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Bacterial plant diseases

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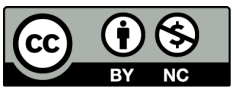

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Introduction

In the presented study texts, comprehensive basic knowledge of phytosanitary bacteriology is summarized. It covers not only the bacterial cell, its cultivation and isolation, but also the causative agents of bacterial plant diseases in the traditional sense, phytoplasmas (mycoplasma-like organisms) and spiroplasmas, which were previously considered as viruses and studied in the framework of plant virology. It is assumed that generalisation of knowledge allows for an easier understanding of specific cases of plant health disruption and facilitates a more comprehensive and targeted approach to the prevention and control of harmful bacterial diseases. The importance of general bacteriological knowledge for plant protection scientists is also becoming more important as the number of biological plant protection products with bacteria as active ingredients is increasing. In the past, phytopathogenic bacteria have only been of interest to a relatively small group of phytopathologists and microbiologists. The increased interest of phytopathologists in phytopathogenic bacteria is due to several factors. While significant advances have been made in direct plant protection against mycoses in recent decades, progress in the effectiveness of direct protection against bacterioses has been relatively small. Protection against bacterioses has become a weak link in plant health care for some crops. From a general biological point of view, new knowledge of the existence of bacteria capable of causing disease not only in the plant but also in man and animals is attracting attention. The mechanisms of pathogenicity and virulence of bacteria pathogenic to plants and humans, as well as the nature of the resistant and susceptible (susceptible) responses of infected plants or mammals, are similar and in some cases identical to the end.

Authors

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1 BACTERIAL CELL

1.1 Internal structure, shape and size of the bacterial cell

1.1.1 Internal structure

All observations have shown that the bacterial (prokaryotic) cell differs from the plant and animal (eukaryotic) cell by a much simpler internal structure. In fact, apart from the colloidal cytoplasm, it has only 4 structures: the nucleus, the ribosomes, the cytoplasmic membrane, and the cell wall (Fig. 1). It is made up of a single, membrane-divided compartment. In terms of complexity and level of organisation of structures, it is an order of magnitude lower than animal or plant cells. The bacterial cell is comparable to the mitochondria and chloroplasts of eukaryotic cells in terms of the complexity of its structures.

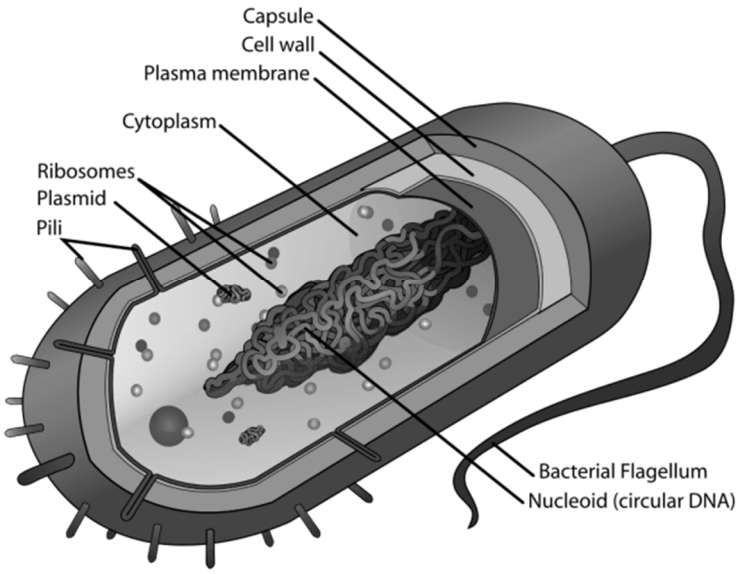

Figure 1 Diagram of a bacterial cell (URL 1)

1.1.2 Size

A bacterial cell is an order of magnitude smaller than an animal or plant cell and is comparable to the size of a mitochondrion. While, for example, a liver cell is approximately spherical in shape with a size of 20 μm, *Escherichia coli* is a rod about 1 μm thick and 2 μm long, and *Staphylococcus aureus* is a spheroid about 1 μm in diameter (Fig. 2). These are typical sizes of bacteria. However, the whole range of sizes in the bacterial kingdom varies from 0.3 x 0.3 μm to 25 x 10 μm (Table 1).

Table 1 Length and width of some bacterial species in μm

Type of bacteria	**Size (μm)**
Chlamydia	0.3 x 0.3
Bdellovibrio	0.8 x 0.3
Rickettsia	1.0 x 0.3
Staphylococcus aureus	0.8 to 1.0 x 0.8 to 1.0
Escherichia coli	2.0 to 3.0 x 0.4 to 0.6
Bacillus subtilis	1.8 to 4.8 x 0.9 to 1.1
Streptomyces	Filament x 0.7 to 1.6
Chromatium	25 x 10

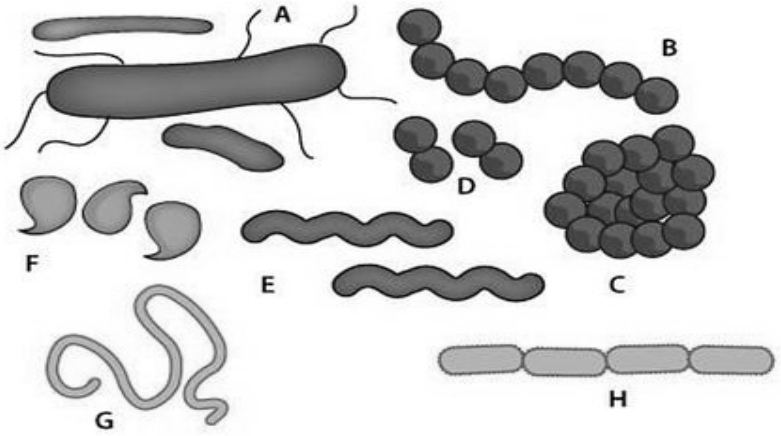

Figure 2 Sizes and shapes of bacterial cells – A – rods (bacilli), B – chains (streptococci), C – irregular clusters (staphylococci), D – pairs (diplococci), E – spirals (spirochetes), F – coryneform bacilli, G – filamentous bacteria, H – chain-like rods (streptobacilli) (URL 2)

The insignificant size of the bacterial cell has its consequences: a large surface to volume ratio (Table 2), hence a large surface area of contact between the cell and the environment, hence a high rate of exchange between molecules, between the cell and the environment. In addition, the times required for intracellular transport of molecules to the site of interaction are reduced to a minimum. The small and membrane-undivided volume of the bacterial cell also allows a high probability of collision of reacting molecules or structures. All this is associated with a high metabolic rate.

Table 2 Ratio of surface area (μm^2) to volume (μm) of some cells

Globular bacteria (coccus)	30 μm : 1
Yeast	0,6 μm : 1
Hepatic cell	0,3 μm : 1

Table 3 Characteristic time required for doubling the biomass mass of growing organisms

Types of organism	**Time**
Bacteria	20-30 min
Yeast	2 h
Seaweed	20 h
Chicken	250 h
Swine	800 h

The density of a cell differs little from the density of water because water makes up more than 70% of the cell's contents.

1.1.3 Shape

Shape diversity is small, though not insignificant, in the bacterial kingdom. The most common shape is that of a spherical (cocci) or a cylinder (rods, bacilli),

sometimes bent or coiled into a coil. Sometimes the cells remain passive after cell division is complete, joined by extracellular cement or by a common sheath in pairs (diplococci, neisseria), chains (streptococci) or clusters (staphylococci). Less frequently, bacterial cells form filaments, sometimes branching and mimicking a mycelium. Only exceptionally are bacteria asymmetrical in shape.

1.2 Elemental and molecular composition

The elemental and molecular composition of bacterial cells does not differ in principle from that of eukaryotic cells (Table 4). Of the more than 90 natural elements that make up the Earth's crust, only 27 are involved in the construction of living organisms. Some of them even only in some species, not in all.

Table 4 Biogenic elements

Forming organic substances	O, C, N, H, P, S
Ions	Na^+, K^+, Mg^{2+}, Cl^-
Trace elements	Mo, I, Si, Sn, Ni, Cr, F, Se

The relative quantitative ratio of the elements composing the living matter and the inanimate environment is quite different. A common characteristic of the living is the ability to specifically select elements from the environment, and it is noteworthy that the elements that are selected are light elements. This is evident from the following table (Tab. 5).

Table 5 Number of atoms of the first four most common elements per hundred atoms of the whole (Kaprálek, 1999)

Universe	H	C	N	O	Total
	90.76	9.08	0.04	0.06	99.97
Lithosphere of the Earth	O	Si	Al	H	
	60.4	20.5	6.2	2.9	90.00
Biosphere of the Earth	H	O	C	N	
	49.8	24.9	24.9	0.3	99.9
Human body	H	O	C	N	
	61.5	25.5	10.5	2.4	99.9

As can be seen, 99.97% of living matter is made up of 4 elements – H, O, C, N, and it is certainly interesting that these are the elements that are most abundant in the universe as a whole. They apparently have properties that make them particularly suited to the construction of the matter we call living. Which properties are they, in fact? What all the elements have in common is that they easily form covalent bonds by sharing electrons: hydrogen needs one, oxygen two, nitrogen three and carbon four electrons to complete their outer electron shells. Their combinations with each other are many. The carbon atom is particularly remarkable; it can take up 4 electrons as well as lose them, it has the ability to bond covalently with 4 other C atoms to form linear, branched or ringed chains of carbon atoms. Because carbon also bonds with hydrogen, oxygen, and nitrogen the preconditions for an almost infinite number of variations of organic compounds are given. The exact elemental composition of a particular organism varies, of course, depending on the type of organism, its functional state and the environment in which it is found. However, we can give a characteristic

representation of the 6 main biogenic elements in the dry weight representing the bacterial cell (C – 50%, O – 20%, N – 15%, H – 8%, P – 3%, S – 1%, total 97%). If we do not count water, the 6 elements thus make up about 97% of the weight of the bacterium. There are about 3 300 different kinds of molecules in a bacterial cell and in total the cell is made up of about 80 million molecules. If we also consider lipids as macromolecules, we can say that 96.7% of the dry weight of the bacterium is macromolecules: proteins, nucleic acids, polysaccharides, and lipids. The function of these macromolecules in the bacterial cell is the same as in all other cells.

Nucleic acids are the carriers of genetic information, they carry out their storage, propagation, transcription, and translation (Fig. 3).

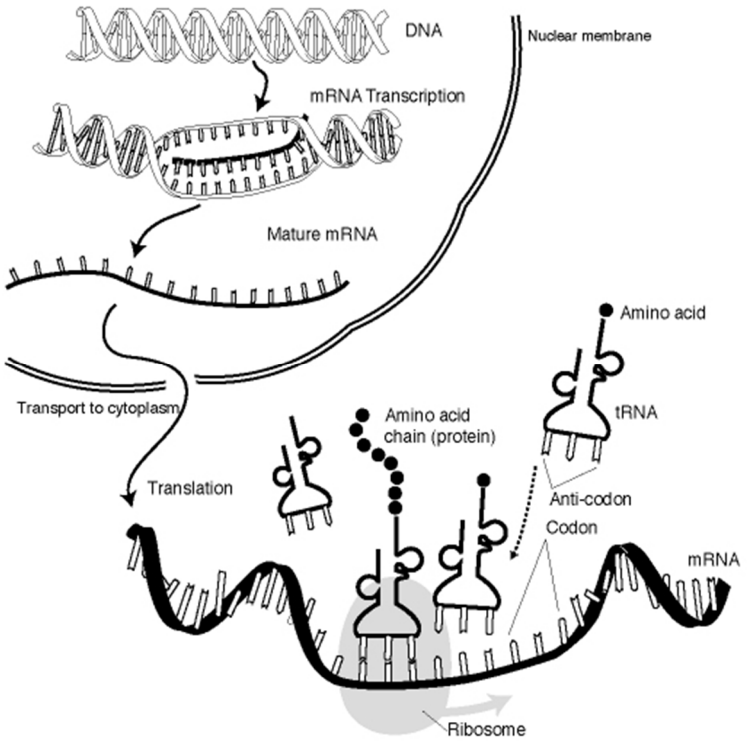

Figure 3 The process of protein formation in a bacterial cell (URL 3 – adjusted)

DNA is present as a single covalently ringed giant molecule with a relative molecular weight on the order of 10^9. In a population of growing and dividing cells, there is on average more than 1 molecule of DNA per cell. In addition to this genetic information, bacteria can contain 100-fold smaller DNA molecules called plasmids. Ribonucleic acids, as in other cells, are present in three types of RNA: mRNA, tRNA, and rRNA. Bacterial mRNA has a very short half-life in a bacterial cell – a few minutes compared to a few hours in eukaryotic cells. This is related to the ability of bacteria to adapt very quickly with new enzyme equipment in a new environment. Another specific characteristic of bacterial mRNA is that it usually encodes more than one protein. For example, 10 enzymes involved in histidine synthesis are encoded by a single mRNA.

Proteins make up the majority of the dry weight of bacteria (50%) and play a primary role in their construction and function. They are the products of genes, but they are also the tools for gene expression, transcription and translation. They convert

genotype to phenotype, converting one-dimensional genetic information into three-dimensional protein molecules and thus a three-dimensional cell with a specific morphology. Most protein molecules have a catalytic function; they are enzymes. In the functioning and production of living matter, enzymes play an important role as operational tools. Through them, specific genetic information is translated into specific cellular activities. The presence or absence of a specific enzyme determines whether or not a given reaction will take place and at what rate. Enzymes determine what a cell can and cannot do. Proteins carry physiology and morphology, order and organization in the cell. Proteins are assembled in cells from amino acids. There are 21 types of these, including selenocysteine. The number of ways to assemble the 21 amino acids is unimaginably large. This number is given by the formula for variations with repetition of elements $V = n^k$. If $n = 20$ and $k = 300$ is $V = 20^{300}$ so 10^{390} variations. With virtually unlimited species variation, proteins are molecules of unlimited possibilities. The evolution of matter by trial and error has therefore had ample time and space to select from an unlimited supply just those protein molecules that have made living systems more successful in survival and reproduction. It is estimated that there are about 3,000 different kinds of proteins in the *E. coli* cell and about 100.000 in the human body. Many of the proteins in *E. coli* and in the human body have the same function, but none are identical. In contrast, each biological species has its own set of protein molecules, and since there are about $1{,}5.10^6$ species of living organisms, this means that there are about 10^{12} different kinds of protein molecules in living matter on Earth. That's a tiny fraction of the 10^{390} possibilities. The number of protein molecules in an *E. coli* cell is estimated to be 2.10^6, so on average there are about $2.10^6/3.10^3) = 670$ copies of a protein molecule per species.

Polysaccharides perform two roles in the bacterial cell. Firstly, they can be intracellular storage substances (glycogen), and secondly, they are structural and functional (peptidoglycan, lipopolysaccharide, teichoic acids) components of the cell wall, as well as of the cell envelope and glycocalyx. They carry the specific antigenicity of the bacterial cell and the ability of specific adherence of parasitic bacteria to specific host cells.

Lipids are molecules insoluble in water. They are divided into simple lipids, which do not contain fatty acids, and complex lipids, which are compounds of fatty acids and alcohol. In bacteria, virtually only complex lipids, specifically phosphoglycerides composed of glycerol-3-phosphate and two fatty acid molecules, are found. Phosphoglycerides are amphipathic molecules. As a result, they spontaneously form bilayers in aqueous environments and are thus a structural element of biological membranes. In the vast majority of bacteria, lipids are found only in the cytoplasmic membrane as phosphoglycerides, specifically phosphatidylethanolamine and phosphatidylglycerol. In exceptional cases, they are part of the cell wall (mycobacteria). Some lipids associate with proteins to form so-called lipoproteins. The bond between protein and lipid is not covalent but hydrophobic: both molecules are attached to each other by their non-polar hydrophobic parts. This type of interaction between lipids and proteins plays a cardinal role in both the structure and function of biological membranes.

Water is a molecule of remarkable properties and a compound exceptional in many ways. It is a stage of life, a space where biomolecules move and meet each other, if the complementarity of their surfaces allows them to interact. Compared to other

chemically similar substances (H_2S), water has extraordinary: high melting and boiling points, large group heat of fusion and boiling, large molar heat capacity, and good thermal conductivity. These properties are responsible for the relatively constant temperature of the oceans and for the small temperature variations even at the surface of the Earth. These conditions were responsible for the origin of life on Earth and its continued maintenance. Water also has a high surface tension, which resulted in life leaving the ocean and spreading on land as well. The properties we have mentioned are indicative of relatively large attractive forces between water molecules. These are then responsible for the asymmetrical structure of the water molecule. This is because oxygen attracts the shared electrons of the covalent bond with hydrogen more strongly than the hydrogen atom and as a result carries a negative partial charge, and the two hydrogens together have the same positive charge. The water molecule is therefore an electric dipole, it is polar. Its individual molecules are electrostatically attracted to each other, and one water molecule can bind with 4 neighbouring molecules. This creates a lattice. The electrostatic bond between the negatively charged oxygen of one water molecule and the positively charged hydrogen of the other water molecule is called a hydrogen bridge. This molecular structure makes water a better solvent than any other liquid. It dissolves salts (e.g. NaCl) well because the electrostatic attractive force between the dipole of the water molecule and the ion is greater than that between the cation and the anion in the crystalline lattice of the salt. Water also dissolves well such organic molecules that contain polar groups (sugars, acids, etc.) because these can form hydrogen bridges with water molecules, thus replacing molecules of separate H_2O in the structural lattice of water. Non-polar molecules cannot penetrate the lattice at all and are therefore insoluble in water. In the water lattice, dissolved molecules replace water molecules themselves imperfectly, and therefore, moving due to thermal motion and encountering other polar molecules or structures, they interact with them only if their surfaces are complementary (e.g. enzyme-substrate), and vacate their place in the water lattice. Such is the fate of, for example, nutrient molecules, metabolic intermediates and metabolic wastes. Water is thus not only a suitable medium for the interactions of molecules in life processes, but also, due to its extraordinary mobility, a transport medium for biomolecules in the space of the cell, the organism, the biocenosis and the entire biosphere.

1.3 Nuclear matter

The nucleolus of a bacterial cell (nucleoid, bacterial chromosome) appears electron optically as a lighter region in the middle of the cytoplasm (Fig. 4). It occupies about 15% of the cell volume, but by weight accounts for only about 3% of the dry weight. Unlike the nucleus of eukaryotic organisms, it is not separated from the cytoplasm by membranes, has no permanent shape, and molecularly is a single giant molecule enclosed in a circular double-stranded DNA helix. In *E. coli*, it has a relative molecular mass of $2.5.10^9$ and contains more than 4.10^6 nucleotides in a single strand. This corresponds to a distance of about 1.4 mm, a thousand times the length of the cell itself. The circular molecule must therefore be folded (spiralized) many times, as in eukaryotic cells (Fig. 5), namely in *E. coli* into more than 50 loops looped into a higher-order structure.

