the plant pathogenic bacteria of each genus

3.1.1. Agrobacterium

The bacterial cells are rod shaped, 0.6–1.0 × 1.5–3.0 μm in size and occur singly or in pairs. They are non spore-forming and Gram-negative. Motility occurs by one to six pertrichous flagella. They are aerobic, possessing a respiratory type of metabolism with oxygen. Some strains are capable of anaerobic respiration in the presence of nitrate. Most strains are able to grow under reduced oxygen tensions in plant tissues. Optimum temperature is 25–28°C. Colonies are usually convex, circular, smooth, and non pigmented to light beige. Catalase positive and usually oxidase and urease positive. *Agrobacterium* species are Gram-negative soil-borne bacteria that can be classified into three biovars based on physiological and biochemical properties, without considering their disease phenotype. The strains belonging to biovar 1 are designated as *A. tumefaciens*, those belonging to biovar 2 as *A. rhizogenes* and the biovar 3 strains as *A. vitis. Agrobacterium* spp. strains efficiently colonize plant roots producing galls in a large variety of plants. During bacterium-plant cell interactions in wounded tissues, tumorigenic strains have the ability to transfer a particular DNA segment from the Ti plasmid of the bacterium to the plant genome, and its expression in the transformed plant cell leads to the development of a crown gall tumour.

3.1.2. Pseudomonas

Pseudomonas a Gram-negative, rod-shaped, motility occurs by one or several polar flagella. In some species lateral flagella of shorter wavelengths may also be formed they are rarely non motile bacterium that can cause severe damage to many plant species, is a significant concern for plant health and crop production. They are aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor; in some cases nitrate can be used as an alternate electron acceptor, allowing growth to occur anaerobically. Xanthomonadins are not produced. Most, if not all, species fail to grow under acidic conditions (pH 4.5). Most species do not require organic growth factors. Oxidase positive or negative. Catalase positive and chemo-organotrophic; some species are facultative chemolithotrophs, able to use H2 or CO as energy sources. It is classified as a hemi biotrophic pathogen that initially feeds on living plant tissues and later causes the death of plant cells. The Pseudomonas phylogenetic group includes more than 60 pathovars and 15 recognized bacterial species. Each pathovar of Pseudomonas infects a distinct group of host plants and is known for its diverse host-specific interactions with the plants. Many species accumulate poly-b-hydroxybutyrate as carbon reserve material, which appears as sudanophilic inclusions. They do not produce prosthecae and are not surrounded by sheaths.

Pseudomonas syringae is responsible for a number of economically important diseases. This bacterium can infect a wide variety of fruits, vegetables, and ornamental plants. A variety of symptoms are associated with woody plants infected by *Pseudomonas syringae*. Symptoms and symptom development depend on the species of plant infected, the plant part infected. More than one symptom can be simultaneously on a single plant. It can ause:

Flower blast: flowers and/or flower buds turn brown to black.

- Dead dormant buds, common on cherries and apricots.
- Necrotic leaf spots.
- Discolored and or blackened leaf veins and petioles resulting from systemic invasion and infection.
- Spots and blisters on fruit.
- Shoot-tip dieback, which appears as dead, blackened twig tissue extending down some distance from the tip.

- Stem cankers: depressed areas in the bark, which darken with age. A gummy substance often exudes from cankers on fruiting and flowering stone fruits (this symptom is referred to as "gummosis"). If cankers continue to enlarge, they may girdle the stem and subsequently kill a branch or the entire plant. If the outer tissues of the canker area are cut away, the tissue underneath shows a reddish-brown discoloration. This discoloration may also occur as vertical streaks in the vascular tissue.
- Seed germination can also be inhibited.

3.1.3. Xanthomonas

The bacterial cells are straight rods, usually 0.4–0.7 × 0.7–1.8 μm in size, predominantly single. Do not produce poly-b-hydroxybutyrate inclusions. Do not have sheaths or prosthecae. No resting stages are known. Cells stain Gram-negative. Motility occurs by a single polar flagellum (except *X. maltophilia,* which has multitrichous flagella). Optimum temperature is 25–30°C. Colonies are usually yellow, smooth, and butyrous or viscid. The pigments are highly characteristic brominated early polyenes, or "Xanthomonadins" (except for *X. maltophilia*, which does not produce xanthomonadins). Oxidase negative or weakly positive. Catalase positive.