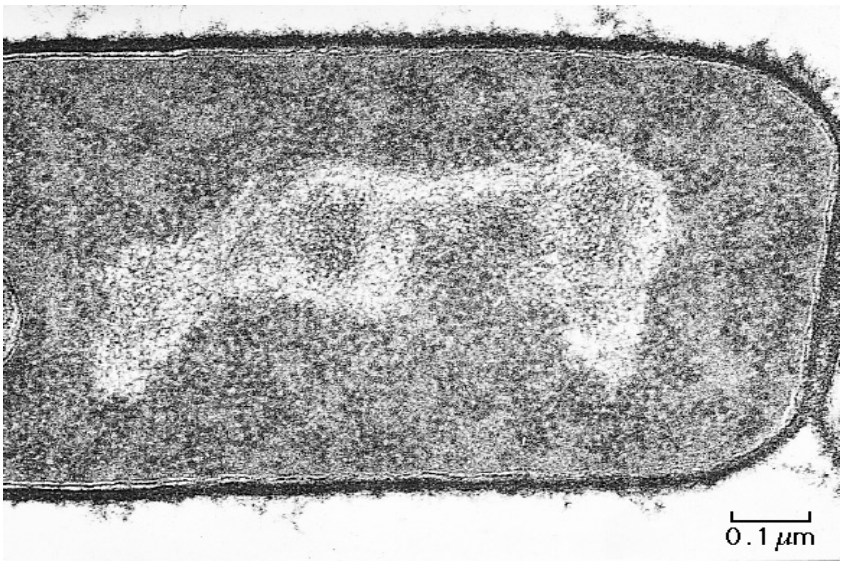

Figure 4 View of the nucleus of a bacterial cell (light part – nucleus) (URL 4)

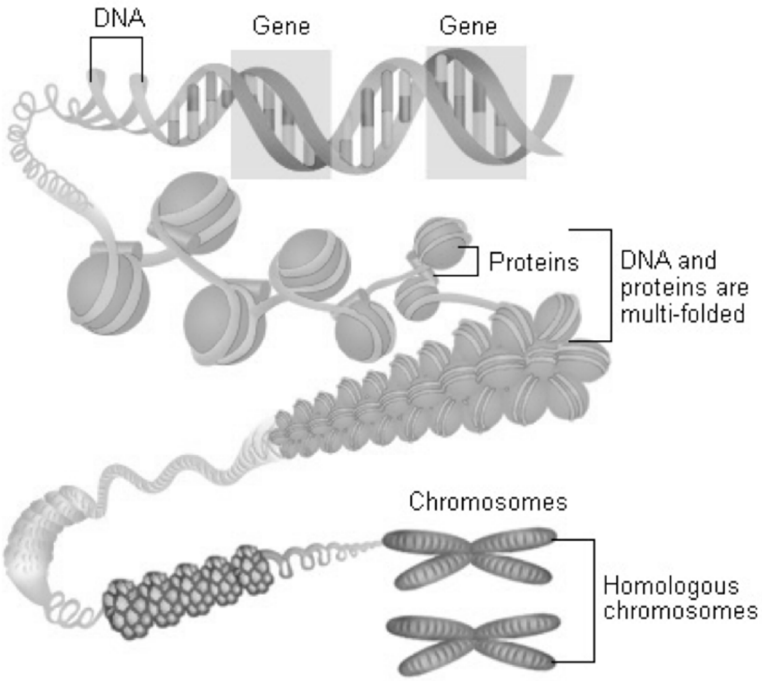

figure 5 DNA superspiralisation in eukaryotic organisms (URL 5 – adjusted)

Unlike the nucleus of eukaryotic organisms, the bacterial nucleus does not contain histones, but about 4 types of "histone-like" proteins are bound to the bacterial DNA molecule. It has been found that there are on the order of 120,000 in their cell, so think 1 protein per 200 base pairs. The best known of these is the HU protein from *E. coli*. It is a dimer of two small (9500 Daltons) base proteins. The function of these proteins is not fully understood. They probably act in transcription (perhaps by inducing knobs on the DNA molecule and thus bringing linearly distant stretches closer together, but which are related to the regulation of gene expression), at the site of specific recombination, and also in the initiation of DNA replication. In each case, the bacterial chromosome is structurally organised and the proteins that do this are called "chromosome organisers". HU protein is a typical representative of these, and DNA topoisomerase is one of them. In addition to these proteins, many catalytic proteins

involved in DNA methylation, recombination and repair, as well as replication and transcription, and regulatory proteins are bound to the DNA molecule. There is only one chromosome in a dormant, non-growing bacterium. In a growing cell, chromosome replication occurs simultaneously with growth, and therefore there is more than one (up to 4) genome equivalent in a single cell. However, such a cell is much larger than a quiescent cell. The nucleus of the cell is where the genetic information of the cell is stored. The instructions for the structure and function of the cell is broken down into functional units, genes, sections of the DNA strand. It is significant that the nucleus of bacteria is haploid and thus contains a given gene in only one allele. In about a dozen bacteria, including *E. coli*, the sequence of the bases of the entire genome has been determined. In *E. coli*, the whole chromosome was found to be composed of 4639221 base pairs and to encode 4397 genes. Of these, 108 genes are for RNA and 4289 for proteins.

Nuclear division is understandably easier in haploid bacteria than in diploid organisms. It consists in making a replica of the DNA and spatially separating the two nuclei, the original and the new one. It is precisely because of its relative simplicity compared to mitosis that the process of DNA replication and repair in bacteria is all the more thoroughly studied at the molecular level. The two equivalents created by nuclear division still need to be spatially separated.

In bacteria, there is no dividing spindle, and the mechanism is different. There is evidence that the bacterial chromosome is attached by its origin to the cytoplasmic membrane and cell wall in about half of the cell. The spatial separation of the daughter chromosomes into the two halves of the growing cell is apparently achieved by intense local growth of the cell envelope in the equatorial region between the two sites of attachment of the chromosomes.

Nuclear division is followed by cell division. Most bacteria divide transversally to form two roughly identical cells. The actual division of the cell is accomplished by the formation of a transverse septum in the equatorial plane of the cell. However, how a cell recognizes that its peptidoglycan envelope is about to stop growing in length and start growing in the perpendicular plane is still an unanswered question by scientists.

During DNA replication, errors in replication accuracy occur, albeit with low frequency, naturally and spontaneously. Their frequency is increased by the presence of certain chemicals or by the effect of UV radiation. These changes in DNA imply changes in genetic information – mutations. To which evolution and the diversity of species are ultimately due.

1.4 Plasmid

In addition to the nuclear DNA itself, bacteria may contain an additional circularly enclosed DNA molecule, about 100 times smaller than a chromosome, called a plasmid (Fig. 6). Probably all species of bacteria have plasmids, but not every individual or population is required to contain them. Plasmids represent additional, not indispensable, genetic information in bacteria. The relative molecular mass of plasmids is on the order of 10^6 to 10^8 (chromosome 10^9) Daltons and thus they can encode from about 3 to 100 genes (chromosome about 3000). The functions they encode are usually not indispensable for life, but under special environmental conditions they may favour their carrier over bacteria that do not have plasmids.

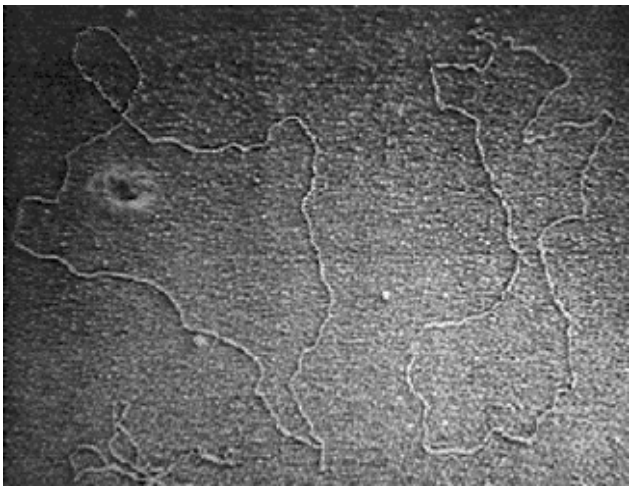

Figure 6 Plasmid in the cytoplasm (URL 6)

Classification of plasmids according to the functions they carry:

1.4.1 Resistance to antibiotics and chemotherapeutics

The mechanism of resistance can either be mediated by the production of enzymes that degrade the antibiotic (e.g. beta-lactamases hydrolysing penicillin) or by chemical modification (acetylation of chloramphenicol, adenylation, acetylation or phosphorylation of aminoglycoside antibiotics such as streptomycin, neomycin, kanamycin and gentamicin), or by the production of an enzyme that modifies the target site of the antibiotic and renders it insensitive to the antibiotic (methylase methylating the adenines in the 23S ribosomal RNA, which are binding sites for macrolide antibiotics such as erythromycin), or by production of a membrane transport protein that exports the antibiotic out of the cell (tetracycline), or by formation of an enzyme that functionally replaces an enzyme blocked by the chemotherapeutic (sulphonamide-blocked dihydropteroate synthase or trimethoprim-inhibited dihydrofolate reductase) (Fig. 7).

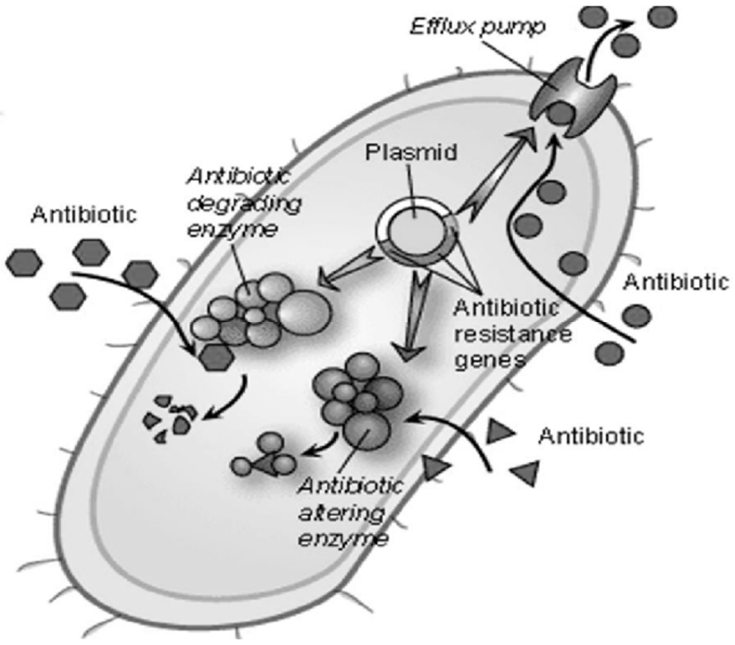

Figure 7 Mechanisms of resistance (URL 7)

1.4.2 Resistance to heavy metals

It is described as plasmid-encoded resistance to mercury, cadmium, silver, antimony, arsenic, bismuth, lead, boron, chromium, cobalt, nickel, tellurium and zinc ions, but the mechanism is only partially known for divalent mercury cations (enzymatic reduction of Hg^{2+} to Hg^0, which is much less toxic and insoluble in water), cadmium (efflux from the cell by a transport protein) and silver (a protein on the cell surface that blocks the cell's ability to extract soluble Ag^{2+} from insoluble forms such as $AgCl_2$).

1.4.3 Production of antibiotics

Through the production of antibiotics, some bacterial species defend themselves against competing species in interspecific struggles for nutrients in the environment. Industrially, many species of streptomycetes are exploited in this way.

1.4.4 Production of toxins

For example, thermolabile (LT) and thermostable (ST) enterotoxin of *E. coli* along with the production of adhesion and colonization factors (Ent plasmid in enterotoxigenic *E. coli* (ETEC) and invasion factor in EIEC strains (enteroinvasive *E. coli*), and also haemolysing of *E. coli.*

1.4.5 Production of bacteriocins

Bacteriocins are plasmid-encoded proteins toxic to other bacteria, but only to strains related to the producer. They are produced by many bacteria, e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus megaterium* also Bacteriocins of *E. coli* are called colicins and are the most extensively studied. The plasmids that encode them are called Col plasmids and there are more types than corresponding colicins. Bacteriocins released into the environment bind to receptors present on the surface of susceptible cells (resistant cells do not have these receptors) and cause them to die. The mechanisms are diverse, the most important being 4: perforation of the cytoplasmic membrane leading to loss of charge and ion gradients on the membrane, endonuclease degradation of DNA, specific cleavage of 16S rRNA leading to arrest of proteosynthesis, and arrest of peptidoglycan synthesis leading to cell lysis.

1.4.6 Degradation and oxidation

Inactivation of biologically inert or toxic organic substances such as hydrocarbons (petroleum), toluene, benzaldehyde, etc. These mechanisms play an indispensable role in the cycling of substances in nature, and bacteria bearing these properties (mainly of the genus *Pseudomonas*) are currently used technologically in the clean-up of oil accidents on land and at sea.

1.4.7 Formation of restriction and modification enzymes

Modifying enzymes covalently (e.g. by methylation) mark their own DNA at specific sites, which is then not cleaved by their own restriction enzymes designed to destroy foreign DNA.

1.4.8 Ability of symbiosis of tuber-forming bacteria

For example, *Rhizobium* with *Papilionaceous* flowers from the Fabaceae family. Many of the genes involved in this very important natural event are encoded by plasmids: nodulation, host specificity, and the ability to fix airborne nitrogen.

Some plasmids are cryptic: they are in the cell; we do not know their phenotypic expression. The number of molecules in the cell ranges from 1 to over 100. The smaller the plasmid, the greater the number of copies of the plasmid in the cell. The number of copies of a plasmid counted per chromosome is a characteristic constant for a given plasmid and host (under given environmental conditions) – the plasmid copy number.

The boundary between plasmids and bacterial viruses (bacteriophages) is not well defined. Plasmids can be viewed as phages that do not have the ability to form their own protein envelope and thus exist outside the cell. Plasmids can be considered as endosymbionts at the genetic level.

1.4.9 Quorum sensing in bacteria

Quorum sensing is a type of purposeful behaviour of bacterial cells that is related to changes in population density. It involves the synthesis and production of chemical signalling molecules called autoinducers. The increase in the extracellular concentration of these substances depends in direct proportion to the increase in population density. Reaching a certain threshold concentration of an autoinducer then results in an effect on gene expression. Both gram-positive and gram-negative bacteria use quorum sensing as a communication cascade leading to alteration of specific cellular physiological expressions. These events are related to e.g. symbiosis, virulence, conjugation, antibiotic production, movement, sporulation and biofilm formation. For communication, gram-negative bacteria mainly use acylated homoserine lactones, whereas gram-positive bacteria use a variety of oligopeptides. Recent findings show that quorum sensing communication is used both within and between species. Reaching a threshold concentration of signalling molecules triggers a specific response of the organism in question. Signalling molecules have different chemical compositions, the mechanism of information transfer through the intracellular environment is different, and genes differ in their expression, which is controlled by the quorum sensing system. Despite these differences, intercellular communication allows bacteria to coordinate their cellular response to a given situation. This process confers on unicellular organisms the behavioural traits that characterise higher organisms. The evolution of quorum sensing systems in bacteria could thus represent an early stage in the evolution of "multicellularity". Single-celled organisms also exhibit other forms of purposeful behaviour that are not mentioned here.

1.5 Cytoplasm

The soluble cytoplasm completely fills the inner space of the bacterial cell, as it usually has no vacuoles. There are 3 types of structural units: chromosome, ribosomes, and storage granules. The cytoplasm itself is a highly concentrated solution of many biomolecules. They are many in species and number, molecules small and large, and huge supramolecular complexes. It is very viscous and resembles a gel rather than a solution. It is the stage of the cell's life, the place where the movement of

molecules and structures in space, their mutual search and interactions take place. The cytoplasm contains more than 50% of all the proteins of the cell and most of them have an enzymatic function. There are enzymes of glycolysis, pentose cycle, glyoxylate cycle, Krebs cycle, also various dehydrogenases, proteases, nucleases, esterases, regulatory molecules (cAMP, guanosine tetraphosphate), repressors, enzymes catalysing the synthesis of amino acids and nucleotides, mRNA and tRNA molecules, enzymes catalysing replication, repair, transcription and translation, as well as low molecular weight intermediates of catabolism and anabolism, nutrients and metabolic products, and numerous cations and anions. Thus, it is not surprising to find that the rate of diffusion of molecules in the cytoplasm and the time required for a specific molecule not to find the complementary surface of a specific partner are factors that place an absolute upper limit on the possible rate of bacterial metabolism.

1.6 Ribosomes

The ribosome of bacteria resembles the ribosome of a eukaryotic cell on the one hand but differs from it on the other. It is composed of two subunits, 30S and 50S, which fuse to form the functional unit 70S (Fig. 8). The S value is the Svedberg unit, which discusses the relative sedimentation value of ribosomes in high-speed centrifugation.

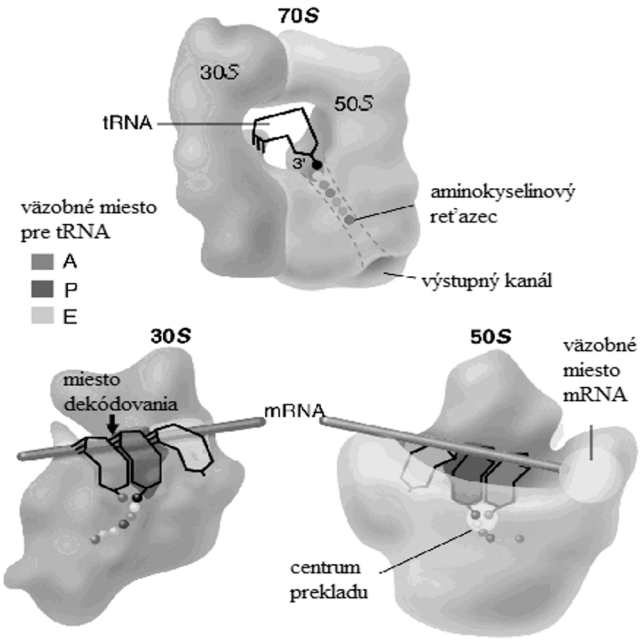

Figure 8 Schematic diagram of the bacterial ribosome (URL 8 - adjusted)

The smaller subunit is composed of 1 16S RNA molecule and 21 protein molecules, the larger subunit of 2 RNA molecules, 5S and 23S, and 34 protein molecules. Of the 55 protein molecules, 53 are distinct and the ribosome is in all respects a supramolecular and asymmetric formation. Its function is to be a surface where dozens of molecules can meet in an oriented manner so that the genetic information carried on the mRNA is translated into the primary structure (amino acid sequence) of the protein. In the bacterial cell, ribosomes are both loose in the cytoplasm, in the nucleus (about 60 %) where they latch onto nascent mRNAs and immediately translate them, and at the periphery of the cell attached to the cytoplasmic membrane (about 30 %) where they synthesize proteins destined for the membrane or

for export. The synthesis of ribosomes in the growing cell is controlled. The rates of all ribosomal components are matched so that none of them accumulate. The total number of ribosomes in a cell is variable and directly proportional to the growth rate: the faster the cell grows, the greater the number of ribosomes. While a quiescent one has only a few dozen, a fast-growing one has 30 000 or more. This is because a higher rate of proteosynthesis can only be achieved by increasing the number of ribosomes. This is because the rate of activity of an individual ribosome is constant and independent of the growth rate. It is about 800 amino acids per minute. By mass, ribosomes account for up to 40% of the dry weight of the cell, and by mass the ribosome is made up of 2/3 ribonucleic acids and 1/3 proteins. The bacterial ribosome and the proteosynthesis taking place on it resemble the ribosome and proteosynthesis of eukaryotes on the one hand, but differs on the other hand. This difference has been exploited over time in the therapy of bacterial diseases by finding substances that inhibit the activity of the bacterial ribosome, not the eukaryotic ribosome. These are substances such as: streptomycin, tetracycline, chloramphenicol, erythromycin and many others.

1.7 Reserve materials

The life processes in each cell take place continuously. Even a quiescent non-growing cell needs energy, at least the energy needed to maintain itself as a system of high order in a disordered environment. The supply of energy must be continuous because the thermal motion of the molecules is continuously operating and the second law of thermodynamics is continuously operating, trying to return the system back to a state of disorder. On the other hand, the organism finds itself in a situation where it temporarily has no external source of energy available. Therefore, a common property of organisms is the ability to build up energy reserves. In bacteria, the reserve energy sources are mainly glycogen and poly-β-hydroxybutyric acid. The latter is specific to bacteria as a reserve material. It is also specific to them that the reserve material is not neutral fats common in eukaryotes. Bacteria do not form reserve substances from nitrogenous substances. It is also important that the reserve substances are osmotically and ionically inactive. An obvious rule of thumb applies: both substances accumulate in cells especially when cells grow in excess of a carbon source or in deficiency of a nitrogen or other nutrient source and, conversely, disappear when the carbon and energy sources are limiting.

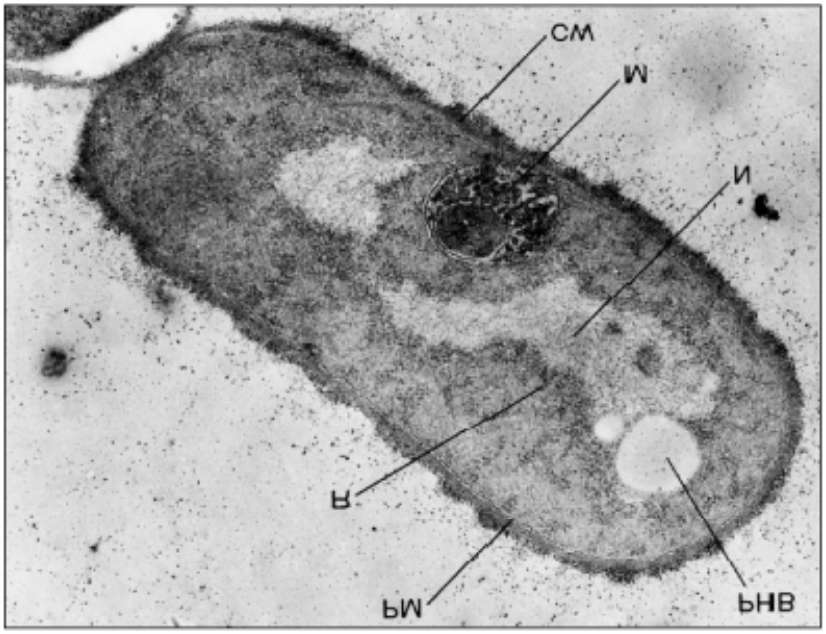

Figure 9 Microscopic cross-section of a bacterial cell (PM-cytoplasmic membrane, R-ribosome, PHB-poly-beta-hydroxybutyric acid, N-nucleus, CW-cell wall, M-mesosomes) (URL 9)

Glycogen is an insoluble polymer of glucose, α-1,4-glucan with numerous branched α-1,6-linkages. The branching occurs on every 8th to 10th glucose molecule in the chain and the molecule is very compact. Glycogen can be up to 50% of the dry weight of the cell. It is randomly distributed in the cytoplasm in the form of bodies invisible in the light microscope.

Poly-β-hydroxybutyric acid forms droplets in the cytoplasm visible only in the electron microscope as oval bodies (Fig. 9). It may constitute up to 60% of the dry weight of the bacterium.

Volutin is a polyphosphate that sometimes accumulates in the form of bodies in some bacteria. It is a reservoir of phosphate molecules. It is formed by chains of up to 500 polyphosphate molecules and is therefore insoluble in water and osmotically inactive. The bond between the molecules is energetic in nature if it requires one ATP molecule to form. Some bacteria have been shown to use volutin as a source of energy in place of ATP-rich phosphate bonds.

Sulphur is the fourth substance occurring as a reserve substance in bacteria, but only in those that use it either as an energy source (chemolithotrophic sulphur bacteria) or as a source of electrons in the process of photosynthesis (phototrophic sulphur bacteria).

1.8 Cytoplasmic membrane

The cytoplasmic membrane surrounds the surface of the bacterial cytoplasm. It is the only biological membrane in bacteria and all those cellular functions and processes that cannot take place except on the membrane, e.g. in solution, are localized on it. At the same time, this means that the entire bacterial cell is a single compartment not divided by other membranes.

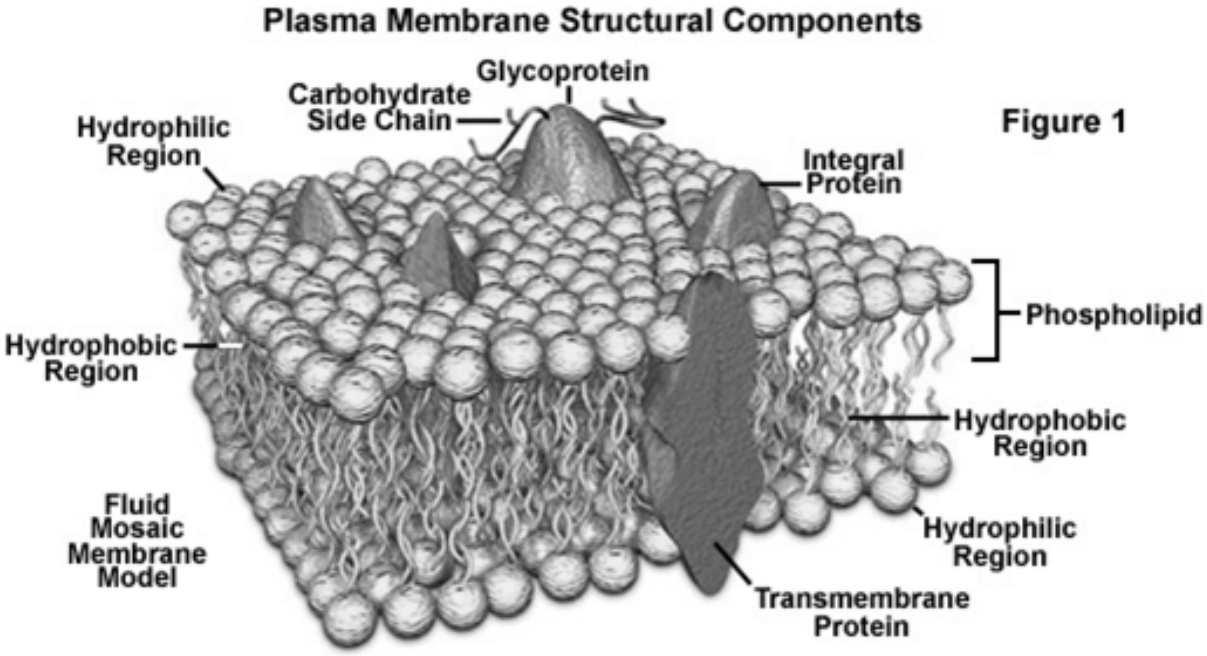

Figure 10 Schematic diagram of the cytoplasmic membrane of bacteria (URL 10 – adjusted)

This is a significantly different situation from the eukaryotic cell, which is interwoven with membranes of the endoplasmic reticulum and multicellular organelles and thus divided into 5 to 6 separate compartments of the cellular compartment. In most bacteria it is smooth and taut over its entire surface. However, there are many bacteria in which we find various depressions and compartments on the surface of the membrane. The bacterial cytoplasmic membrane constitutes about 16 – 26% of the dry weight of the bacterium. Its thickness is about 8 nm and all indications are that its structure is not fundamentally different from that of other biological membranes. It is based on a more or less fluid continuum of phospholipid bilayers, with protein molecules floating on either side, more or less submerged and tethered by hydrophobic and electrostatic forces, singly or joined together in a supramolecular functional complex. Viewed as a surface, the membrane appears as a mosaic made up of lipid molecules and various proteins, and because the lipid bilayer is fluid, such a membrane structure is called the fluid mosaic model (Fig.10).

The chemical composition of the membrane varies over a wide range depending on the species of bacteria and the conditions of their environment. Roughly, proteins make up about 70% of the membrane mass and lipids about 30%. Of the lipids, phospholipids, namely: phosphatidylglycerol and phosphatidylethanolamine, are the main ones. A characteristic feature is the absence of cholesterol. The ratio of saturated to unsaturated acids varies depending on the culture temperature (a particular bacterium can grow and multiply in the range up to 30 °C). A remarkable feature of the cytoplasmic membrane of bacteria is its structural and functional asymmetry. This also determines the vectoriality of metabolic reactions on the membrane. Some integral protein molecules are on the inner side of the membrane, others on the outer side, and those that penetrate the membrane are embedded asymmetrically within it. Also, the bilayer of phospholipids forming the membrane is asymmetric.

The bacterial cytoplasmic membrane is a polyfunctional device and a number of enzymatic activities are located on it. Typically, these are processes that are spatially oriented and cannot take place in solution. A prerequisite for the membrane to be functional is that it must form a typologically closed unit. Biologically and evolutionarily, the primary function of the membrane is to isolate the internal environment of the cell from the external quite distinct environment. The inner hydrophobic layer of the membrane carries this property because it is impermeable to polar molecules. However, the insulation must not be absolute. A living cell is an open system exchanging matter, energy, and information with its surroundings. Therefore, on each cytoplasmic membrane of a bacterium there is a molecular device allowing only those molecules for which the device is specific to penetrate the membrane, either outwards or inwards. The second major life function localized on the cytoplasmic membrane of bacteria is energy transformation. In photosynthetic bacteria the membrane carries the transformation of light energy into proton gradient energy, in chemotrophic bacteria gaining energy by oxidation of reduced matter by oxygen or other electron acceptor it carries the respiratory chain generating the proton gradient, there is an ATPase on the membrane transforming the energy of the proton gradient into the energy of the ATP molecule, and there is a flagellum anchored in the membrane driven by the proton gradient.

1.9 Bacterial cell wall

Above the cytoplasmic membrane of bacteria is the cell wall. It is the only solid formation in the bacterial cell. It plays the role of the cell skeleton, gives the cell its shape and, as a rigid shell, protects it mechanically. It protects it very efficiently: if we want to break the cell mechanically, a rather brutal and long-lasting action is needed. The wall gives the cell chemical resistance, resistance to radiation, to adverse osmotic conditions, etc. At the same time, it compensates for the rather considerable osmotic overpressure inside the cell: 500 kPa (5 ATM) in gram-negative bacteria and up to 2500 kPa (25 ATM) in gram-positive ones. The cell wall is not impermeable to molecules; it has the character of a sieve. We can see all these functions by a simple experiment if we deprive the cell of its cell wall. For example, by the action of lysozyme, which hydrolyses the wall, or penicillin, which blocks its synthesis without affecting the physiological functions in the cell (hence penicillin acts only on growing bacteria). Thus, we obtain bacterial cells without a cell wall, called protoplasts or spheroplasts, which are always spherical in shape and must be immediately in a solution with a high osmotic pressure (e.g. 20% sucrose) in order not to burst. They can only survive in an environment with a suitable osmotic pressure, but sometimes they can also grow and reproduce. In this case they are called L-form bacteria. Some L-forms of pathogenic bacteria have also been isolated from diseased humans, but their role in pathogenesis is not clear. Laboratory-produced L-forms of pathogenic bacteria are not pathogenic. The cell wall is of diverse structure and there are varying degrees of complexity in different species of bacteria. However, this does not mean that it is different in every bacterium. In fact, we can distinguish two basic types of cell wall. The cell wall of gram-positive and gram-negative bacteria (Fig. 11).

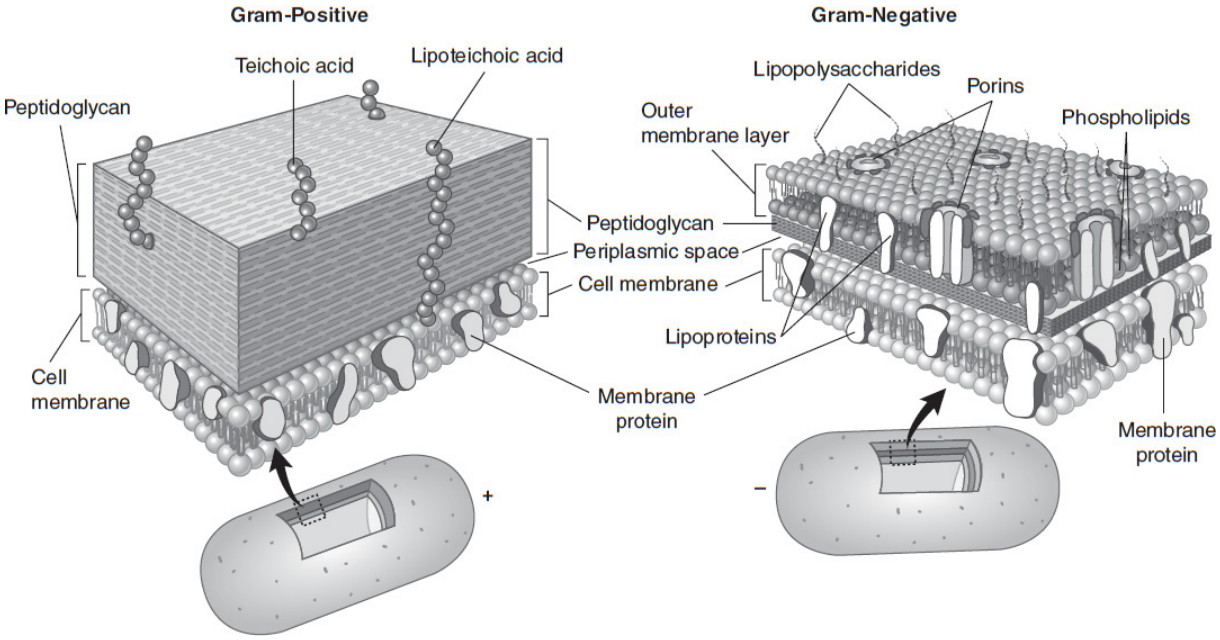

Figure 11 Differences between G^+ and G^- cell walls (URL 11 – adjusted)

However, they both have one essential thing in common: peptidoglycan (mucopeptide, murein). Its presence in the cell wall is the ticket to the club of domain Bacteria. However, there are organisms called archaea that belong to domain Archaea but do not have peptidoglycans in the cell wall. Nowhere else but in bacteria has peptidoglycan been found. It is a linear polymer of two alternating amino sugars: N-acetylglucosamine and its 3-O-Dlactyl derivative called N-acetylmuramic acid linked together by β-1-4 bonds. The carboxyl of N-acetylmuramic acid is followed by a chain of 4 amino acids. A typical combination is L-alanine-D-glutamic-R-D-alanine, R is a variable amino acid, most commonly L-lysine, L, L-diaminopimelic acid (DAP), L-ornithine.

The cell wall of gram-positive bacteria is formed:

The cell wall of gram-positive bacteria is about 20 nm wide and is made up of a massive layer of peptidoglycan. Linear chains of teichoic acids penetrate through it to the surface. Teich acids are basically water-soluble linear polymers of glyceryl phosphate or ribitol phosphate with glycosidic linked sugars. They are covalently bound to peptidoglycan. Their function is still not precisely understood, but they do not contribute to cell wall strength. Their main role seems to be the binding of cations, especially divalent cations (Ca^{2+}, Mg^{2+}), which are indispensable for the integrity of the cell wall and membrane. What is certain, however, is that they are the major surface antigen of gram-positive bacteria. Such bacteria, such as mycobacteria, corynebacteria and nocardia, may contain many lipids and waxes bound by ester linkages to peptidoglycan. The cell wall does not contain proteins. However, when it does contain them, exceptionally, e.g. in streptococci, it is as a continuous, covalently bound layer on the surface of the wall, which gives the bacteria specific antigenic properties. In streptococci, there is an additional layer of polysaccharides between the peptidoglycan and the surface layer. The polysaccharide and protein layer is sometimes considered to be another part of the bacterial cell envelope, the envelope, and because of its thinness it is called the microcapsule.

The cell wall of gram-negative bacteria is formed:

It is considerably different from that of gram-positive bacteria. It is thinner (about 10 nm), has less mass, but is more complex. It is made up of an inner wall – a thin layer of peptidoglycan (2 – 3 rows) and an outer membrane, which is made up of a lipid bilayer composed of phospholipids mainly localized in the inner layer of this outer membrane; lipoproteins, which connect the outer membrane with the peptidoglycan layer; lipopolysaccharides (LPS), which are localised on the outer layer of the inner membrane and which are composed of three parts (lipid A, coronal part and polysaccharide O antigen); proteins that are sex- and species-specific, act as enzymes, adhesins, invasins and resist phagocytosis. The outer membrane, like the cytoplasmic membrane, is semipermeable, permeable to small molecules through pores that are composed of proteins (porins). It prevents the escape of some enzymes and the entry of some toxic substances (PNC, lysozyme). LPS adds strength to the outer membrane and acts as a PAMP by stimulating innate immune mechanisms that manifest as inflammation, increased temperature, phagocytosis. It also activates the alternative complement pathway. Biologically, it manifests itself by endotoxin activity. The periplasmic space is formed by the gelatinous material between the outer membrane, peptidoglycan, and cytoplasmic membrane. It contains enzymes and binding proteins that allow extracellular nutrients to be broken down into smaller molecules and transported across the cytoplasmic membrane. The total phospholipid content is less than that of the cytoplasmic membrane itself. Instead of phospholipids, there are lipopolysaccharides in the inner membrane. The proteins are different from those of the cytoplasmic membrane. Significant are specific phage receptors, called porins, which form micropores for the penetration of small specific hydrophilic molecules. The inner membrane is anchored to the peptidoglycan by lipoprotein molecules.

1.10 Capsule, glycocalyx and S-layer

Above the cell wall of gram-negative and gram-positive bacteria there may be an additional layer, of varying thickness, varying structure and varying sharp interface to the environment. It bears the name capsule, glycocalyx (made up of fibrous proteins) or mucus layer. It is not found in all bacteria and is not indispensable for bacterial life. Its formation is influenced by the environment. The only exception is the case of *Bacillus anthracis*, which is formed by poly-D-glutamic acid; all other bacteria form a case of polysaccharides. If there is a well-defined layer on the bacteria that separates the cells from each other it is called a sheath. If the cells fuse into a single unit, the mass around the cells is called the mucus layer, and if there are individual single filaments sticking out of the bacteria, this layer is called the glycocalyx. In addition, the bacterium may carry on its surface the recently discovered S-layer. The bacterial envelope carries antigenic properties and varies in composition even in the same species of bacteria. In pathogenic species, it contributes to virulence and invasiveness. The glycocalyx is composed of individual long polysaccharide filaments and plays a key role in the adherence of bacteria to different surfaces (stones in a stream, tooth enamel, the wall of the intestine or the wall of the urogenital tract). The ability to adhere to eukaryotic cell surfaces is crucial for parasitic and symbiotic microorganisms.

1.11 Fimbriae (pili)

Fimbriae or otherwise called pili are relatively short, rigid, straight filaments sticking out in all directions from the surface of the bacteria. They are very fragile and

break off easily. They occur only in gram-negative bacteria. There are several dozens of them on a single cell. Their building blocks are protein subunits.

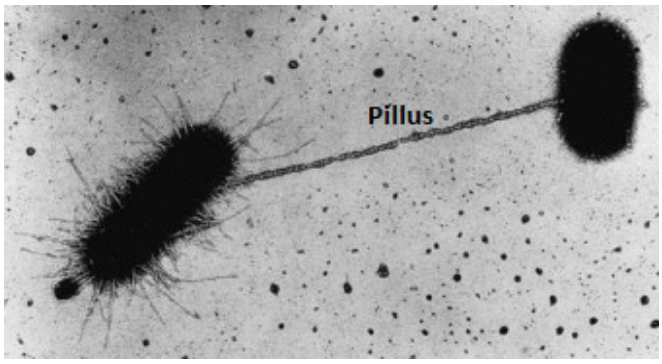

Figure 12 Bacterial pili (URL 12 – adjusted)

They give the bacteria the ability of specific adhesiveness. For example, in the enteropathogenic bacterium *E. coli*, toxin production is related to the formation of adherence fimbriae. In addition to adhesion fimbriae, there are also so-called sex fimbriae, which are encoded by a conjugative plasmid. In most cases, only one such plasmid is found per cell and is formed between the donor and recipient cells. It is essentially a hollow bridge by which plasmid DNA passes from one cell to the other (Fig. 12).

1.12 Flagellum

Some bacterial cells have flagella; these may be located at one of the poles of the cell (monotrichous), one flagellum at each pole of the cell (amphitrichous), or it may be a bundle of flagella at one pole or both poles of the cell (lophotrichous) or over the entire surface of the cell (peritrichous) (Fig. 13). The flagella are much larger to the dimensions of the bacteria and serve as organs of locomotion. The speed of movement of the bacterium is considerable, up to 50 μm per second, due to the flagella. The flagellum is composed of three parts: the flagellar filament, the hook, and the basal part. The flagellum filament is up to 20 μm long and measures between 10 and 30 μm in diameter. Its thickness is about 20 nm. It is therefore invisible under a light microscope. It is composed of protein molecules called flagellin and is species specific. The flagellin molecules are arranged in a whorl in the flagellum, making the filament hollow in the middle. The flagellum is thus not straight but coiled.

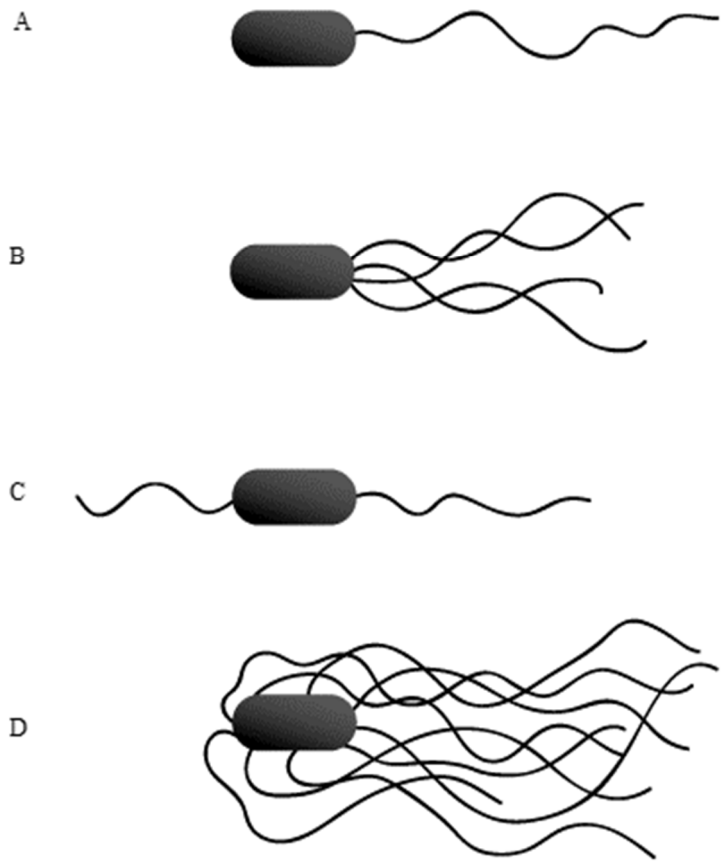

Figure 13 Location of flagella: A-monotrichous, B-lophotrichous, C-amphitrichous, D-peritrichous (URL 13 – adjusted)

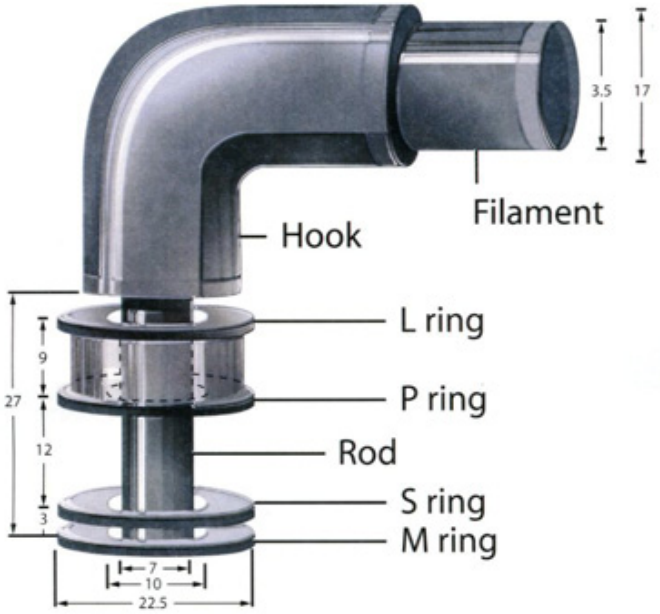

Figure 14 Basal part of the flagellum in a gram-negative bacterium (URL 14 – adjusted)

The bacterial flagellum of gram-positive bacteria has a similar arrangement to that of gram-negative bacteria, but without the L and P rings. This is related to the different structure of the membranes of gram-negative and gram-positive bacteria. The hook is a short so-called sleeve that stiffens the lower part of the filament and attaches it to the basal part of the flagellum. It is of a different composition than the flagellum itself. Its function has not yet been elucidated, but it is thought to be a flexible connection between the fixed basal part and the rigid filament. The basal part of the flagellum anchors the flagellum to the cell wall and cytoplasmic membrane. The entire basal part is composed of 9 different proteins. The mode of movement of bacteria has been elucidated recently. It has been shown that bacteria do not move in a wave-like motion like a snake, but it is a complex mechanism similar to that of a ship's propeller. Where a static flagellum with a hook and basal layer acts as a rotor and a so-called stator is located in the cell membrane (Fig. 14). The energy source is the gradient of protons in the membrane. It has been calculated that the bacterium consumes 256 protons per revolution. The bacterium can use the flagellum to change direction depending on its own need. It can spin clockwise or anticlockwise. If the bacteria need to swim straight in the medium, the whip is spun in a counterclockwise direction if the bacteria are wiggling or rotating the whip is spun clockwise. Bacteria moving in this way are attracted or repelled by certain chemical compounds. We thus speak of positive or negative chemotaxis. The molecular device for receiving and processing the chemical signal from the environment is located on the surface of the cytoplasmic membrane and is called a chemosensor. These are distributed over the entire surface of the cytoplasmic membrane of the bacterium. It is shown that the signal to the chemosensor is not induced by the substance itself, but by its concentration in the medium.