The genus *Xanthomonas*; is a well-studied group of plant- associated Gram-negative bacteria that belong to the family *Xanthomonadaceae* subclass Gammaproteobacteria. An estimated 27 species is pathogenic to approximately 400 plants. The life cycle of *Xanthomonas* has two stages: epiphytic and endophytic. The epiphytic stage starts once bacteria colonize the surfaces of new plant using adhesion ligands such as bacteria surface polysaccharides, adhesion proteins, and type IV pili. After colonization comes biofilm formation, which then protects the bacteria from environmental stress factors. The endophytic stage is characterized by bacterial entry into plant tissue via lesions or stomata and eventual movement throughout the vascular system. The bacteria re-emerge onto the plant surfaces once their population reaches the threshold, transmission occurs to new hosts and the infection cycle repeats. The plant pathogenic bacterium *Xanthomonas campestris* is the causal agent of the serious diseases that denominated Xanthomonas-leaf spot which has been recorded on many araliaceous plants worldwide. Bacterial leaf spot or blight disease important diseases of foliage and flowering ornamental plants as well as vegetables plants. These bacterial diseases may destroy leaves, petioles and stems rendering infected plants

unsightly and unsalable. The disease is characterized by circular, gray to black, water-soaked lesions on the leaves. The lesions coalesce, become irregular in shape

3.1.4. Streptomyces

Streptomyces is the largest genus of Actinobacteria and the type genus of the family *Streptomycetaceae*. Over 500 species of *Streptomyces* bacteria have been described. *Streptomyces* species are chemoorganotrophic, filamentous gram-positive bacteria but not acid-alcohol fast, not fungi and occur in the same habitats as fungi and are superficially similar. The filaments and spores are very small usually 1 μm or less in diameter. The spores are formed by the fragmentation of the filaments and are borne in straight, wavy, or helical chains. The colonies are slow-growing and often have a soil-like odour because of production of a volatile metabolite, geosmin. *Streptomyces* species are nonmotile, catalase positive, reduce nitrates to nitrites and degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and L-tyrosine.

The filamentous bacterium *Streptomyces scabiei* causes excrescences, called scab, on potato (sugar) beet, radish and carrot. The bacterium often penetrates the plant via young, not yet suberized lenticels or wounds. *S. scabiei* produces pectinases, enabling the hyphae to grow between cells. After plant cells have been killed, intracellular growth also takes place. Because *S. scabiei* does not produce toxins that rapidly kill plant cells, cork layer formation can take place in tissue at a distance from the lesions.