1.13 Spores and sporulation

Bacteria of the genera *Bacillus* and *Clostridium* can under certain conditions, generally speaking under unfavourable conditions, switch from a vegetative form to a dormant form, which is characterised by zero metabolism but extreme resistance to the external environment. Such formations are called spores or endospores, which are produced by a process called sporulation inside the cell. Among the spore-forming bacteria we also include the genera *Alicyclobacillus*, *Geobacillus*, *Sporocytophaga*, etc.

The sporulation of growing bacterial cells begins at the end of the exponential growth phase when nutrients disappear from the environment. Spore formation itself takes about 10 hours. The process of sporulation is well studied. It has been found that the expression of silent genes is switched on and the expression of previously active genes is switched off on the basis of environmental stimuli. Morphologically, there are 7 distinct stages in the sporulation process, which are generally denoted by Roman numerals. The vegetative cell is referred to as stage 0. In stage I, the morphology of the bacterial nucleus changes from a spherical formation to a filamentous formation. Stage II is characterized by the division of the nucleus and the subsequent division of the cell by the septum of the double cytoplasmic membrane into two equal halves, the future spore and the original vegetative cell, the sporangium. This is a process very similar to cell division, as each of the two cells has a complete genome, but the cells are not the same size and face different fates. During stage III, the smaller half of the original cell, the future spore, travels into the interior of the mother cell, with the original septum growing poleward, so that near the end of stage III there is a separate,

double-membrane-enclosed special cell inside the mother cell - the prespore. Once the prespore is formed, the process of sporulation is irreversible; sporulation is complete regardless of any change in environmental conditions. At stage IV, a cortex is formed in the space between the inner and outer membranes of the prespore, which is actually a peptidoglycan with a specific structure. In addition to this, dipicolinic acid begins to form and the spore begins to accumulate calcium. At stage V, protein coatings form over the outer membrane, which together form the spore capsule. Dipicolinic acid formation and calcium accumulation continues. At stage VI, the spore already has the typical and definitive characteristics: light refractivity, dehydration and resistance to chemical and physical influences. The lysis of the mother cell and the release of the spore is referred to as stage VII (Fig. 15). The energy required for the synthesis of spore structures is obtained by the cell through the oxidation of the storage intracellular poly-β-hydroxybutyric acid. For sporulation of aerobic bacteria, the presence of oxygen is essential, whereas in anaerobic bacteria, oxygen acts as an inhibitor of sporulation. The presence of certain cations (NH_4^+, Mn_2^+, Ca_2^+, Co_2^+, Ni_2^+) and anions (PO_4^{3-}, SO_4^{2-}, NO_3^-) in the environment is required to initiate the sporulation process. By analogy, the presence of calcium cations is necessary as it is a signal in the sporulating cell for the synthesis of dipicolinic acid.

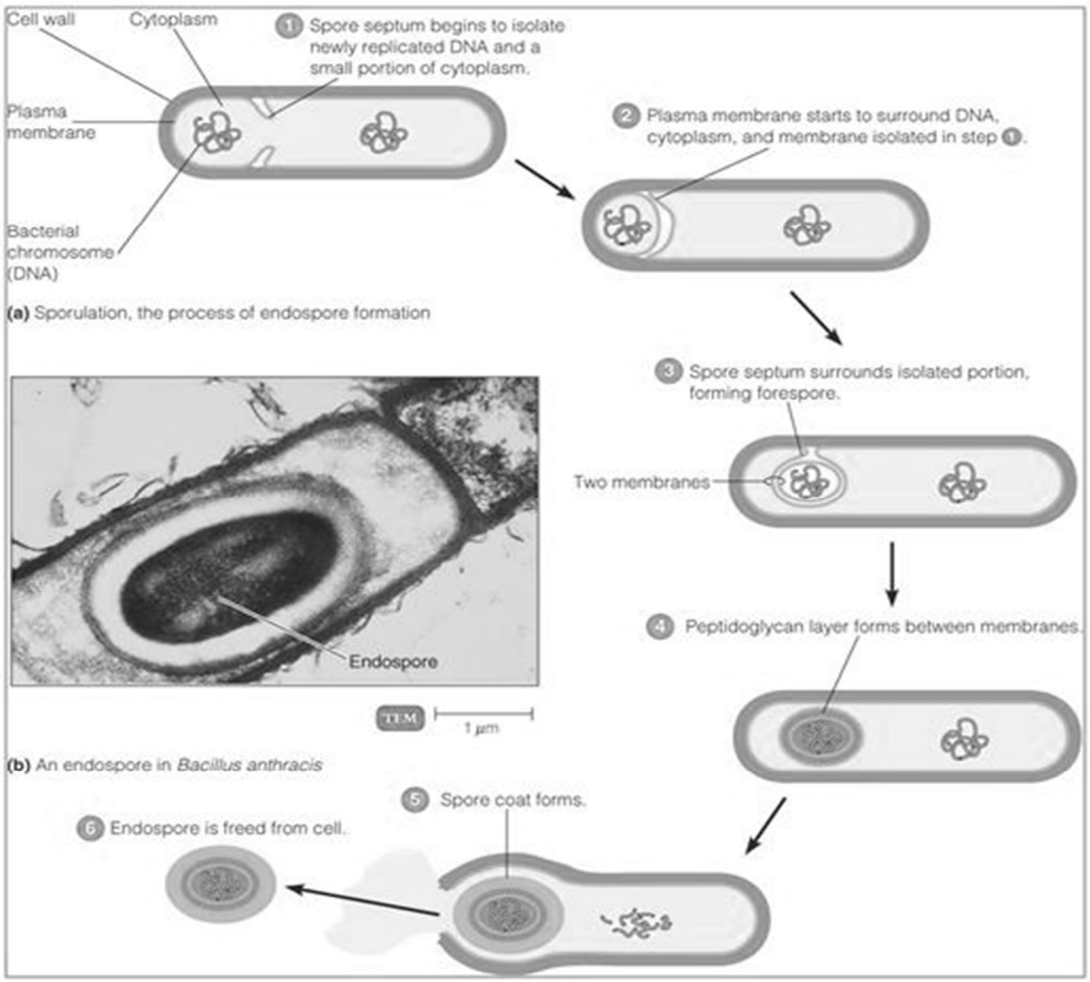

Figure 15 Sporulation in a bacterial cell (a) and endospores in *Bacillus anthracis* (b) (URL 15)

With morphological changes come physiological changes in the process of sporulation. In general, these physiological changes can be divided into three groups:

1. the formation of completely new molecules and structures that are not present in the vegetative cell, only in the sporulating cell and are indispensable for the cell. An example is dipicolinic acid, or peptidoglycan, which is completely different in composition from the peptidoglycan of the vegetative cell, also a protein of the spore capsule.

2. there is a substantial change in the activity of some cellular functions that are indispensable for successful sporulation. Examples include an increase in the activity of Krebs cycle enzymes and the content of cytochromes associated with increased rates of energy production by aerobic respiration. If changes in this category are prevented in progress, sporulation will not take place.

3. changes in physiological activities that are not related to sporulation itself but to nutrient depletion at the end of the exponential phase. Examples are an increase in the activity of proteases or amylases.

Among the biochemical changes occurring in parallel with sporulation is mainly the production of hydrolytic enzymes (proteases, amylases, etc.) released into the environment. Also, in some production strains, antibiotics (e.g. bacitracin) are produced.

The finished dormant spore is a bacterial protoplast enveloped by multiple layers. The protoplast itself contains primarily the genome, then also a small amount of the complete proteosynthetic apparatus, including ribosomes, tRNAs, accessory enzymes, and protein factors. A characteristic feature of the spore is its low, almost zero free water content and the associated high light refractivity of the spore and its high resistance to elevated temperature.

The innermost layer surrounding the protoplast is the cytoplasmic membrane. Above it is a thin layer of peptidoglycan identical to the peptidoglycan of the vegetative cell wall. It is the basis of the future cell wall. This is followed by a massive layer called the cortex, specific to the endospore, unknown elsewhere, made up of concentric layers of special peptidoglycan. Cortex carries extreme resistance to mechanical and other damaging influences. Above it is the spore capsule made up of proteins. The mantle carries resistance to chemicals and also to UV and ionizing radiation. The very surface of the spore may still have an exosporium, which is a membrane composed of lipids and proteins. However, it is not indispensable.

The resistance and viability of bacterial endospores is indeed extreme. In this respect, they surpass all known quiescent life forms. Water scarcity does not bother them even after centuries. They also tolerate boiling for hours, so that an autoclave is necessary for sterilisation. They are resistant to all forms of radiation, acids and organic solvents. This is undoubtedly linked to the other extreme: virtually immeasurable metabolism. A bacterial endospore can be transformed back into a growing and multiplying vegetative cell. The impetus is the presence of chemical and physical conditions suitable for growth and multiplication.

2 TAXONOMY OF DOMAIN BACTERIA

Taxonomy in a broad sense is the science of classifying (classification), naming (nomenclature) and determining (identification) of organisms. Thus, taxonomy includes three separate but interrelated fields. Classification deals with the sorting of organisms into taxonomic (systematic) groups (taxa) on the basis of their characteristics (characteristic features and properties). A taxon is a general name for any systematic (taxonomic) unit (e.g. species, genus, family, order, class, division, phylum, kingdom) formed by a group of organisms that is designated by a scientific name. Nomenclature deals with the establishment of names for defined taxa according to internationally accepted rules. Identification is the determination of the affiliation of an unknown organism to one of the already established and named (i.e., known) taxa.

2.1 Classification

Classification is the arrangement (sorting) of organisms into groups made up of very similar or closely related individuals according to a pre-selected system. Each group is to be homogeneous and to be distinct from other groups.

In classifying organisms into taxa, both natural, i.e., based on genetic and phylogenetic relatedness, and artificial or practical, based on phenotypic similarity, systems are applied. A phenotype is a set of characteristic apparent traits and properties of a particular strain of bacteria. It is the result of the interaction of its genotype and environment. The genotype is the set of all the genes of an organism, regardless of their location in the cell.

The bacterial classification system is the result of complex analyses that take into account the overall degree of similarity and dissimilarity of both phenotypic and genotypic characteristics of the organisms. For each taxon, a set of characteristic properties (markers) must be established by which one taxon can be distinguished from another. The description of the characteristic properties and distinguishing features of a taxon is called a diagnosis in biology (meaning in medicine and phytopathology the recognition, detection and nominal determination of a disease). Diagnostic (distinctive) character is for distinguishing one taxon from another. Classification is a subjective activity. Taxonomists may differ in their views on the similarity and dissimilarity of organisms. They may disagree on which characteristics are important or forgettable in distinguishing organisms. Each group of taxa can be classified in several ways.

The responsible agency for the nomenclature of bacteria is the International Committee for Systematic Bacteriology (ICSB) and its adjudicating committee, the Judicial Commission. The classification evolves in accordance with the corresponding degree of knowledge of the properties of bacteria and the development of the methods used to study them. The internationally accepted ('official') system for the classification of bacteria is the so-called orthodox Bergey system, published in 'Bergey's Manual of Determinative Bacteriology'. The first edition was published in 1923. Since then, the grading system has always been refined and supplemented after a certain period of time. It deals exclusively with the identification of those bacteria which have been described and cultured. This differs from the previous Bergey manuals, which attempted to give a summary of both the system and the determination of bacteria. In the future, information on the system of bacteria will be given by "Bergey's Manual of Systematic Bacteriology" and on their identification by "Bergey's Manual of Determinative Bacteriology".

2.2 Methods used in classification

The classification of organisms is based on knowledge of their characteristics and properties. In domain bacteria, this knowledge has been acquired both by observation with the eye, light and electron microscopes (morphological characters) and by experiment (physiological, biochemical and genetic characters).

In contrast to botany and zoology, in bacteriology the object of classification is not an individual (a single bacterial cell) but – with the exception of morphological and structural characters – a population of identical individuals (over many generations). Microscopic, cultural, physiological, biochemical, chemical, ecological, molecular biological, genetic, serological and numerical taxonomic methods are used in the classification of bacteria. The methods used in classification are also applied to the identification of bacteria. At this point we will only mention the characterisation of the bacterial genome and numerical taxonomy.

The bacterial genome can be characterized by the total nitrogenous base content of DNA, which is commonly expressed as the molar percentage (mol %) of guanine (G), i.e. purine base, and cytosine (C), i.e. pyrimidine base, of the total nitrogenous base content (mol % G + C). This figure is sufficient for the characterization of the genome; it is not lawful that the total amount of guanine and cytosine is equal to the total amount of the remaining two nitrogenous bases, adenine and thymine. Thus, the sum of guanine and cytosine content indirectly reflects the representation of nucleotides in DNA. The different genera and species differ from each other in the representation of nucleotides in DNA, this representation of nucleotides being constant. Organisms that differ by more than 18-30 % in the total percentage of G+C usually have no common nucleotide sequences (identical sequence). A convention has been adopted that organism showing 70 % or greater DNA relatedness belong to the same species.

Numerical taxonomy is based on the quantification of similarities and differences between organisms. Based on the analysis of a larger number of characters (at least 50), taxonomic relatedness is expressed by a **similarity coefficient**, which is calculated according to the proportion of characters shared out of the total number of characters observed. According to the calculated percentage of relatedness, the tested isolates are placed in similarity **dendrograms** (tree-branching graphs) or triangles. While the traditional bacterial classification system (monotheistic system) gave different importance to individual characters, in numerical taxonomy all characters are considered equally important.

In numerical taxonomy, special terms are used, which, in addition to those already mentioned, include phenon and cluster. **A phenon** is a taxonomic group representing an operational unit that replaces the term species, genus, etc. For example, if a taxonomic group has a similarity of 85%, it is said to be 85% phenon. **A cluster** is a group of strains with more or less similar characteristics that is formed when strains are grouped by percent relatedness into similarity dendrograms rather than triangles. A cluster does not have to agree with the taxon definition.

The criteria of the modern taxonomy of culturable bacteria, including a summary of genotypic, phylogenetic and phenotypic information, cannot be applied to phytoplasmas (which have so far failed to be cultured) because their phenotypic characters are difficult to determine. Therefore, phylogenetic pedigrees constructed by analysing the sequences of the 16S rDNA gene or the gene encoding 16S rRNA (ribosomal RNA), which is a universal **phylogenetic trait for classifying** the basic groups of all bacteria, have become the basis for the classification of phytoplasmas.

Sequence analysis of the conserved gene encoding the elongation (elongation) factor TU (tuf-gene), which is commonly involved in the process of translation and is commonly used as a phylogenetic marker, is beginning to be applied as a complementary means of classifying phytoplasmas, suitable for differentiation of closely related phytoplasmas. Molecular characterization of conserved genes represents only a partial solution to classification, as it allows the construction of a classification scheme that is independent of phenotypic features.

2.3 Taxonomic units and hierarchy

The usual criteria for defining a bio logical species cannot be applied to domain Bacteria. In eukaryotic organisms, a species is understood as a set of individuals capable of interbreeding and having a common phylogenetic origin. In bacteria, however, sexuality does not exist in the strict sense of the word. A bacterial species is defined as a collection of strains that share many features that distinguish it from strains of another species. There is not the same sharp boundary between different species of bacteria as there is for eukaryotes. A bacterial species is sometimes subdivided into subspecies on the basis of minor but stable phenotypic differences within the species or on the basis of genetically determined clusters (clusters) within the species. Of the higher taxa, considerable importance is attached to the genus because there is considerable divergence between genera. Taxonomic units at the level of family, order and class are rarely used in bacteriology.

The taxonomy of phytoplasmas is still not settled. Previous attempts to classify the phytoplasmas and whether the strains of phytoplasmas known so far have confirmed the validity of the classification of phytoplasmas in the class Mollicutes. Phytoplasmas are more closely related to mycoplasmas than to bacteria having a cell wall. Within the class Mollicutes, phytoplasmas form a distinct monophyletic unit (clade) formed by cladogenesis, i.e. the process of splitting and isolation of developmental branches in the phylogenetic development of organisms. At the same time, phytoplasmas have been shown to be a much more diverse group of organisms than previously thought. Phytoplasmas are included in 14 to 20 groups (subclades) and several dozen subgroups. It is thought that each group probably corresponds to at least one species.

Many of the diseases that scientists believed to be caused by phytoplasmas were described before phytoplasma groupings were made on the basis of DNA analysis. However, it is now possible to identify genetically defined phytoplasmas with specific plant diseases. For each phytoplasma strain, it will be possible to determine the presence, absence or extent of a phenotypic trait and to compare it with such traits as the range of insect or plant hosts and the ability to induce a particular type of symptom on the host plant.

2.4 Additional terms in taxonomy

A. General terms

Phylum. The offspring of a single (single, unique) isolation from a specific source (e.g. an infected plant or soil sample) in pure culture. Usually the strain is composed of a number of cultures derived originally from a single initial colony. However, most phylum are not known to be clones.

Type phylum and **reference phylum**. A type phylum is a live pure culture of micro-organisms derived from the phylum according to which the nomenclatural type

has been established by the respective author. A culture of this phylum, the so-called type culture, must be deposited in an official culture collection with an appropriate description. A reference phylum is a culture of micro-organisms used as a standard for comparative studies. It is not identical to the type phylum.

Clone. A population of bacterial cells derived from a single mother cell**.**

Culture. A population of bacterial cells at a given location (e.g. on nutrient agar) and at a given time; may also be of long duration, e.g. as a lyophilisate.

Pure culture. A population of bacterial cells from a single strain of bacteria. It is not specified whether it is not also a clone.

Mixed culture. A population of bacterial cells that is made up of several strains of bacteria.

Isolate. A single pure culture obtained by direct isolation from fresh material and a subculture established therefrom. Sometimes considered synonymous with strain.

Individual. This term has no place in bacteriology; it could refer to a single bacterial cell as well as to a phylum.

B. Terms relating to strains of bacteria

New name (older, incorrect name)	**Characteristic feature**
Biovar (biotype)	special biochemical or physiological properties
Serovar (serotype)	special antigenic properties
Pathovar (pathotype)	pathogenicity to certain host organisms
Fagovar (fagotype)	susceptibility to lytic infection with certain bacteriophages
Morphovar (morphotype)	special morphological characters (growth R- and S-forms)

C. Terms relating to phytopathogenic domain Bacteria

Pathovar (pv.). A phylum or group of phylums with the same or similar characteristics differentiated at the subspecies level from other phylums of the same species or subspecies on the basis of specific pathogenicity to one or more host plant species. Classification of a taxon as a pathovar does not preclude the existence of differences in biochemical, serological, or other non-pathogenic characteristics between that pathovar and other pathovars of the same species or subspecies, but merely indicates that, at the subspecies level, these differences are considered to be taxonomically less significant than differences in pathogenicity. Not every pathovar can be correctly identified by routine biochemical tests. Usually, pathovars are distinguished on the basis of demonstrated differences in the host range. However, pathovar designation can also be used in those cases where clear differences in disease symptoms in the same host species are expressed.

The division of species into pathovars is mainly applied to the genera *Xanthomonas* (more than 140 pathovars known) and *Pseudomonas* (more than 50 pathovars). No pathovars have been established in the genera *Agrobacterium*, *Streptomyces*, *Rhodococcus*, or in the phytoplasmas and spiroplasmas.

Although pathovars have no place in the official taxonomy of bacteria, from a phytopathological point of view the established pathovar system is useful. However, it has its shortcomings. In most cases, the host range of the strains of a given pathovar is not reliably known, because no large-scale study has yet been carried out that includes the numerous cross-inoculations necessary to precisely define the host

specialization. There is demonstrable genetic heterogeneity within pathovars. Non-pathogenic xanthomonads that have been isolated from both healthy and diseased plants cannot be classified within the pathovar system.

Race. A set of phylum of strains that differ from other phylum within a bacterial species or pathovar in their host specialization to cultivars (varieties) or other germplasm (germplasm). Races are identified by differentiating plant hosts, which may be cultivars or other identifiable germplasm. Sorting into races is not subject to the rules of the bacterium logic code. Races may be indicated by letters or numbers.

Forma specialis (f. sp.; plural formae speciales, abbreviated ff. sp.). Designation for pathogens characterized by specificity for a special host. In phytopathogenic bacteria it is not actually used or only exceptionally used. In no case does this designation replace the terms pathovar or race.

Phylum. In contrast to the concept common in general bacteriology, in phytopathology this term can also be applied to refer to isolates of the same pathovar but isolated from different hosts. Thus, for example, one can speak of bean or cherry strains of *P. syringae* pv. *syringae*, etc. The subdivision of species or pathovars into races is e.g. applied to *Pseudomonas syringae* pv. *phaseolicola* (9 races), *P. syringae* pv. *pisi* (6 races), *Xanthomonas vesicatoria* (3 races) and *Ralstonia solanacearum* (3 races).

2.5 Taxonomic classification of phytopathogenic bacteria

According to the current state of knowledge (1999), phytopathogenic bacteria are part of the superkingdom Bacteria. They are subdivided into three main groups, the Gracilicutes, Firmicutes and Tenericutes, differing in cell wall structure and the presence or absence of an outer membrane. The inclusion of some genera (e.g. *Rhizobacter* and *Liberobacter*) in the higher taxa has not yet been decided.

2.6 Nomenclature

Nomenclature can be considered as a product of taxonomy. A name must be chosen for each organism assigned to a particular taxon. We distinguish between scientific nomenclature (formal, legitimate, in accordance with internationally agreed rules) and informal nomenclature (professional, but not subject to internationally binding rules).

2.7 Scientific nomenclature

The scientific nomenclature of bacteria is governed by the "International Code of Nomenclature of Bacteria", the latest revised edition of which dates from 1992. It is subject to the nomenclature of subspecies and higher bacteria. The “Bacteriological Code” of 1975, which gave rise in 1980 to the publication of the seminal document entitled "Approved List of Bacterial Names". It contains all the scientific names of bacteria that have retained their nomenclatural validity from the past. Each additional name is considered valid if it has been published or validated (cited in the validation list) in the International Journal of Systematic Bacteriology (USB), since 2000 in the International Journal of Systematic and Evolutionary Microbiology (USEM). However, this does not mean that all names published in USB or USEM are correct. Validity is not the same as correctness (see below). If scientific names are in quotation marks, it means that they have not been validly published.

The nomenclature of pathovars of phytopathogenic bacteria follows the international standards called "International Standards for Naming Pathovars of Phyto pathogenic and List of Pathovar Names and Pathotype Strains". It is a recommendation issued in 1980 by the Commission on Taxonomy of Generic Bacteria of the International Society for Plant Pathology. A revised edition of these standards was published in 1991.

The official nomenclature has to be seen as a compromise between the need to stabilise nomenclature on the one hand and the need to reflect the inevitable changes that occur in the classification of bacteria on the other. It should also be respected that different classification schemes may exist for a given group of organisms**.** The Bacteriological Code ensures that the correct names of organisms are determined (in terms of legitimacy, publication validity, and name priority), but it does not determine which taxonomic interpretation is correct and which is not. It follows that there may be several different valid names for the same organism. Fortunately, there are not many such cases. Among the phytopathogenic bacteria, this applies, for example, to the pectinolytic erwinia, for which there is a nomenclatural correct generic name, *Pectobacterium* (Walder 1945), but which is rarely used by phytopathologists.

In accordance with the peculiarities of classification, the nomenclature of the phytopathogens of the class Mollicutes has also evolved differently. The nomenclature of spiroplasmas follows the International Nomenclatural Code of Bacteria. In contrast, the nomenclature of phytoplasmas has its own specificities.

2.8 Nomenclature of bacteria

The species name is formed by a binary combination (a concatenation of two words) of the genus name and the species attribute (epithet). In addition to the genus and species names, a complete correct citation must also include the name of the author who proposed the name and validly described the bacterium or the author who renamed the bacterium, and the year of publication (in taxonomic works, the year of publication is additionally followed by the page of the original text where the taxon was described).

Example: *Erwinia carotovora* (Jones 1901) Bergey, Harrison, Breed, Hammer & Huntoon 1923.

The subspecies name is a ternary combination of the genus name, species epithet, abbreviation "subsp." and the subspecies epithet.

Example: *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff 1914) Davis, Gillaspie, Vidaver & Harris 1984.

The name of a pathovar is formed by a ternary or quaternary combination (a combination of three or four words), namely, the genus name, the species epithet, or the subspecies epithet (preceded by the abbreviation "subsp."), and finally the pathovar epithet, which is preceded by the abbreviation "pv." (patovar). If a scientific name is given for the first time in a publication, it should be given in its full form. Thereafter, when there is no longer a risk of confusion, the pathovar name may be used in abbreviated form.

For example: *Pseudomonas syringae* pv. *lachrymans* (Smith & Bryan 1915) Dye, Bradbury, Goto, Hayward, Leliott & Wilkie 1978.

Abbreviated forms of the name: *Pseudomonas syringae* pv. *lachrymans*; *P. s. lachrymans*; pv. *lachrymans*.

The scientific name should be understood as a label or marker and not as a description of the organism. Scientific names are not to be abbreviated in the text.

Exceptions are the generic names of some very well-known organisms, which are sometimes used in a counted form. In such cases, they are inflected and written with a small initial letter (e.g. „with clavibacter", „on pseudomonads", etc.).

2.9 Nomenclature of phytoplasmas

The international nomenclature for phytoplasmas was initially identical to that used for phytoviruses. It is based on the common English name of the host plant (often the name of the plant on which the phytoplasma was first described) together with the characteristic type of symptom that the phytoplasma in question causes. The Czech nomenclature of phytoplasmas has also followed this.

Example: Apple proliferation phytoplasma.

In 1995, the International (Committee on Systematic Bacteriology) approved the use of the provisional category *Candidatus* to describe bacterial nonculturable entities for which not only the DNA nucleotide sequence is available, but other requirements are also met, including the deposit of the sample in a gene bank and the indication of the natural environment in which the organism occurs, and others by which the organism can be identified by *in situ* hybridisation or other techniques for cellular identification. The category *Candidatus* represents an intermediate stage to the usual binomial (binary) nomenclature.

Example: "*Candidatus* Phytoplasma fraxini". It is a name encompassing the earlier two phytoplasmas, i.e. Ash yellows phytoplasma and Lilac witches-broom phytoplasma (lilac witches'-broom phytoplasma).

2.10 Non-scientific nomenclature

Non-scientific nomenclature is used for:

a) Species (possibly several related species):

- putrefactive bacteria = *Erwinia carotovora*;
- fire blight bacteria = *Erwinia amylovora*;
- tumour bacteria = *Agrobacterium tumefaciens*;
- vascular bacteria = *Clavibacter michiganensis*;

b) phylum, e.g. *A. radiobacter* K84, producer of bacteriocin;

c)races, e.g. race 1, 2 and 3 for bacteria *Pseudomonas syringae* pv. *phaseolicola*;

d) serovars with certain antigenic properties, e.g. „la";

e) genetic mutants requiring valine for growth, marking „val".

3 IDENTIFICATION

The same methods shall be used for identification as for classification. Identification is dependent on the perfection of the classification system. However, the characters used for identification are not completely identical to those used to classify a particular group of bacteria. For example, classification may be based on the results of nucleic acid hybridisation, whereas identification is based on phenotypic characteristics known to correlate with the genetic information of the organism. Whereas classification takes into account a large number of traits, a few traits are often not sufficient for identification. Serological reactions are usually of limited value for

classification of bacteria, whereas their value in identification is enormous because of their simplicity, speed and usually high specificity.

Due to the statistical nature of the observed traits, identification of an unknown bacterial organism is always possible only with a certain degree of probability. Conventional bacteriological tests used in bacterial classification are often sufficient to identify pathovars in phytopathogenic bacteria.

Identification is usually preceded by isolation and obtaining a pure culture. In many cases it is possible to identify phytopathogenic bacteria without isolation, i.e. directly in host tissues or in plant homogenates.

The identification of bacteria relies primarily on cultural, serological and genetic traits or characteristics. Most bacteria should be identifiable without knowing from what environment they originate. However, knowledge of the environment from which the bacterium originates is an important landmark to facilitate and speed up the identification process. In fact, certain environments (soil, watercourses, plants, animals, etc.) are characterised by the presence of a particular microbiota (this is known as the 'indigenous microbiota'). Some phytopathogenic bacteria are very closely associated only with certain plant species, where they can cause typical symptoms of disease.

A special approach and special methods are applied in the identification of phytoplasmas that cannot be cultured. In the past, identification could rely mainly on symptoms on host plants, observation of phytoplasmas in sieve plates using electron microscopy and bioassays on indicator plants. More recently, staining of phytoplasma DNA with the fluorochrome DAPI, which is used for specific staining of nucleic acids followed by observation of fluorescent dots at the DNA site using a fluorescence microscope, has been used with success in some cases. Since the 1990s, analysis of the 16S-segment of rDNA by polymerase chain reaction (PCR) and restriction fragment length polymorphism, abbreviated as restriction fragment length polymorphism (RFLP), has been applied for identification.

3.1 Isolation and obtaining pure culture

Isolation

The basic prerequisites for the successful isolation of phytopathogenic bacteria from diseased plant tissue are: the use of appropriate isolation techniques (e.g. it is a mistake to place larger tissue segments on nutrient agar, as is practiced for fungal isolation); the selection of the right sample of infected tissue for isolation (depending on the type of symptoms); the use of appropriate culture media (preferably selective or semi-selective).

Non-pathogenic phytopathogenic bacteria survive best at the edge of infected plant weeds. Attempts to isolate the pathogen from dead weeds are often unsuccessful. The most suitable site for taking weeds for isolation is the interface between necrotic and living tissue. For isolations of bacteria of the genus Agrobacterium, the causative agents of tumorigenicity, it is recommended to collect for isolation to the overlying tissues of tumors. For isolations of vascular wilt pathogens, it is best to take a portion of the stem about 2 – 3 cm below the newly wilted leaves or petals. A small piece of the infected stem or petiole is placed in sterile water in which the bacteria at the site of the incision wound from the vascular bundle will drain out. For some vascular pathogens (*Clavibacter michiganensis* subsp. *sepedonicus* on potato; *Xanthomonas campestris* pv. *campestris* on brassica; *Erwinia tracheiphila* on cucumber) it is best to cut the tuber or stem and squeeze with the fingers until droplets of mucilage appear on

the surface of the surface at the site of the vascular bundles, from which it is not infrequent to obtain a pure culture of the pathogen.

Isolation and cultivation on artificial media are not yet feasible with phytoplasmas but is possible with spiroplasmas.

3.2 Differentiation and selective media

Various special media are recommended for the isolation of different phytopathogenic bacteria. Many bacteria grow and survive well on yeast-peptone medium at pH 6.8-7.2. Growth can be improved by adding sugars and alcoholic sugars (mannitol, sorbitol, etc.). However, there is a danger that in this case the bacteria grow rapidly and if the pH of the medium drops significantly, the culture will die. Slime also forms abundantly on media with a higher sugar content. Many phytopathogenic bacteria grow on simple media with an inorganic nitrogen source. However, certain species require one or more growth factors, most commonly nicotinic acid, thiamine or biotin. For the growth of some xanthomonads, most coryneform pathogens require amino acids, especially those containing sulphur (e.g. L-methionine).

Differentiation media provide the ability to identify bacteria by characteristic colonies. The most commonly used differentiation medium is the so-called King B-medium, on which most phytopathogenic pseudomonads form a characteristic fluorescent pigment.

Selective media allow the identification of certain bacteria by suppressing the growth of other microorganisms. Such media are difficult to develop. A special approach is used in the preparation of selective media for the challenging bacteria *Xylella fastidiosa* and for spiroplasmas. The essence of selectivity is not the content of inhibitors effective against 'unwanted organisms', but the content of special nutrients without which 'no organisms' can be cultivated.

Semi selective media combine the properties of differentiating and selective media. In practice, semi selective media are more widely used than selective media; more than 350 of them have been developed for phytopathogenic bacteria. For example, semi-selective media for isolation of *Ralstonia solanacearum*.

3.3 Pure cultures

The basis of a pure culture is bacterial cells forming a single colony, which is assumed to be derived from a single bacterial cell. If doubts arise as to the purity of the isolate obtained, one single colony is again taken from the culture, and this gives rise to another subculture. From a mixed culture it is usually pointless to carry out identification tests, which applies without exception to physiological and biochemical tests.

Various bacteriological techniques are used to obtain a pure culture. Among the most widely used are both the so-called cross-spotting method on agar medium and various modifications of the Koch method by dilution in liquid medium (broth, isotonic solution) or in molten agar medium. It is not desirable to rush the inoculation of selected colonies on solid media, as some contaminating bacteria may only appear on the culture plates after prolonged incubation.

Selective media cannot be used for the preparation of pure culture for physiological, biochemical, or other identification tests. A culture growing on selective media may contain contaminants which, although not growing on the soil, remain alive within or near the colonies of the desired organism and may enter the next subculture

together with the selected (desired) organism. In addition, the selective media may contain special inhibitory substances which may also affect to some extent the characteristics of the culture of interest.

For phytoplasmas agents, identification tests must dispense with pure cultures, as these organisms have not yet been cultured on artificial media.

3.4 Methods used for identification

Since the late 1980s, new methods have been gradually applied to the detection and identification of bacteria, which have in common that they analyse structural elements of target organisms, i.e. nucleic acids, polyamines, proteins, lipids, fatty acids, polysaccharides and lipopolysaccharides. The new methods are rather quickly sidelining in particular those conventional identification procedures which are very laborious and time-consuming. New methods for the detection and identification of phytoplasmas that cannot be cultured on nutrient media are of revolutionary importance.

3.4.1 Microscopic methods

The shape, size and grouping of bacterial cells, their motility, number and location of flagella, cell wall structure (Gram staining), presence of sheaths, endospores and inclusions are observed. The morphological characteristics (detectable by staining techniques) of individual bacteria are not sufficient for accurate determination, because the relatively simple morphological and structural structure of bacteria provides a relatively narrow scope for species differentiation. In many cases, morphological identification is also hampered by the shape variability of the bacterial cells.

Electron and fluorescence microscopy of ultra-thin sections of plant tissues is of considerable importance for the diagnosis of phytoplasmas. Good results are obtained by a special DNA staining technique. The dye DAPI (4,6-diamino-2-phenylindolepyridine hydrochloride) is used for staining. The stained thin sections of the weft are viewed with a fluorescence microscope. The intensity and location of the fluorescent spots can be used to distinguish phytoplasma DNA from host plant DNA.

3.4.2 Cultivation methods

Colony size, shape and pigmentation (macroscopic colony morphology) on solid media (e.g. nutrient agar), growth in liquid media (e.g. broth), growth rate and slime production, and possibly culture odour are recorded.

White colonies are typical for bacteria of the genus *Agrobacterium*, yellow colonies for the genus *Xanthomonas*, red colonies for the species *Erwinia rhapontici*, yellow-green fluorescent colonies for the group of fluorescent bacteria of the genus *Pseudomonas*, yellow-pink colonies for *Clavibacter flaccumfaciens* pv. *poinsettiae*. On soils of special composition, the blue pigment indigoidin is formed by *Clavibacter michiganensis* subsp. *insidiosus* and *Erwinia chrysanthemi*. Actinomycete colonies resemble micromycete colonies in appearance.

3.4.3 Physiological methods

Relationship to oxygen, temperature range for growth, resistance to extreme temperatures, tolerance to sodium chloride, sensitivity to antibiotics, and mode of

energy acquisition (e.g. by fermentation, aerobic or anaerobic respiration) are delineated.

3.4.4 Biochemical methods

The utilization (utilization) of carbon sources (in all genera) is determined, and evidence of nitrate reduction (in *Pseudomonas*), starch hydrolysis (in *Xanthomonas*), gelatine (in *Xanthomonas*, *Pseudomonas*, and *Erwinia*), and esculin (in *Xanthomonas*) is carried out; the production of indole (in *Erwinia*), hydrogen sulphide (in *Erwinia* and *Xanthomonas*), acetoin and lysine or ornithine decarboxylases are monitored; evidence of enzyme production (catalase, oxidase, phosphatase, urease, 8-galactosidase and others) is carried out.

Biochemical and physiological tests were originally carried out in liquid media in test tubes, less frequently on slant or perpendicular agar plates. In the last decade, standardized miniaturized multitest systems, called microassays, have been developed instead of the classical tube assays. A number of commercially produced miniaturized diagnostic kits are available, especially for the needs of medical, veterinary and food microbiology. These are actually transparent plates in which microtubes are moulded containing dehydrated (lyophilised) media with the appropriate substrates for each test.

A suspension of cells prepared from a pure culture is inoculated into the microtubes. Results are read 18 – 24 hours after inoculation. An identification register or computer programs are used to interpret the results. However, these are usually not designed to identify phytopathogenic bacteria. However, for the purposes of plant bacteriology, commercially produced miniaturized multi-testing systems can be used to rapidly perform some biochemical tests.

The best known of the commercially produced diagnostic kits are the so-called API systems (API System SA). For phytopathogenic bacteria, the special Biolog diagnostic system (Biolog Inc.) is useful. It differentiates bacteria based on their ability to utilize carbon sources using 96 microtiter plates. At 24 hours after inoculation with pure culture, the staining of the medium in the microtubes is evaluated. The biochemical profile of the test bacterium is analysed by computer and an identification is made with an indication of the probability of correct determination.

3.4.5 Chemical methods

The composition of the cell wall (presence of various peptidoglycans, teichoic acids, etc.) is detected. In high-tech workplaces, the fatty acid content is determined by gas chromatography.

Gram staining and cell wall solubility in potassium hydroxide

The nature of the distinction of bacteria according to the result of Gram staining into two groups, i.e. gram-positive (stainable) and gram-negative (non-stainable), after staining with triphenylmethane dye (crystal violet) and after staining with potassium iodide solution (Lugol's solution), is not fully elucidated. It is probably due to the different structure of the cell wall, which in gram-positive bacteria prevents leaching of the resulting complex of crystal violet and potassium iodide by ethanol or acetone, whereas in gram-negative bacteria it does not. Reliable positive controls are species of the genus *Bacillus* or *Lactobacillus*. Any species of the genus *Pseudomonas* may serve as a negative control.

Distinction of bacteria by Gram staining results correlates with aminopeptidase activity. Gram-negative bacteria (G-) show aminopeptidase activity (AP+), whereas gram-positive bacteria (G+) lack aminopeptidase activity (AP-).

Gram staining can be replaced by a cell wall solubility assay in 3% potassium hydroxide solution. If the bacterial suspension becomes viscous under the influence of KOH so that it sticks to the handle after 5 – 10 seconds and forms a string of slime, the test is positive (e.g. species of the genera *Agrobacterium*, *Pseudomonas* and *Erwinia*) and the bacterium tested is gram-negative. Conversely, if the cell wall is not lysable (e.g. in species of the genus *Bacillus* or coryneform bacteria), the test is negative and the test bacterium is gram-positive.

Content of fatty acids

Gas chromatography is used to detect qualitative differences in the content of poisonous fatty acids in bacterial cells and their relative proportions. Fatty acids include all aliphatic monocarboxylic acids derived from the hydrolysis of natural lipids. They are contained in complex lipids (liposaccharides) of the plasma and outer membranes and occur in smaller amounts as free ones. Each taxon is characterised by a particular fatty acid profile (composition). The fatty acid profile obtained for a particular bacterial species may be influenced by the composition of the culture medium, the temperature of the culture, the age of the culture and the technical equipment used to analyse the sample. A highly standardised fatty acid extraction procedure, analytical procedure and identification software has been developed (Microbial ID, Newark, USA). The database of the system is currently one of the largest in the world, including aerobic and anaerobic bacteria, actinomycetes, mycobacteria and yeasts. Cells are grown on standard medium. 40 mg wet weight of cells is sufficient for analysis. The identification itself takes place within a few hours. The disadvantage is the need for expensive instrumentation and experienced personnel.

3.4.6 Serological methods

Serological methods are a very useful means of identifying bacteria at the species level and lower taxa. They are based on the specific *in vitro* reaction (binding) of specific antibodies with the type of antigen that gave rise to the production of these antibodies in the body of the immunized animal. Specific antibodies are for theins (immunoglobulins) that are formed in the blood serum of vertebrates in response to the presence of a foreign substance, called an antigen. The specificity of the antigen response by homologous (paired) antibodies is used to detect and identify bacteria of plant weeds, in mixed or pure cultures. The advantages of serological identification are its speed, specificity, sensitivity and the affordable availability of the necessary instrumentation. Specificity is increased when a monoclonal antibody (homogeneous antibody against a single antigenic determinant) is used instead of a polyclonal antiserum (containing antibodies against different antigenic determinants).