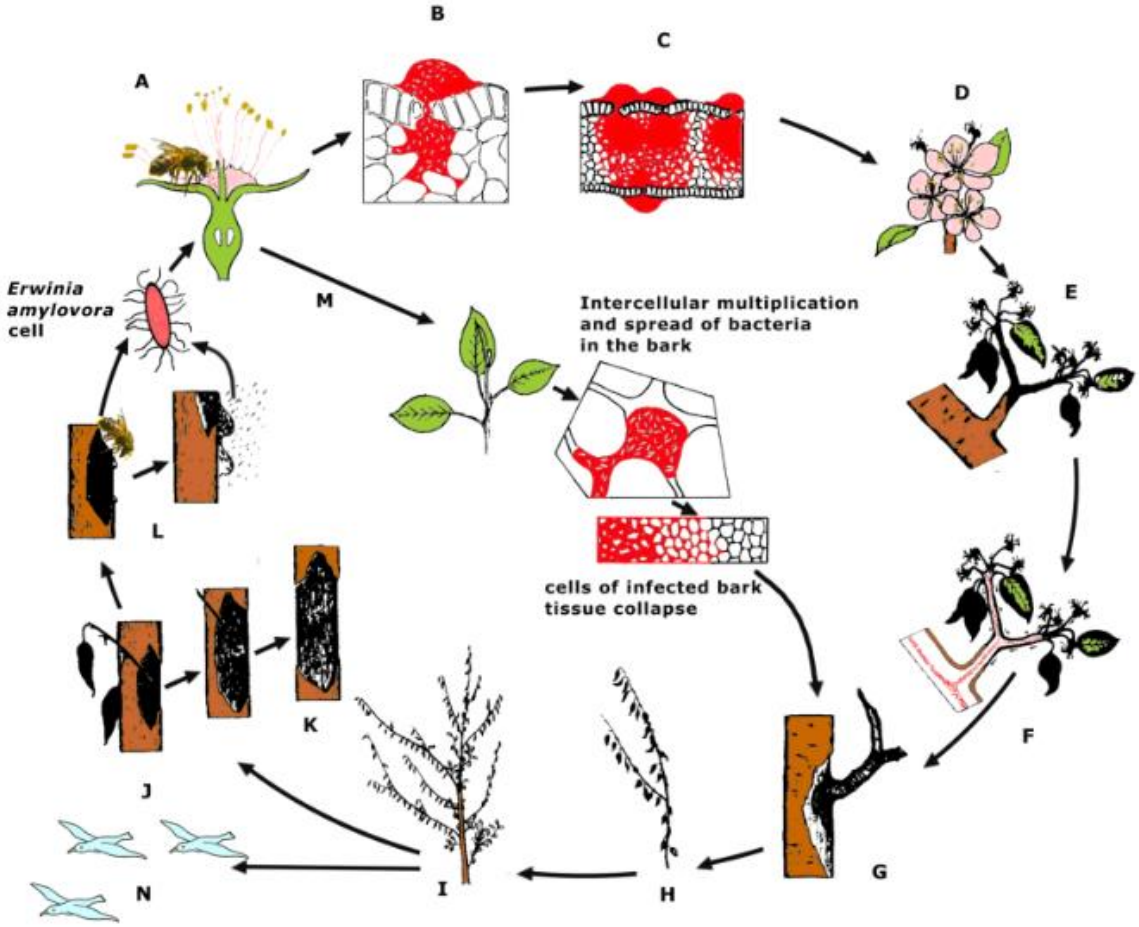
3.1.5. *Erwinia*

The bacterial cells are straight rods, 0.5–1.0 × 1–3 μm in size, occur singly, in pairs, and sometimes in short chains. Gram-negative. Motile by peritrichous flagella (except *E. stewartii*). Facultatively anaerobic. Chemoorganotrophic, having both a respiratory and a fermentative type of metabolism. Optimum temperature is 27–30°C. D-Glucose and other carbohydrates are catabolized with the production of acid; most species do not produce gas. Oxidase negative and catalase positive; they are lysine decarboxylase, arginine dihydrolase, and ornithine decarboxylase negative.

Three soft rot coliforms, *Erwinia carotovora* ssp.*carotovora* (Ecc), *E. carotovora* ssp. *atroseptica* (Eca) and *E. chrysanthemi* (Ech), they have recently been classified in the

resuscitated genus Pectobacterium as P.ssp. *carotovorum, P. carotovorum* ssp. *atrosepticum* and *P. chrysanthemi*.

The bacteria are Gram-negative, non sporing, facultative anaerobes, characterized by the production of large quantities of extracellular pectic enzymes. They rely mainly on the production of these enzymes together witha wide range of other plant cell wall-degrading enzymes to cause disease. Additional pathogenicity-associated characters have been identified, which could also be involved in the establishment of the bacteria in plant tissues and in a free-living or saprophytic life phase. They are the main cause of tuber decay in store



and blackleg or stem rot in the field.

Figure 24: In (A) honeybees transmit bacteria to flowers, where they subsequently penetrate nectarthode (nectaries) and leaves through wounds and stomata and start multiplication and spread through tissues. (B, C). The infected flowers become black, shrivel and die (D) and the infection spreads to other flowers, leaves and twigs of the same tree (E). Disease becomes extensive (F) and cankers are formed on branches and stems (G). Eventually whole

twigs or trees are diseased and killed (H, I). Bacteria may overwinter at margins of old cankers (J) that may enlarge and girdle trees (K). Bacteria in exudates on cankers can be spread by insects and rain (L) to flowers again and from flowers direct infection of young twigs is also possible (M), where spread to larger branches and stems can take place. Long-distancespread of bacteria in exudates on legs of birds has become very plausible for fire blight (N). After Goto (1992)