The specificity of the antiserum is evaluated by whether it reacts positively not only with the type of antigen that was used to prepare the antiserum, but also whether it shows a so-called cross-reaction, i.e. it reacts positively with other bacteria within or outside the species. Monoclonal antisera are potentially more specific than the usual polyclonal sera. However, cross-reactions with non-target organisms also occur with monoclonal antisera. The sensitivity (potency) of an antiserum is expressed as the lowest level (concentration) of bacteria that can be detected using that antiserum.

Serological methods have a high detection efficiency (they are very sensitive) because they can detect the presence of pathogenic bacteria from natural sources (in plant material, etc.) at a concentration of about 10^3-10^5 cells in 1 ml, which is at the level of the detection efficiency of the polymerase chain reaction **(PCR)**.

In practice, the following serological tests are used to identify bacteria: slide agglutination, double immunodiffusion in agar according to Ouchterlony, ELISA and immunofluorescence. Sometimes serological methods are used in combination with nucleic acid analyses. For example, the target bacteria are first bound from the solution by antibodies attached to magnetic beads, which are then captured by a magnet. Subsequently, the polymerase chain reaction can be performed on the concentrated antigens.

Bacterial agglutination

Test bacteria suspended in a drop of saline on a slide are mixed with a specific antiserum. The reaction is evaluated using a light microscope in 3 – 5 minutes. If the antigen meets the homologous antibody, the reaction is positive, i.e. the bacterial cells aggregate (agglutinate, flocculate).

Enzyme-Linked Immunosorbent Assay – ELISA

This standard serological method uses enzymes (alkaline phosphatase) conjugated (chemically bound) to immunoglobulins separated from the antiserum to visualize the reactions of the antigen of the test bacterium with the homologous antibody.

In a first step, the walls of the wells in the polystyrene plates are rubbed with antigen (contained in the substrate to be tested, e.g. plant homogenate). The antiserum conjugated to the enzyme is added to the wells. After washing off the excess antiserum, the enzyme substrate (i.e. p-nitrophenylphosphate sodium solution) is added to the wells. This substrate is hydrolysed by the enzyme bound to the antiserum. The action of the enzyme releases chromogens (i.e. p-nitrophenol) which change the colour of the reaction mixture. The intensity of staining is directly proportional to the concentration of the antigen-binding enzyme. The colour change can be evaluated visually, the intensity of the staining photometrically (spectrophotometer).

Immunofluorescence Assay – IFA

To visualize the reactions of the antigen on the surface of the test bacterium with the homologous antibody, dyes (e.g. fluorescein isothiocyanate – FITC) are used. The dye conjugates (binds) either to the respective homologous antibody (in so-called direct fluorescent staining) or to another antibody that specifically binds to the first-applied homologous antibody (in so-called indirect fluorescent staining). In both cases, the presence of antibodies on the surface of the homologous bacteria is reflected by a bright green fluorescence. The method is characterised by its high sensitivity, coupled with the ability to detect bacteria directly in host tissues. It also has the advantage of being able to determine the size and shape of bacterial cells. The method is suitable for routine testing for the presence of a particular pathogen in a large number of samples of plant material.

Double diffusion in agar (gel immunodiffusion)

The Ouchterlony double radial diffusion test is performed by cutting wells in a flat layer of agar in a petri dish and filling them with antisera and antigens. The well in the middle usually contains the antiserum and the wells around it contain the antigens to be tested (a suspension of unknown bacteria). The antiserum and antigens

diffuse against each other. If the reaction is positive, a precipitate is formed at the site of the encounter, visible to the eye as one or more white precipitation lines.

3.4.7 Molecular-biological methods

Among the molecular biological methods, nucleic acid hybridization, polymerase chain reaction and length polymorphism are mainly applied in the determination of bacteria. These new methods are expected to have higher specificity and more reliable test reproducibility than conventional methods. However, even with these new molecular biological methods, false positive and false negative results cannot be excluded. It also remains an open question how to proceed with phytoquarantine measures when DNA of a target organism is detected, if we are not sure whether it is DNA from living cells or not.

Dot hybridization

The principle of this method is the hybridization (pairing) of complementary single-stranded stretches of DNA. A molecular probe is used to detect complementary stretches of DNA of the test (target) organism. A probe is a different length molecule of labelled dsDNA, ssDNA or RNA of known sequence. It is a defined (known) oligonucleotide or polynucleotide prepared from the DNA of a known defined bacterial strain. The probe hybridizes only with denatured nucleic acid chains that are complementary. Hybridization is limited to only those regions where the DNA of the probe and the DNA of the test sample share a homologous nucleotide sequence. Hybridisation of DNA probes with fragments of test DNA can be detected autoradiographically on X-ray film if radiolabelled probes are used, or also by colorimetric or chemiluminescence systems if the probes are labelled non-radioactively (digoxigenin, biotin, photobiotin).

Dot hybridization allows the detection of homologous DNA sequences in bacterial cells *in situ* without prior purification of the DNA of the tested bacterium. The actual detection assay is performed by applying a homogenate of the tissues in which the pathogen to be detected, or a suspension of pure or rabid test bacterial culture (3 μL at a concentration of 10^8 CFU* in 1 ml), to the inside of a cellulose or nylon membrane. This is immediately followed by lysis of the cells and denaturation of the DNA (i.e. disruption of the hydrogen bonds and cleavage of the double-stranded DNA) by exposure to NaOH and a temperature of 80 °C. After incubation with the probe and removal of the excess probe (washing away unbound parts of the probe), the hybridization result (number of pairings) is assessed autoradiographically or dosimetrically if the probe was radiolabelled (32P), or colorimetrically if an enzyme-labelled probe was used.

Polymerase Chain Reaction – PCR)

The essence of this method is the *in vitro* amplification of specific DNA fragments characteristic of the pathogen to be detected and identified.

Repeated cycles of DNA synthesis include: thermal denaturation of the dsDNA of the test bacterium; thermal hybridization (annealing) or attachment of primers (two short single-stranded specific 'matrix' oligonucleotides derived from the DNA of a defined bacterial strain); synthesis of double-stranded DNA in the presence of a temperature-stable DNA polymerase (usually tag-polymers of phosphates, the building blocks of nucleic acids.

Because of its high sensitivity (theoretically, one copy of DNA can be detected in a sample), PCR is probably best suited for the detection of those bacteria and

phytoplasmas that are present in plant tissues at low concentrations. Nested PCR (two consecutive PCRs, where the primers used in the second reaction must be 'nested' inside the amplified segment using the first primers) can be applied to increase sensitivity. PCR can also be performed in combination with immunomagnetic capture and fluorescent labelling.

If primers encoding those stretches of DNA that encode bacterial pathogenicity were available, the PCR technique could eliminate the need for pathogenicity tests. However, PCR is an instrument- and material-intensive method. Commercially produced, automated, easy-to-use instruments for thermal amplification of DNA by polymerase chain reaction are available. The disadvantage of the standard PCR method is that it does not allow the presence of the test organism or virus to be quantified. Newer PCR methods allow DNA quantification.

Restriction Fragment Length Polymorphism (RFLP) Analysis)

Typical RFLP analysis involves first isolation and purification of genomic or organelle DNA, followed by cleavage of the purified DNA with restriction endonucleases. These enzymes cleave the DNA at specific sites into fragments of different lengths. Commercial preparations are available for cell lysis and for the isolation, purification and cleavage of chromosomal DNA. The cleaved DNA fragments can be separated by one-way electrophoresis in agar gels, in which different length fragments have different mobility. The localisation of the fragments in the agar gel can be visualised by staining with ethidium bromide. The electropherograms show a spectrum of bands (so-called RFLP-profile) corresponding to fragments of different lengths. If a particular endonuclease is used for cleavage, the identity or dissimilarity of the restriction profiles can be used to infer the identity or dissimilarity of the analyzed genomic or organelle DNA. Either the whole chromosome (less frequently) or only certain DNA sequences (more frequently) can be subjected to restriction analysis. In whole genome analysis, the fragments are usually many to very many and overlapping, making evaluation with a densitometer difficult. For this reason, probes are used that allow visualization of only those fragments that hybridize to the probe used. A procedure known as Southern transfer or Southern blotting is used. Denatured DNA is transferred from the gel to a membrane filter, where it can then hybridise with a complementary nucleic acid sequence.

RFLP analysis is used to differentiate bacteria at the level of lower taxa, time to strain level. For routine detection and determination of bacteria, RFLP analysis is not suitable because it is relatively complex, expensive and time consuming.

Random Amplified Poly morphic DNA (RAPD) Analysis

In this method, profiles are obtained by polymerase chain reaction amplification of genomic DNA using short primers (about 10 base pairs) of random (arbitrary) sequence. The permissible temperature for DNA denaturation ranges between 36 – 45 °C. The obtained profile of amplification products allows the resolution of lower taxa, not infrequently at the strain level. The advantage of this method is that it is very fast and requires no prior data on target DNA, probe, gel-to-membrane filter transfer and hybridization or restriction sites.

3.4.8 Electrophoretic methods

Electrophoresis is a physicochemical method of separating substances based on the differential velocity of movement of particles carrying an electric charge in an electric unidirectional field. The rate of movement depends on the charge and the size

of the particles. In microbiology laboratories, electrophoresis is used to separate proteins extracted from bacterial cells. 40 – 50 mg wet weight of isolated cells is taken for analysis. Subsequent electrophoresis of the proteins is carried out in a polyacrylamide gel by the so-called SDS-PAGE (Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis) method. A protein electrophoresis is obtained, which is evaluated visually or densitometrically. The obtained protein profile, formed by bands in the gel (protein patterns), is compared with the profiles of the standards and evaluated by computer. By electrophoresis of cellular proteins, taxa can be distinguished at a subspecies level. The results correlate well with data obtained by nucleic acid hybridization.

Polymorphic enzyme electrophoresis (isozyme analysis) is also used to determine bacteria at the species level (less frequently) and for lower taxa (more frequently). Different strains of bacteria within the same taxon (e.g. serovars) may differ genetically in that the same enzyme is encoded by different alleles of its structural gene in different strains. The produced variants of the same enzyme (called isoenzymes or isozymes) differ in some physicochemical properties, including mobility during gel electrophoresis. The analysis of isozymes is relatively inexpensive but time-consuming. Standardisation is not easy. Isozyme profiles can sometimes be consistent with RFLP profiles, other times not.

3.4.9 Phage typing

Phage typing uses so-called monovalent phages (bacterial viruses) that are typical of a particular species or strain of bacteria whose cells are capable of lysing. The lytic properties of specific phages are used in the detection and identification of phytopathogenic bacteria of the genus *Xanthomonas* (*X. axonopodis* pv. *citri* is determined when checking consignments of lemons) and *Pseudomonas* (*P. syringae* pv. *tomato*). The procedure is that a virulent specific phage (called indicator or detector) is introduced into the bacterial culture to be tested (in semi-solid or liquid medium). In the case of a culture of a susceptible strain, the virulent phage will manifest itself by the formation of a so-called sterile spot or plague (in semi-solid medium) or by clearing (in liquid medium) as a result of lysis of the infected cells. Compared with biochemical, serological and molecular biological identification methods, the range of applications of phage typing is relatively small. This is probably because cases of absolute phage specificity are sparse.

3.4.10 **Biological tests**

The most important characteristic of microorganisms causing plant diseases is their pathogenicity. This property cannot yet be determined except by artificial infections on living plants. Methods that mimic natural infection are used.

For identification purposes, artificial plant infections are particularly applicable in the following cases:

- when it comes to recognising pathovars and bacterial races;
- in the determination of the causative agents of phytoplasmas;
- for the rapid differentiation of phytopathogenic pseudomonads, erwinia and xanthomonads from saprophytes by the so-called hypersensitivity test;
- to ensure the pathogenicity of certain polyphagous on indicator plants;

- to distinguish virulent and avirulent strains of pathogenic bacterial species.

Artificial plant infections are also of great importance in determining the level of resistance or susceptibility of host plant species and varieties and in testing the efficacy of plant protection products.

Tobacco hypersensitivity test

To quickly distinguish phytopathogenic pseudomonads and erwinia from saprophytes, the tobacco hypersensitivity test can be used ev. Burley (but also other cultivars). An aqueous suspension of bacteria with a concentration of more than 5.10^6 cells in 1 ml is injected by *P. syringe* into the intercellular spaces of the interosseous (interveinal) plexuses of the tobacco leaf. Phytopathogenic bacteria induce necrosis at the injection site within 24 hours, while saprophytes do not produce any symptoms. After inoculation of the plexuses with *P. syringae* pv. *tabaci*, necrotic symptoms appear only a few days after injection. The hypersensitivity test for xanthomonads is more appropriately performed on paprika or tomato plants. If tobacco plants are used, it is recommended to adjust temperatures to 16 °C 4 days before and during inoculation to 33 °C after inoculation. The hypersensitivity test is one of the most commonly applied tests in the identification of phytopathogenic bacteria. It is applied immediately after isolation of the bacteria. It reduces the need for further physiological and biochemical tests. Its advantage is its simplicity and time-saving nature. It does not require sterile facilities. The results obtained are relatively reliable.

Pathogenicity tests on indicator host plants and weeds

The following tests have found wider application:

- vacuum infiltration of pea seeds to verify the pathogenicity of *Rhodococcus fascians* isolates;
- eggplant (*Solanum melongena* ev. Black Beauty) plants are used to verify the pathogenicity of isolates of the causal agent of potato ringspot (*Clavibacter michiganensis* subsp. *sepedonicus*) and to determine the presence of the pathogen in homogenates prepared from potato tubers;
- the so-called pear test on immature fruit has proved useful for determining the degree of virulence of isolates of the causal agent of scarlet fever (*Erwinia amylovora*);
- tomato, durman, sunflower, or fresh discs of fleshy roots of carrot, turnip, parsnip, beetroot, Jerusalem artichoke, and tubers have proved to be good indicator plants to verify the tumorigenic activity of *Agrobacterium tumefaciens* isolates;
- immature detached cherry fruits are inoculated to distinguish isolates of *Pseudomonas syringae* pv. *syringae* from *P. syringae* pv. *morsprunorum*;
- *Catharantus roseus* (syn. *Vinca rosea*) plants are commonly used to harbour phytoplasmas; this host is capable of harbouring most of the known phytoplasmas, which not infrequently induce specific symptoms in it, so that it also serves as an indicator plant. Transmission of phytoplasmas to indicator plants is carried out by insect vectors or by grafting;
- woody indicator for the causal agent of phytoplasma proliferation is the apple cultivar Golden Delicious, for the causal agent of phytoplasma pear proliferation is the pear cultivar *Pyrus communis*;
- *Nicotiana tabacum* plants proved to be an experimental host species for studying plant-*Xylella fastidiosa* interactions.

When evaluating the result of infection tests, it is necessary to avoid considering the hypersensitive reaction of the test plant as a manifestation of typical disease symptoms. Most phytopathogenic bacteria are capable of inducing atypical symptoms (necrosis of the weeds at the site of inoculation and in the immediate vicinity) in many non-compatible plant species (i.e. those that are not host plants under natural conditions). On the other hand, a negative species or variety, or that the conditions for inoculation were not suitable, should also be evaluated judiciously. If the pectinolytic activity of the bacteria is determined on discs from potato tubers, it should be taken into account that the result of this test may not be indicative of the maceration potential for the tissue of other plants.

3.5 Choice of detection and determination procedure

By detection we mean both the search for or detection of the presence of a hidden disease agent and the result of this activity. Determination (identification) is the determination of the affiliation of an organism to a known named taxon on the basis of its characteristics.

Presumptive (probable, presumptive) diagnosis of phytopathogenic bacteria relies on: assessment of symptoms on plants; knowledge of the spectrum of potential pathogens on the host species concerned; the weather pattern that preceded the disease; evaluation of a few identification tests. For subsequent **confirmatory** (confirmatory, confirmatory) diagnosis, the principle applies that it must be based on the results of several different identification tests.

Plant diagnostic laboratories do not aim to identify all the bacteria present in diseased tissues, but in the first place those that are the causal agents of the disease. It is therefore advisable to exclude from further investigation those companion bacteria which are clearly not true phytopathogens. However, some sporulating rods, e.g. certain species of the genera *Bacillus* and *Clostridium*, are also considered to be opportunistic phytopathogens.

The results of identification tests may vary depending on the concentration of inoculum used, the temperature during inoculation and incubation, the length of the incubation period, the composition of the culture medium, the ratio between surface area and volume of the medium and, last but not least, the criteria used to define "positive" and "negative" reactions. **It is therefore important that the conditions under which each trait is to be tested should be internationally standardised.**

In diagnostic practice, it is important to select from the dozens of tests for the identification of bacteria, only the most appropriate and necessary ones, which allow the isolate under study to be identified quickly and by a simple procedure (economically acceptable). **A specific identification strategy has been developed for each genus of bacteria and a set of appropriate differentiation tests has been recommended.** Diagnostic protocols are mandatory for the Member States of the European and Mediterranean Plant Protection Organisation and are published in the OEPP/EPPO Bulletin as 'Approved EPPO Standards'.

In some cases, **isolated bacteria** can be tentatively assigned to a genus by the morphological features of the colonies on certain agar media and by the result of Gram staining. More complicated is the determination of the species and the pathovar. Thus, for example, no further tests are necessary for assignment to the genus Pseudomonas for those isolates which we have found to be polar flagellated gram-negative rods, to produce a green, fluorescent pigment, and to be oxidase-negative or oxidase-positive. Similarly, the yellow coloration of colonies on culture media (xanthomonadin

production), extracellular polysaccharide mucilage, and the ability to degrade starch are determinants for tentative placement in the genus Xanthomonas.

The results of the identification tests are compared with diagnostic differentiation tables published in bacteriological manuals. Identification keys (usually dichotomous or polyclonal) are less well established. Computer processing of biochemical tests has been applied, e.g. in the Biologist method. The diagnostic differentiation tables give an overview of the presence (+), absence (-) or variable occurrence (D) of a particular set of characters for a given taxon. With a larger number of characters for a larger number of bacterial taxa and a greater variability of characters, the tables become cluttered and do not always allow unambiguous guidance for identification.

Identification keys are based on a sequential sorting according to the presence or absence of individual features. The most commonly used is the so-called **dichotomous key**, where one of the two states is considered in each step, because the characters tested are not qualitative but quantitative; characters occur at a certain frequency in one taxon, namely greater than 0% and less than 100%.

The polychotomous key is constructed as a polynomial division procedure. Computer-assisted identification is mostly based on the principle of numerical identification. A fixed number of tests are performed, and the results are evaluated by computer so that the final identification is given with a calculated probability. If the chosen identification value is reached, then the test strain is considered to be identified.

The diagnosis of phytoplasmas relies mainly on the detection of the presence of characteristic pathogen cells in the host cells, on the results of pathogenicity tests, i.e. the determination of the ability to induce characteristic symptoms in specific host plants, and on the confirmation of the link between the occurrence of the disease and certain vectors. *Phytoplasmas* and *Spiroplasmas*, unlike other phytopathogenic bacteria, are resistant to beta-lactamase antibiotics such as penicillin.

3.6 Collections of bacterial cultures

Departments involved in practical bacterial determination, but also in other activities (e.g. bactericide testing) for which they need certain bacterial strains as standards, cannot do without the services provided by bacterial culture collections. The most important international collections of phytopathogenic bacteria include: the International Collection of Phytopathogenic Bacteria (ICPB), Davis, USA; the National Collection of Plant Pathogenic Bacteria (NCPPB), Har penden, UK; the International Collection of Microorganisms from Plants (ICMP), Plant Disease Division, DSIR, Auckland, New Zealand; and the American Type Cult re Collection (ATCC), Manassas, Va., USA. A considerable number of phytopathogenic bacterial strains are preserved in the Czech Collection of Microorganisms (CCM) of the Faculty of Natural Sciences, Masaryk University, Brno, Czech Republic.

4 CHARACTERISTICS OF PHYTOPATHOGENIC BACTERIA

In total, about 4 000 organisms from domain Bacteria are known. More than 300 bacteria exhibit phytopathogenic properties. Most of the most serious pathogens belong to one of the following genera: *Agrobacterium*, *Burkholderia*, *Clavibacter*, *Curtobacterium*, *Erwinia*, *Pseudomonas*, *Streptomyces* and *Xanthomonas*. A smaller

number of economically important phytopathological genes are represented in the genera *Ralstonia*, *Pantoea*, *Xylella* and *Xylophilus*. Less severe or opportunistic phytopathogenic bacteria are found in the genera *Acetobacter*, *Acidovorax*, *Bacillus* and *Paenibacillus*, *Clostridium*, *Enterobacter*, *Herbaspirillum*, *Rathayibacter*, *Rhizobacter*, *Rhizomonas* (*Sphingomonas*), *Rhodococcus*, *Proteus*, *Serratia* and others. In the overview that follows, we restrict ourselves to the characterisation of bacterial genera that include economically important phytopathogens.