3.1.6. Clavibacter (Corynebacterium)

It was noted in Bergey's Manual of Systematic Bacteriology that the traditional genus Corynebacterium was heterogeneous and that there were proposals to divide it into several genera. A number of such proposals have now received general assent, and in particular the aerobic plant pathogens containing 2,4-diaminobutyric acid in the cell wall are treated under the new genus Clavibacter. The bacterial cells are straight or slightly curved, slender rods have tapered or sometimes clubbed ends and are 0.3–0.8 × 1.5–8.0 μm in size. One species (*C. Matruchotii*) has a whip handle–shape. Cells are usually arranged singly or in pairs, often in a V formation or in palisades of several parallel cells. Gram-positive, though some cells stain unevenly, giving a beaded appearance. Nonmotile, non sporing, non acid-fast. Facultative anaerobes, commonly requiring nutritionally rich media on which colonies are usually convex and semi opaque, with a mat surface. Bacterial canker disease is caused by *Clavibacter michiganensis* subsp. *michiganensis*. This pathogen is seed-borne, and is also able to survive in soil for more than two years in infected plant debris. Typical canker symptoms develop at 23-28°C, when relative humidity is 80 % or more). The first disease symptom in case of greenhouse grown tomatoes was the reversible wilting of leaves during hot mid-day hours. Later, wilting becomes irreversible and the whole plant dies. Under field conditions; the edge of the leaflets of lower leaves start drying out; and then small whitish pustules were produced on leaf veins and petioles. During the advanced stages of the disease, a reddish brown discoloration of the vascular tissue can easily be recognized at the union of stems and branches; petiole and peduncle. The disease affects the fruits, so they may fail to develop and fall, or ripen unevenly. The incidence of symptomless latent infections and the invasion of tomato seeds by *Cmm* are widespread. Pathogenicity is mediated by virulence factors and transcriptional regulators encoded by the chromosome and two natural plasmids. The virulence factors include serine proteases, cell wall-degrading enzymes (cellulases, xylanases, pectinases) and others.

3.1.7. *Xyllela*

The genus was created in 1987 by Wells et al. It includes only one species, *X. fastidiosa*. The species *X. fastidiosa* is a gammaproteobacterium within the family Xanthomonadaceae, order Xanthomonadales. There are four subspecies of *X. fastidiosa*: subsp. *fastidiosa*, *multiplex, sandyi*, and *pauca*. This classification is based on DNA relatedness and multilocus sequence typing (MLST) results.

X. fastidiosa are single, aflagellate, rod-shaped cells, with a rippled cell wall and estimated dimensions of 0.25–0.5 μm in diameter and 0.9–4.0 μm in length. These estimates were obtained from different subspecies and media conditions, in addition to cells colonizing plant tissue. Although cells are devoid of flagella, they possess both short (type I pilus) and long fimbriae (type IV pilus); in fact, it has been shown that type IV pili are responsible for twit Colonies are of two types: Convex to pulvinate, smooth, opalescent with entire margins and umbonate, rough with finely undulated margins. Cells stain Gram- negative. Non motile. Oxidase negative and catalase positive. Strictly aerobic, nonfermentative, non-halophilic, non pigmented. Nutritionally fastidious, requiring a specialized medium such as BCYE containing charcoal or glutamine-peptone medium (PW) containing serum albumin. Optimal temperature for growth is 26–28°C. Optimum pH is 6.5–6.9. Habitat is the xylem of plant tissue.ching motility in *X. fastidiosa*. These traits are also supported by genomic sequences.

The majority of *X. fastidiosa* diseases, irregular chlorosis evolves in mature leaves recognized by interveinal yellowing on the upper side of leaf and corresponding brownish gum like material over the side. Later on, brown spots coalesce and necrosis becomes evident, eventually leading to leaves dropping from branches. Zinc and iron like deficiency can be frequently observed in the affected leaves. Stunted trees show twig dieback and fruits reduce in size and harden, becoming unsuitable for the juice industry as well as for the fresh fruit market. Severely infected plants do not die but become economically non productive.

3.1.8. Ralstonia

Bacterial wilt caused by *Ralstonia solanacearum* was reported for the first time at the end of the 19th century on potato, tobacco, tomato and groundnut in Asia, southern USA and South America. The bacterium was described for the first time as *Bacillus solanacearum*. In the

years following, at least five pathogenic races and five biovars have been discriminated. Ralstonia is a Gram-negative bacterium with rod-shaped cells, 0.5–1.5 μm in length, with a single or tuff of polar flagellum. The positive staining reaction for poly-b-hydroxybutyrate granules with Sudan Black or Nile Blue distinguishes. *R. solanacearum* from many other (Phytopathogenic) Gram- negative bacterial species. The colonies often produced non fluorescent but diffusible brown pigment. On a general nutrient media, virulent isolates of *R. solanacearum* develop pearly cream white, flat, irregular, and fluidal colonies often with characteristic whorls in the center. *R. solanacearum* are entirely deep red Foliage symptoms include rapid wilting of leaves and stems, usually first visible at the warmest time of day. Eventually, plants fail to recover, become yellow and brown necrotic and die. As the disease develops, a streaky brown discoloration of the stem may be observed on stems above the soil line, and the leaves may have a bronze tint. Epinasty of the petioles may occur. A white, slimy mass of bacteria exudes from vascular bundles, when broken or cut. This slime oozes spontaneously from the cut surface of a potato stem in the form of threads, when suspended in water. Such threads are not formed by other bacterial pathogens of potato.

4. Management of Bacterial Diseases

Plant pathogenic bacteria do not form endospores. In principle, therefore, they can be easily controlled. However, only a few effective (systemic) bactericides are commercially available. Moreover certain antibiotics are not allowed to be used in plant health in many countries. Due to the epidemic occurrence of bacterial diseases, the quarantine status of many of them and the substantial losses caused, control is often regulated and executed by governmental bodies. In practice it is tried to reduce damage of bacterial diseases by a combination of protective and preventive control measures.

4.1. The chemical management

The potential for the chemical management of individual bacterial diseases has been largely driven by factors such as the availability of effective modes of action, the opportunity to access the pathogen on plant surfaces, the susceptibility of the pathogen to the specific chemical, the economic value of the crop threatened and the market potential of the use of the chemical from an industrial perspective. Initial forays into the chemical management of bacterial diseases focused on a ‘kitchen sink’ approach, involving the testing of a wide range of available compounds against a wide range of diseases. From these types of study, copper

compounds and the antibiotic streptomycin proved to be the most efficacious, and have been the most commonly used bactericide spray treatments for bacterial disease management on plants, mainly targeting *Pseudomonas* spp., *Xanthomonas* spp. and *E. amylovora.*

Although, in general, these bactericides have been relatively successful disease management tools, the use of both copper and streptomycin has been impacted by the evolution of resistance in populations of plant pathogens (Cooksey, 1990; McManus et al., 2002).

A few additional antibiotics have been used as alternatives to streptomycin either because of resistance or in some pathosystems in which streptomycin is not effective. Oxytetracycline has been used on pome fruit trees to control fire blight (*E. amylovora*) in the USA and Mexico, on peach and nectarine targeting bacterial spot (*Xanthomonas arboricola pv. pruni*) in the USA, and on vegetable crops targeting Pseudomonas spp. and *Xanthomonas* spp. In Latin American countries (McManus et al., 2002). Gentamicin has been used in Latin American countries to control fire blight and various vegetable diseases, and oxilinic acid has been used in Israel to control fire blight (Shtienberg et al., 2001). More recently, kasugamycin has been registered for use in the USA to target fire blight, especially in orchards containing streptomycin-resistant *E. amylovora* (McGhe and Sundin, 2011).

4.2. Biological control

Biological control can be broadly defined as the use of beneficial microbes or their by products or by products/extracts from plants or animals in the suppression of plant disease. Strategies to manipulate populations of biological control agents (BCAs) in field situations are typically either in native, in which the BCA is introduced in a sufficient quantity to suppress disease, even without reproduction, or augmentative, in which the BCA is introduced in sufficient quantity to generate a stable, replicating population suitable for disease suppression. The best example is the use of *Agrobacterium radiobacter* strain K84 or its genetically engineered form K1026 for control of the crown gall bacterium *Agrobacterium tumefaciens*. This soil-inhabiting saprophytic bacterium is very closely related to *A. tumefaciens*, but does not possess a tumour-inducing (Ti) plasmid. *A. radiobacter* is a good root colonizer (better than *A. tumefaciens*) and produces a bacteriocin, Agrocin 84, which is toxic for the crown gall bacterium *A. tumefaciens*.