4.1 *Acidovorax*

Straight to slightly bent gram-negative rods that are 0.2-0.7 x 1.0-5.0 µm in size. They are motile with one polar flagellum, rarely 2 – 3 polar flagella. Colonies on nutrient agar are round with smooth or slightly wavy margins, convex, smooth to slightly granular, beige to slightly yellow. Some phytopathogenic strains produce a yellow to slightly brown diffuse pigment. The optimum growing temperature is between 30 –35 °C. Species of the genus *Acidovorax* are found in soil, water, clinical specimens and infected plant material. The phytopathogens include *A. avenae* with three subspecies, of which subsp. *avenae* (causal agent of yellow and brown streak disease of cereals and forages) and subsp. *cattleyae* (causal agent of leaf spot and bud rot of orchids of the genera *Cattleya* and *Phalaenopsis*) are worthy of mention. Due to the relatively high optimum growth temperature of pv. *cattleyae*, this pathogen is more likely to occur under greenhouse conditions or in warmer areas.

4.2 *Agrobacterium*

Short rods, 0.6-1.0 x 1.5-3.0 µm in size, gram-negative, non-sporulating. Movable with 1-6 peritrichous flagella; if only 1 flagellum is present, it is more often located laterally than polarly. Occur singly or in pairs. They are aerobic, having an aerobic respiratory metabolism. Colonies on agar soils are round, convex, smooth, unpigmented to light beige. Extracellular polysaccharide mucilage is usually abundant on carbohydrate-containing media. The optimum growth temperature is 25 – 28 °C. *Agrobacteria* are found in the soil, water and on the surface of the roots. Some strains of this genus have the ability to infect a wide range of dicotyledonous plants. Less frequently, they infect monocotyledonous plants (e.g. asparagus, daffodils, gladiolus) and also gymnosperms (e.g. pine and larch). Through wounds they penetrate into root necks, roots and stems. They cause transformation of plant cells, which then autonomously enlarge and divide rapidly. Tumours are formed on the plants or the number of roots increases. The ability to induce tumour formation is conditioned by the presence of a large plasmid, the so-called Ti-plasmid (tumour inducing). The ability to induce root formation depends on the so-called Ri-plasmid (root inducing). Strains that do not carry Ti- or Ri-plasmid are non-pathogenic (saprophytic). Tumorigenic and rhizogenic strains occur mainly in soils that have been previously contaminated with diseased plant material. Non-phytopathogenic strains of the genus *Agrobacterium* have been isolated from clinical samples taken from diseased humans.

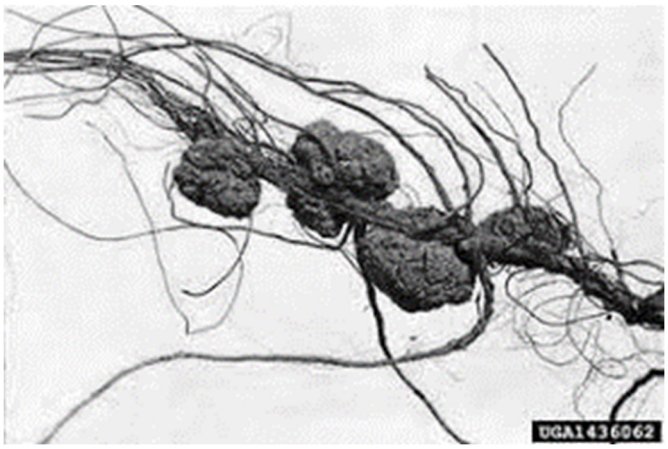

Figure 16 Damage of bacteria *Agrobacterium tumefaciens* (URL16)

4.3 *Burkholderia*

Straight rods, 0.5-1.0 x 1.5-3.0 μm in size, gram-negative, non-sporulating. They move by one or several polar flagella. Only bacteria of the species *B. mallei*, which are pathogenic to humans and animals, are immobile. They are aerobic, metabolism is aerobic respiratory. Colonies on live agar soils are white or pale yellow. The optimum growth temperature is 30 – 35 °C. Bacteria of the genus *Burkholderia* were included in the genus *Pseudomonas* until 1992, where they formed a separate homologous group II. Strains of *B. cepacia* are widespread in nature, not only in association with plants and soil. They are also frequently found in clinical specimens (urinary tract, bronchial washings, ear and blood). Phytopathogens include: *B. cepacia* (causal agent of onion skirt rot); *B. caryophylli* (causal agent of knotweed wilt); *B. gladioli* (causal agent of sword flower stem base and tuber rot); *B. andropogonis*. Many species of the genus *Burkholderia*, in particular strains of *B. cepacia*, *B. gladioli*, *B. glumae* (and also *Acidovorax avenae*), show inhibitory activity against some phytopathogenic organisms, such as *Clavibacter michiganensis* subsp. *michiganensis*, *Agrobacterium tumefaciens* and *Fusarium oxysporum* f.sp. *cepae*.

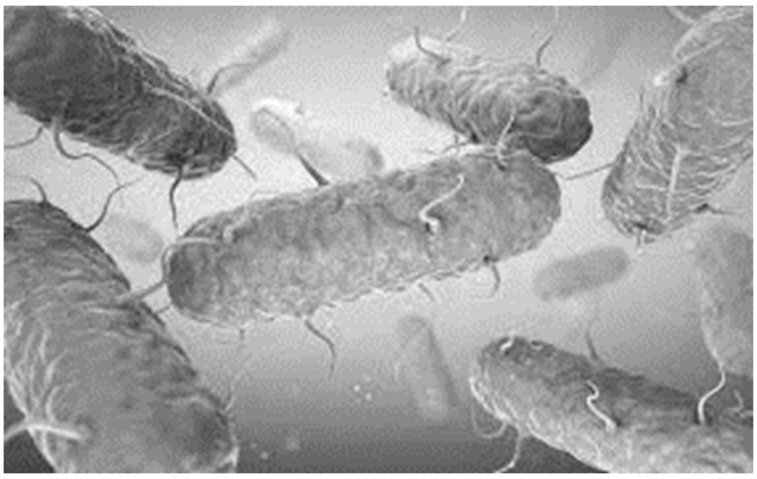

Figure 17 *Burkholderia cepacia* (URL17)

4.4 *Ralstonia*

The phenotypic characteristics of bacteria of the genus *Ralstonia* are similar to those of the genus *Burkholderia*, to which they were assigned until 1993. They are gram-negative rods with several flagella at one pole, non-fluorescent. They often form a diffuse brown pigment on agar medium. The phytopathogens include *R. solanacearum*.

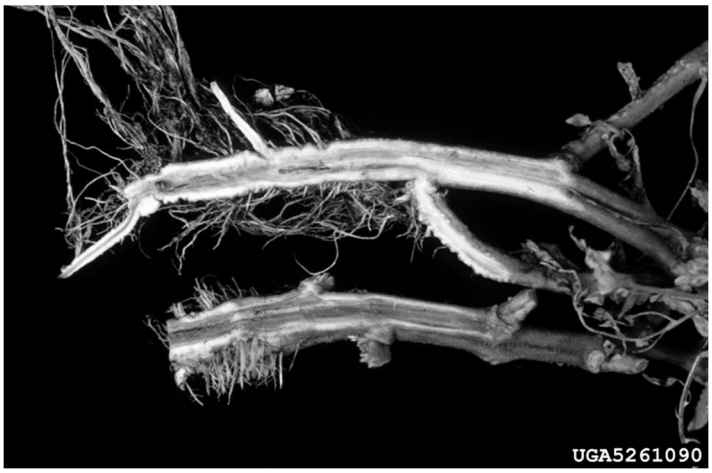

Figure 18 Damage of bacteria *Ralstonia solaneceum* (URL 18)

Bacteria of this species attack the xylem portion of vascular bundles in a wide range of host plants (more than 450 species and 30 families), including potato and tomato plants. *R. solanacearum* is a heterogeneous species comprising strains with different biology and host species. It is a complex of varieties that have been described as groups, races, biovars, biotypes, subraces and strains. The various subdivisions of the species used in the literature sources have caused much confusion. According to the host species, they are divided into races:

- Race 1 – infests tobacco, tomato, potato, eggplant, diploid banana and many other leafy crops and weeds. It has a high temperature optimum (35 – 37 °C). In temperate climates, race 1 can occur on plants grown in greenhouses, such as *Curcuma zanga*, *Anthurium* or *Epipremnum*.
- Race 2 – attacks triploid banana tree (causing the so-called Moko disease) and *Heliconia* spp. Like race 1, it has a high temperature optimum (35 – 37 °C).
- Race 3 – attacks mainly potato and tomato, with poor virulence for other leafy crops. It has a lower temperature optimum (27 °C). Other hosts are bulbous weeds, such as the bittersweet bulb (*Solanum dulcamara*), black bulb (*S. nigrum*), *S. cinereum* (in Australia), the compound weed *Melampodium perfoliatum* (in Costa Rica) and species of the genus *Pelargonium*. The status of the other two races, one (race 4) attacking ginger (*Zingiber officinale*) and the other (race 5) attacking mullein (*Morus* spp.), is uncertain.

4.5 *Erwinia*

Straight short rods, 0.5-1.0 x 1-3 μm in size, gram-negative, non-sporulating. Motilated by several peritrichous flagella. Colonies on nutrient agar have a creamy white colour. They are facultatively aerobic, have aerobic respiratory and fermentative type of metabolism. The genus includes about 24 phytopathogens, which can be classified into two groups according to their ability to hydrolyze pectins:

- The group of non-spectinolytic *Erwinia* includes a diverse group of phytopathogens and saprophytes. Among the phytopathogens are necrogens, e.g. *E. amylovora*, the causal agent of rose scab, and *E. pyrifoliae*, the causal agent of necrotic symptoms on the leaves and branches of the Asian pear (*Pyrus pyrifolia*). It also includes the less important phytopathogen *E. rhapontici*, which, among other things, is the causal agent of wheat grain pinking.
- More recently (in 1999), non-pigmented *Erwinia*-like isolates originating from cortical necroses, scorched flowers and immature fruit of pear, apple, cherry, hawthorn and elm, originally related to *Erwinia herbicola* and later to *Pantoea agglomerans*, have been shown to form a distinct species, *E. billingiae*. Strains of *E. billingiae* are considered to be secondary invaders rather than primary pathogens.

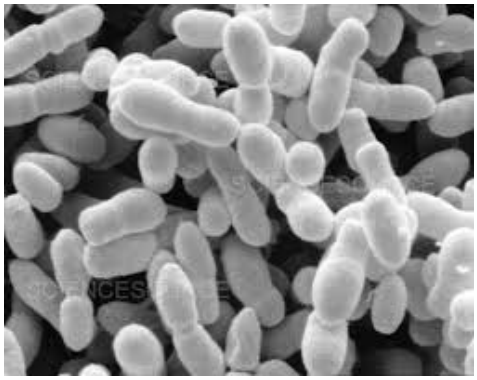

Figure 19 *Erwinia carotovora* (URL19)

Some pathovars of the predominantly saprophytic species *E. herbicola* are considered or thought to be phytopathogenic, causing tumours on plants of the genera *Wisteria* (bean lianas) and *Gypsophila paniculata* (gypsophila, an ornamental plant). The species *E. herbicola* and *Pantoea agglomerans* are closely related or identical and their nomenclature is not yet settled. *E. carotovora* (with several subspecies, e.g. subsp. *carotovora* and subsp. *atroseptic*a) and *E. chrysanthemi*, causing soft rots on a wide range of host plants.

4.6 *Phytoplasmas* and other bacteria localised in the phloem

4.6.1 Phytoplasmas

Phytoplasmas, formerly known as mycoplasma-like organisms (MLOs), are polymorphic organisms that do not have a cell wall. Its function is at least partially replaced by a three-layered, 7 – 10 nm thick plasma membrane. The cells of phytoplasmas are spherical to oval with a diameter of 100-400 (600) nm. They resemble mycoplasmas, but unlike mycoplasmas, they are very often found in filamentous and branched forms. They have not yet been cultured on artificial media. They are susceptible to antibiotics mainly of the tetracycline group. In plants, phytoplasmas are localised in the sap of sieve worms or in the cytoplasm of infected phloem parenchymatous cells. They cause more than 300 plant diseases. In nature, phytoplasmas are transmitted by insect vectors belonging to the families Cicadellidae and Fulgoridae. According to their phylogenetic relationship, phytoplasmas have been divided (in 1998) into 14 groups and 38 subgroups. For Europe, the following groups of strains are relevant: aster yellows group; apple proliferation group; X-disease group; elm yellows group; elm yellows group; stolbur group. The largest number of pathogens is in the aster yellows group.

4.6.2 Spiroplasma

Pleomorphic organisms without a cell wall, helical to spherical or ovoid. The helical forms are 3-5 μm long and 100-200 nm wide. Spherical forms are 300-800 nm in diameter. They are facultatively anaerobic. Colonies are often diffuse because the cells are mobile by twisting, jerking and turning movements. Non-diffuse colonies are 200 μm in size or smaller and have a typical bud-like shape (a 'set-egg' shape). The genus contains 3 species, of which *Spiraplazma kunkelii* is worth mentioning, originating from stunted maize in the tropical regions of the American continent. Like phytoplasmas, spiroplasmas are localized in the phloem, namely in the sap of sieve worms. The vectors of spiroplasmas are species of straight-winged and flatworm insects.

4.6.3 „Liberobacter"

Bacterial cells are elongated, 0.25-0.5 x 0.8-4.0 μm in size. They are surrounded by a 'corrugated' envelope consisting of two individual membranes (8 nm wide) and a cell wall (20 – 32 nm). They are similar to gram-negative bacteria. According to phylogenetic analyses, they belong to the subdivision Proteobacteria. Initially (in 1971) they were considered as MLO (mycoplasma-like organisms), but when they were found to have a true cell wall, they were designated as "bacterium-like organisms" (bacterium-like organisms-BLO). These organisms have also been mislabelled as rickettsiae-like organisms. They have not yet been cultivated on artificial media. The name *'Liberobacter'* was proposed (in 1994) for this new group of organisms, which is provisional pending further data needed for official classification and naming. Their way of life is linked to the phloem of plants. They are transmitted by vectors of the family Meridae. They cause around 20 different diseases. They are manifested by stunting, yellowing of young leaves, curling and twisting of leaves, uneven leaf development, greening of the crown petals and many times premature death of the plant.

4.6.4 Rickettsia-like organisms – RLO

Since 1973, several bacterial phytopathogens resembling *Rickettsia* have been described. According to their localization in the vascular bundles and the symptoms they showed on infected plants, RLOs were divided into two groups, namely bacteria localized in the xylem vasculature and bacteria localized in the phloem sieve vessels. Later, the bacteria localized in the xylem were shown to be bacteria of the genus *Clavibacter* or *Xylella*. Some bacteria localized in the phloem also do not belong to the *Rickettsia* but to the provisional genus "*Liberobacter*". In the 1980s and 1990s, publications suggesting or confirming a link between the presence of RLO in the phloem of plants and disease symptoms appeared only sporadically.

4.7 Coryneform bacteria

The term coryneform bacteria is used for a broad species of gram-positive, non-spore-forming, coryne-shaped bacteria (Greek coryne = sticks, club). Among phytopathogenic bacteria, this includes species of the genus *Clavibacter* (of great economic importance), *Arthrobacter*, *Curtobacterium* and *Rhodococcus* (of relatively minor importance). All phytopathogenic coryneform bacteria are strict aerobes,

catalase-positive and oxidase-negative. Metachromatic granules (storage substances that stain with basic dyes) are often found in the cells. In the exponential phase, the size and shape of the rods is variable, and includes straight more or less bent, and club-shaped forms. The cells tend to group in pairs and clusters in V, Y or palisade shapes. Mycelial outgrowths do not usually form, but rudimentary outgrowths may occur. In the stationary phase of culture, the cells are usually less variable in shape, are shorter and coccoid cell shapes may appear in varying proportions. They are either immobile or motile with 1 – 2 polar or lateral flagella. Immobile rods are typical of slow-growing species of the genus *Clavibacter*, motile ones for fast-growing species of the genera *Arthrobacter* and *Curtobacterium*. Colonies are generally tinged yellow or orange (especially on nutrient-rich soils); non-pigmenting species are a minority (e.g. *Clavibacter michiganensis* subsp. *sepedonicus*). *Many strains of Clavibacter michiganensis* subsp. *insidiosus* are characterised by the production of the blue-grey pigment indigoidine.

4.7.1 Clavibacter

In this genus, typical occludogens belonging to the species *Clavibacter michiganensis* and *C. xyli* are represented, specialised in colonisation of blood vessels (tracheae). The optimum growth temperature is 20 – 29 °C; the maximum is 29 – 35 °C. Individual subspecies attack a particular host plant species, e.g. subsp. *michiganensis* tomato.

Figure 20 Damage of bacteria *Clavibacter michiganensis* (URL20)

4.7.2 Rathayibacter

Three species of coryneform bacteria that had previously been placed in the genus *Clavibacter* were placed in this new genus in 1993. They are the causal agents of yellow slime mold and inflorescence (ear) deformities in cereals and annual forage crops. Symptoms arise under the co-action of nematodes of the genus *Anguina*. In European conditions, *Rathayibacter rathayi* can occur on forage grasses.

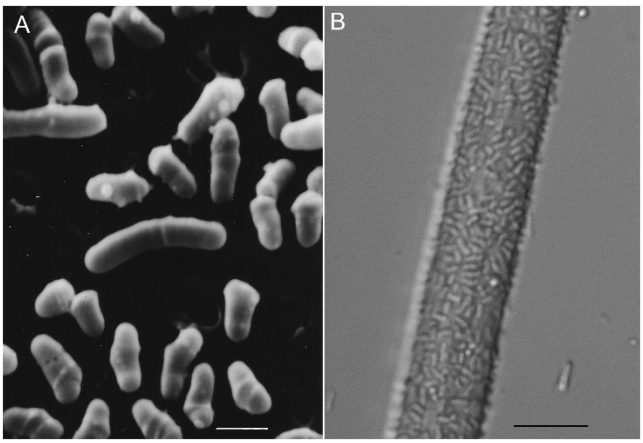

Figure 21 *Rathayibacter rathayi* (URL 21)

4.7.3 Arthobacter

Only the species *Arthrobacter ilicis*, which is the causal agent of leaf and flower scald on holly (*Ilex opaca*) plants, has phytopathogenic properties. The pathogen does not occur in Europe.

4.7.4 Curtobacterium

Several soil bacteria are included in this genus. The optimum temperature for growth is 24 – 27 °C; the maximum is 35 – 37 °C. Four pathovars of *C. flaccumfaciens* are phytopathogenic, namely: pv. *betae*, the causal agent of leaf silvering in beet; pv. *flaccumfaciens*, the causal agent of bean wilt; pv. *oortii*, the causal agent of a systemic disease in tulip, which is manifested by yellow blisters on the bulbs and silvery grey blistering spots and subsequent cracking of the leaf surface; pv. *oortii*, the causal agent of leaf wilt; pv. *oortii*, the causal agent of a systemic disease in the tulip, which is manifested by yellow blisters on the bulbs and silvery grey blistering and subsequent cracking of the leaf surface; pv. *poinsettiae*, the causal agent of leaf spot, yellowing of cortical plexuses and darkening of vascular plexuses of woody shoots of the Mexican rose (poinsettia) *Euphorbia pulcherrima*. *C. plantarum* is a non-pathogenic species that occurs on the leaves of many plants. It is transmitted by soybean seeds and corn kernels.

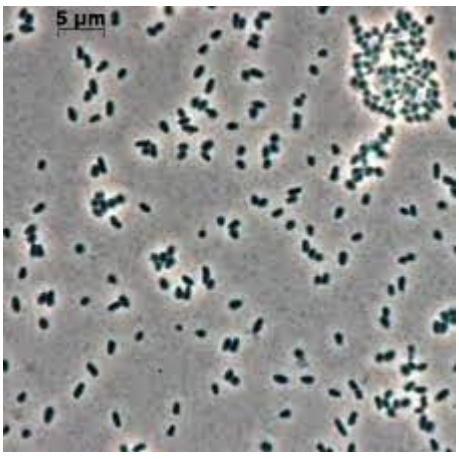

Figure 22 *Curtobacterium flaccumfaciens* (URL 22)

4.7.5 Rhodococcus

Bacteria of the genus *Rhodococcus*, belonging more recently to the group of nocardioform actinomycetes, may be cocci, rods or branching filaments. They are gram-positive, non-sporulating. Colonies on solid culture media are small, convex, mucoid, pale orange, red, sometimes colourless. Colonies of *Rhodococcus fascians* are creamy yellow. Bacteria belonging to this genus are mainly found in soil and water. The only phytopathogenic species is *R. fascians*, the causal agent of bud multiplication or shortened deformed stems with misshapen leaves on a wide range of host plants, especially those growing in the northern hemisphere. The optimum temperature for growth is 24 – 27 °C; maximum 37 °C.

4.8 *Pantotea*

Straight rods, 0.5-1.0 x 1-3 µm in size, gram-negative, non-sporulating. Mostly motile with peritrichous flagella, some are immobile. Colonies on nutrient agar are smooth, translucent and more or less convex, with straight margins. The colour of the colonies is yellow, pale cream to pale reddish yellow. However, strains with non-pigmented colonies also occur. They are facultatively anaerobic. They grow best at 30 °C on agar soils. They are common on plant and seed surfaces, in soil and water, but also on animals and humans (in wounds, blood, urine and internal organs). They can therefore be isolated from different geographical areas and different ecological sources (hence the name of the genus: from the Greek adjective pantoios = coming from different sources). Saprophytic strains of *P. agglomerans* (formerly known as *Erwinia herbicola*) are a common component of the epiphytic microbiota of green plants. The taxonomy and nomenclature of closely related or identical species of *P. agglomerans* and *Erwinia herbicola* is not yet settled. The causal agent of vascular wilt of maize is *P. stewartii* subsp. *stewartii* (formerly known as *Erwinia stewartii*). Strains of *P. ananas*, more recently referred to as *P.* a. pv. *uredovora* (formerly known as *Erwinia uredovora*), are attacked by *Puccinia graminis* rust.