Bacteriophages are abundant in most if not all ecosystems on Earth, and phage capable of lysing plant-pathogenic bacterial species can be readily isolated from host plant tissue or

soil. Inundative application strategies are typically used for disease management, and the two main limiting factors affecting bacteriophage efficacy are stability in the environment and spontaneous resistance mutations in target bacterial pathogens.

Small antimicrobial peptides (AMPs; typically 50 amino acid residues or smaller) are synthesized by bacteria, fungi and oomycetes, functioning in inter-microbial competition, and by animals and plants, as part of the innate immunity system in response to microbial challenge. Most of these peptides are cationic and can either insert into and disrupt cell membranes or can be taken up by cells and inhibit nucleic acid or protein synthesis (Brogden, 2005). Investigations of the potential for peptides in the management of bacterial plant diseases have been ongoing for more than a decade with strategies mainly involving the inundative application of AMPs to plant surfaces, the use of native fungal or bacterial antagonists that express and secrete AMPs as a BCA, the expression of an animal defensin, such as cecropin, in a transgenic plant or the use of a modified synthetic analogue of an animal or plant defensin in a transgenic situation (Montesinos, 2007).

4.3. Treatment by breeding for resistance

Probably the most durable form of plant protection is breeding for resistance. Especially in the case of bacterial plant diseases, where (systemic) pesticides are rare or absent, resistance breeding is the only option apart from hygiene and other phytosanitary measures. Breeding for disease resistance has been a desirable method for bacterial disease management for many years, even up to 100 years ago.

The deployment of specific R genes into agronomically important crop plants has generated positive momentum for disease management in a wide variety of bacterial disease pathosystems. However, in many cases, the resistance is not durable, as mutational modification of effector targets results in the evolution of new pathogen races that can overcome the resistance. Although most known examples of durable resistance are quantitative (polygenic), this type of resistance is typically too difficult to transfer by breeding. Thus, the pyramiding of groups of R genes into new cultivars has been a very common breeding strategy, and success in durability is predominantly dependent on combinations containing R genes that have been known to be highly effective over long periods of years, such as the Xa3, Xa4 and Xa21 genes in the rice–bacterial blight (X. *oryzae pv. oryzae*) system.

4.4. Curative treatment

A curative treatment is sometimes possible by heat treatment (thermotherapy) or chemical treatment of planting material. Thermotherapy may be performed by a) hot water treatment, usually 50-54°C for 5-30 min, b) aerated steam at 50°C for 1 h). Thermotherapy has been applied to a number of bacterial diseases in different plant parts with reasonable success for true seed, cabbage seed, bulbs; (4-6 weeks 30ºC, 2 weeks 38ºC, 3 days 44ºC); rhizomes, ginger and plantlets or cuttings, sugarcane and grape.

4.5. Disinfection and incineration

Good sanitation is extremely important in the case of contagious bacterial diseases. Plant pathogenic bacteria can survive on many different materials, sometimes for many years. These materials can be sanitized with disinfectants. Success of disinfection is dependant on the concentration of the compound, duration of the application, nature of the material to be disinfected and especially the amount of organic material present. Organic material inactivates many compounds very quickly. Disinfection of (surface) irrigation water, also when recycling is applied in greenhouses, is possible using (combinations of) filtration, UV irradiation, chlorine dioxide and/or hydrogen peroxide with peracetic acid. Diseased crop residues can be removed and burned in a safe place, or disposed of in a safe waste disposal. In many countries waste can only be left in licensed disposals and landfills.

5. Conclusion

Plant pathogenic bacteria produce consistent crop losses from such diseases most frequently and severely in many countries in particularly severe in developing countries, where they are aggravated by the paucity of resources devoted to pathological studies. Plant bacterial diseases are generally characterized by plant morphological symptoms such as leaf and fruit spots, cankers, blights, vascular wilts, rots, and tumors. Phytopathogenic bacteria provoke diseases in plants by penetrating into host tissues. Microbial pathogenicity has often been defined as the biochemical mechanisms whereby pathogenic microorganisms cause disease in a host organism. Microbial virulence is defined as the degree or measure of pathogenicity shown by one or more plants.

This overview party has provided an overview of plant pathogenic bacterial diseases. We hope that the discussions and reviews that comprise a wide range of aspects of plant pathogenic bacterial pathogenicity, epidemiology, and taxonomy tools these can be a help to control. This document is powerful for students, plant pathogenic bacterial researchers

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