Figure 23 *Pantoea agglomerans* (URL 23)

4.9 *Pseudomonas*

Straight short rods, 0.5-1.0 x 1.5-5 µm in size, gram-negative, non-sporulating. Motilated by one or more polar flagella. They are aerobic, have aerobic respiratory metabolism. Colonies are mostly greyish white, transparent. Most species are common

inhabitants of soil and water, some are pathogens to animals (including humans), others to plants or fungi. The genus Pseudomonas also includes some rhizobacteria that promote plant growth. Pseudomonads can be divided into fluorescent and non-fluorescent depending on whether they produce the yellow-green diffuse pigment fluorescein on iron-poor soils. At the same time, fluorescent species are characterised by the fact that they do not accumulate poly-8-hydroxybutyrate as an internal carbon source inside their cells, whereas non-fluorescent species do. Fluorescent species contain a large number of economically important pathogens. Depending on the type of syndromes they induce on plants, they can be referred to as hyperplasia, tumours or hyphae, which are caused by *P. savastanoi* pv. *savastanoi* (syn. *P. syringae* pv. *savastanoi*).

Within *P. syringae*, about 48 different pathovars are known, which can be classified into 9 genomic groups (likely future separate species) based on DNA relatedness. The most important of these are: causal agents of aerial organ spotting (pv. *syringae*, pv. *coronafaciens*, pv. *phaseolicola*, pv. *tomato*, pv. *lachrymans* and others); bark necrosis in woody plants (pv. *mors prunorum*, pv. *persicae* and others). Necrosis on mushroom fruiting bodies is caused by *P. tolasii*. *P. syringae* pv. *syringae* bacteria are a common component of the epiphytic microbiota on a wide range of plants. They act as biotic condensation nuclei for condensation of water vapour in the air and prevent hypothermia at temperatures of -2 to -5 °C. From a phytopathological point of view, an important feature of some fluorescent pseudomonads, namely the polyphagous species *P. marginalis* and *P. viridiflava*, is their pectinolytic activity. Non-fluorescent species are less frequently used as phytopathogens. It is worth mentioning *P. corrugata* as the causal agent of marrow necrosis in tomato.

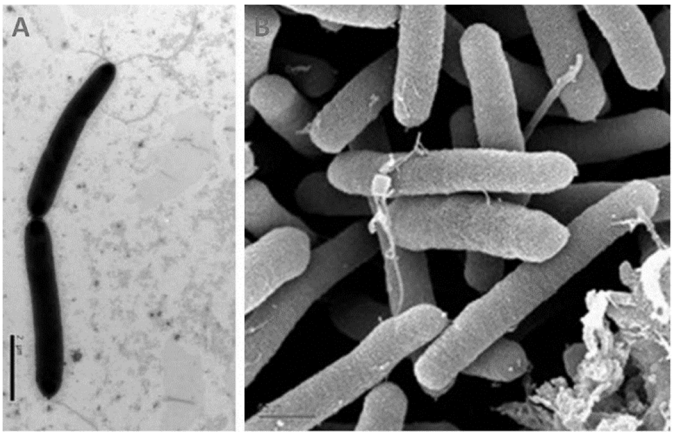

Figure 24 *Pseudomonas syringae* (URL 24)

4.10 *Streptomyces*

Thin richly branched hyphae 0.5-2 μm in diameter. On aerial hyphae (sporophores) spirally coiled chains of three to many spores are formed, the size of which is equal to the diameter of the hyphae. They are gram-positive, obligately aerobic. Metabolism is aerobically respiratory. When growing on a solid substrate, the unicellular organism produces three distinct types of hyphae: substrate, surface, and aerial. On nutrient media, colonies are initially small, 1 – 10 mm in diameter. At first, they are almost smooth, but later an aerial coating of mycelium forms, which may appear granular, powdery, velvety or flaky. Many strains produce a variety of pigments, colouring the mycelium and substrate. In nature, they are widespread in soil

and composts. They are producers of a wide variety of bioactive compounds. *S. scabies* is considered to be the predominant species responsible for scab on potato tubers, beet tubers and roots of various crops. Other phylogenetically distinct species of the genus *Streptomyces* may cause similar symptoms. *Streptomyces scabies* is considered by some phytopathologists to be only an opportunistic pathogen. Phytopathogenic strains of *S. scabies* are characterized by melanin production, smooth grey spores formed on spiral chains, and utilization of specific sugars. Not all strains of *S. scabies* are phytopathogenic. An *in vitro* method has been developed to separate pathogenic strains of *Streptomyces scabies* from non-pathogenic strains according to the production of the phytotoxin thaxtoxin A.

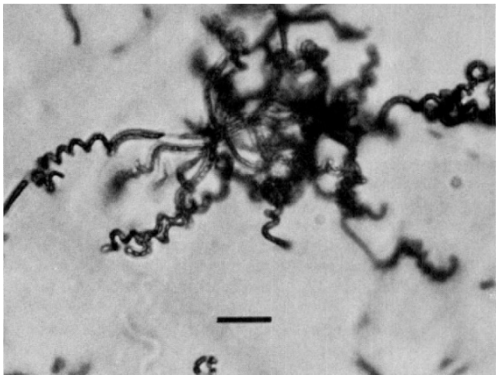

Figure 25 *Streptomyces scabies* (URL 25)

4.11 *Xantomonas*

Straight short rods, 0.4-0.6 x 1.0-1.8 µm in size, gram-negative, non-sporulating. They are motile and move with a single polar flagellum. They are aerobic, having an aerobic respiratory metabolism. Most xanthomonads form yellow mucoid smooth colonies. The yellow pigments called xanthomonadins and the exopolysaccharide xanthan are characteristic of this bacterial genus. It does not form poly-B-hydroxybutyrate inclusions. The xanthomonads known so far are phytopathogens and are only found in association with plants or plant material. Some xanthomonads, such as *X. vesicatoria*, show pectinolytic activity. Many phytopathogenic xanthomonads may occur as epiphytes. The genus *Xanthomonas* contains the largest number of phytopathogens (about 140), included in 20 species and several dozen pathovars. Bacterioses caused by xanthomonads are particularly damaging in areas with tropical and subtropical climates. In temperate climates, some pathovars of importance are *X. campestris* (e.g. pv. *campestris*), *X. arboricola* (e.g. pv. *pruni*, pv. *juglandis*), *X. axonopodis* (e.g. pv. *phaseoli*), *X. bromi*, *X. hortorum* (e.g. pv. *pelargonii), X. hyacinthi*, *X. translucens*, *X. vesicatoria* and *X. populi*. Among the xanthomonads, necrogens, i.e. the causal agents of necrotic canker and necrotic canker, predominate. However, there are also occludogens colonising the xylem vasculature (e.g. *X.* c. pv. *campestris* on brassicas or *X.* c. pv. *graminis* on grasses) and also *macerogens*, e.g. *X. hyacinthi* on hyacinth. The causal agents of blight include *X. fragariae* on strawberries, *X. translucens* pv. *translucens* on cereals and grasses, *X. axonopodis* pv. *phaseoli* on beans, *X. vesicatoria* on tomato and paprika, *X. hortorum* pv. *pelargonii* on geraniums. *X. arboricola* pv. *pruni* on stone fruit, *X. arboricola* pv. *juglandis* on walnut and *X. populi* on poplar can be identified as the causal agent of bark necrosis. So-called opportunistic xanthomonads are often isolated from plant material. These are

xanthomonads living in close association with plants but causing no obvious disease symptoms on the host. In the past, this group of non-pathogenic xanthomonads has been mostly overlooked. The non-pathogenic xanthomonads form a heterogeneous population containing numerous strains that could not be assigned to any of the described species.

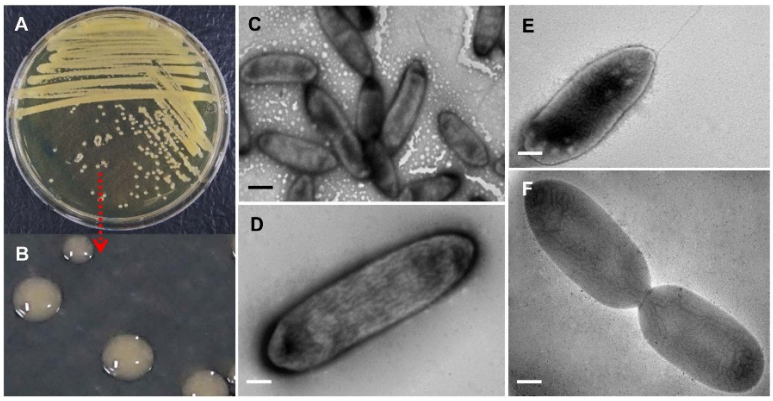

Figure 26 *Xantomonas arboricola* (URL 26)

4.12 *Xylella*

Straight rods, 0.25-0.35 x 0.9-3.5 μm in size, gram-negative, immobile. They are oxidase-negative and catalase-positive. The cell wall is usually wavy in appearance. In certain culture conditions they form long extracellular filamentous cords. It is thought to serve to attach the bacteria to the substrate, e.g. tracheal walls. They are strictly aerobic, non-fermentative. They have specific requirements for the composition of the culture medium (until recently they could not be isolated from plants and cultured on artificial substrates). The optimum temperature for growth is 26 – 28 °C, the optimum pH is 6.5-6.9. The colonies are of two types: hemispherical to convex, smooth, opalescent (iridescent) with straight margins, and convex, rough, with gently undulating margins.

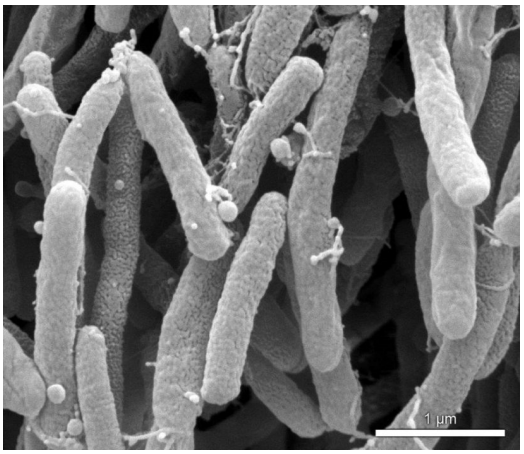

Figure 27 *Xylella fastidiosa* (URL 27)

Pathogens of the genus *Xylella* are typically occludens on a wide range of host plants. They are transmitted by grafting and by insects. Diseases caused by bacteria of the genus *Xylella* were originally considered as viruses, later as rickettsioses. After the pathogen could be cultured and its properties determined, it was placed in the genus

Xylella with a single species, namely *X. fastidiosa*. The most serious diseases caused by this pathogen include Pierce's disease of grapevine, plum leaf blight, peach stunting and others. *X. fastidiosa* bacteria colonise numerous plant species without causing any disease symptoms.

4.13 *Xylophilus*

Straight to weakly curved gram-negative non-sporulating rods, 0.4-0.8 x 0.6-3.3 μm in size. In older cultures, filamentous cells 30 μm longer may appear. They are moved by a single polar flagellum. They are strictly aerobic, having an aerobic respiratory metabolism. Colonies are yellow, nonmucoid, smooth, round, with straight margins, 0.4 – 0.8 mm in diameter. Even at the optimum growth temperature of 24 °C, growth is very weak and slow (unlike bacteria of the genus *Xanthomonas*, to which they were until recently assigned). *Xylophilus* grows on L-glutamine but not on calcium lactate (in contrast to strains of the genus *Xanthomonas*). Phytopathogens include *X. ampelinus*, the causal agent of leaf and flower scald and scarring or necrosis of vine shoots. It is so far the only representative of the genus *Xylophilus*. It is listed as a quarantine pest in the Slovak Republic.

4.14 *Bacillus*, *Paenibacillus* and *Clostridium*

A common feature of the above bacterial genera is that they are gram-positive rods that have the ability to produce endospores. Bacteria of the genera *Bacillus* and *Paenibacillus* are obligately aerobic or facultatively anaerobic organisms, whereas bacteria of the genus *Clostridium* belong mostly to obligately anaerobic organisms. All three genera of bacteria are of interest to phytoanalysts because they include several opportunistic phytopathogens. The bacteria of the genus *Bacillus* have also been the focus of attention because the populations of some species of this genus contain strains useful in the biological control of insect pests or fungal pathogens. The primary habitat of most species of the genera *Bacillus* and *Paenibacillus* is soil. Through their metabolic activity, they are thought to play an important role in the carbon and nitrogen cycle. *Clostridia* are found in soil, water, food and the digestive tract of many animals. Although some species of the genus *Bacillus* (*B. cereus*, *B. megaterium*, *B. subtilis*) and *Paenibacillus* (*P. macerans*) are common on plants (or inside plants), they have not yet been safely demonstrated to be active phytopathogens. Exceptions may include *Bacillus megatherium* pv. *cerealis*, which was described in 1982 in North America as the causal agent of light brown streak on leaves of wheat, barley and oats. Often, however, bacteria of the genera *Bacillus* and *Paenibacillus* are the first colonisers of dying weeds. When attempts are made to isolate bacterial phytopathogens, they are therefore often found on nutrient soils. Some are involved in the decomposition of plant material, especially after harvesting. In soil, *P. polymyx* and *B. pumilis* are common species.

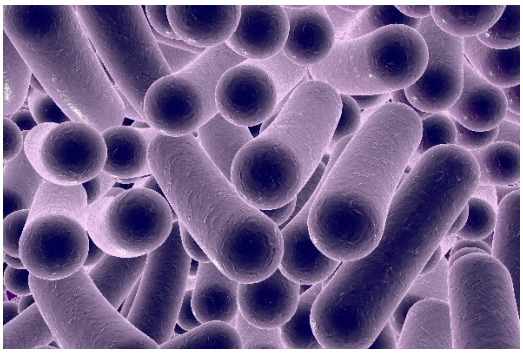

Figure 28 Rod *Clostridium* (URL 28)

B. mycoides is sometimes included among the pathogens of key plants such as canola but is usually considered a saprophyte. *Paenibacillus polymyxa* is reported to cause rots of various plant weeds (potato, melon, paprika, tomato, cabbage, onion), usually under unfavourable field conditions and especially for stored plant products, i.e. high temperatures or reduced access to atmospheric oxygen. The species *B. pumilus* is a contributor to potato tuber rot and secondarily to cotton capsule rot. The action of *B. subtilis* is associated with corn grain rot, poor germination of soybean, and capsule rot of cotton; the bacteria are found many times on paprika seeds. Some species of the genus *Clostridium* are found in association with decaying plant material under poor storage conditions. The pink-pigmented species *C. puniceum* is characterised by its strong pectinolytic properties. Undescribed clostridia occur together with *Erwinia carotovora* on potato plants with symptoms of blackening of the base of the stem and on rotting tubers. Species of the genus *Clostridium* are also reported to be involved in the development of complex disease of carrots.

4.15 Non-parasitic harmful rhizobacteria

Non-parasitic harmful rhizobacteria (deleterious rhizobacteria) belong to the genera *Pseudomonas, Enterobacter, Klebsiella, Flavobacterium, Achromobacter* a *Arthrobacter*. Their species affiliation is mostly not yet specified. Non-parasitic rhizobacteria are found in the rhizoplane and rhizosphere. In the past, their harmful effects on plants were not known. It was not until around 1982 that it was shown that their deleterious effect on the yield of some crops (such as potatoes, beans, lettuce, etc.) could be equal to or greater than that of known parasitic pathogens attacking the roots of plants. The overgrowth of harmful rhizobacteria also explains the so-called specific soil fatigue, which occurs when a particular crop is grown repeatedly in the same plot.

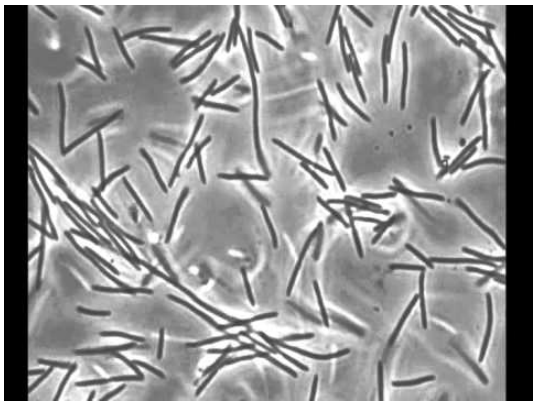

Figure 29 *Flavobacterium* (URL 29)

5 POLYPHAGOUS AGENTS OF BACTERIOSES

5.1 *Agrobacterium tumefaciens* (Smith &Townsend) Conn; *Agrobacterium rhizogenes* (Riker, Banfield, Wright & Sagen) Conn

Characteristics of pathogens. The pathogenicity of agrobacteria is determined by plasmids. Strains carrying Ti-plasmid induce tumorigenicity, strains carrying Ri-plasmid cause hairiness. Avirulent (saprophytic) strains are those that do not contain either plasmid. Both plasmids are perhaps transmissible from one strain to the other. According to the biochemical properties, 3 biovars are distinguished within the genus *Agrobacterium*. Biovar l is identical to *A. tumefaciens*, biovar 2 to *A. rhizogenes* and biovar 3 to *A. vitis*. Biovars 1 and 2 have a wide host distribution. This means that it is not possible to conclude whether *A. tumefaciens* or *A. rhizogenes* is the causative agent on the basis of symptoms and host plant alone without knowledge of the biochemical properties of the isolated bacterial strains. The affiliation of a strain to one of the biovars does not correspond to its pathogenic properties. Genes encoding virulence genes, genes encoding opines catabolism and genes controlling the conjugative transfer of Ti-plasmid between cells of the genus Agrobacterium, among others, are found on the Ti-plasmid. Opines are substances formed by the condensation of amino acids and ketoacids. Depending on the type of opines encoded by the genes on the 11-plasmid, nopaline, octopine and atropine-type plasmids are distinguished. Only part of the Ti-plasmid is integrated into the plant genome, the so-called T-DNA segment, which contains genes encoding auxin and cytokinin production (these are involved in plant metabolism) and opines production (which are catabolisable only by agrobacteria). Similarly, the Ri-plasmid carries a segment of T-DNA having the ability to insert into the plant genome. Two genes responsible for auxin synthesis and four genes coding for the production of substances affecting root hair morphology are localized on this segment.

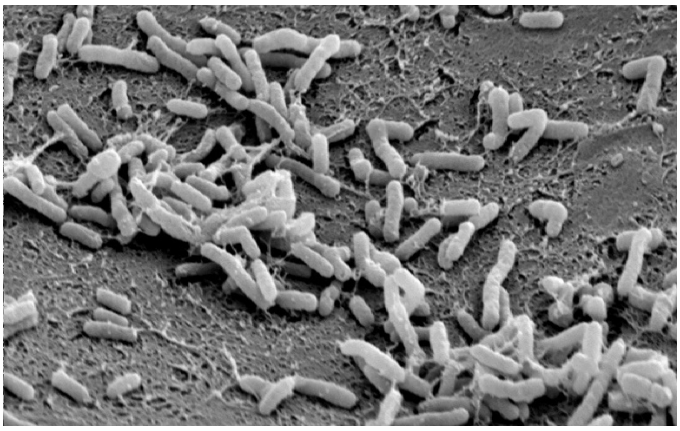

Figure 30 *Agrobacterium rhizogenes* (URL 30)

Host plant. Hosts of *A. tumefaciens* and *A. rhizogenes* include many, if not most, dicotyledonous plants (data on 643 species from 331 genera are available). Fruit trees (especially pear), stone fruits and roses are most commonly and heavily infested. Beet, rape, sunflower, walnut, hops, honeysuckle, chrysanthemum, rhododendron, etc. are also affected. Of the forest tree species, deciduous trees are more likely to be affected than conifers. Different species and plasmid types of agrobacteria have different host distributions. *Agrobacteria* belonging to different species may be found on the same host plant. Infestation by Ri-plasmid-carrying agrobacteria is quite rare in nature. It occurs in perennial dicotyledonous plants (not monocotyledons) and in woody species such as apple, aspen and *Spirea*.

Harmfulness and geographical distribution. Agrobacterium is widespread throughout the world. Tumours caused by them are becoming economically important in fruit and forest nurseries, as cuttings with tumours are unsaleable. The impact of tumours on plant vigour, productivity and survival depends on where the plants are located, how many tumours there are or how large they are. In stone fruit orchards, pome fruit orchards and berry plantations, the damage caused by most agrobacteria is economically insignificant. Inoculation with Ri-plasmid-carrying agrobacteria is sometimes used as a means of inducing adventitious root formation in hard-to-seed trees.

Symptoms. Small tumours induced by Ti-plasmids can appear on any part of the plant, but most often occur on the root collar, near the soil surface, at the grafting point and on the roots. In some plants, tumours on aerial organs are also quite common. New tumours usually appear during the growing season. They are spherical, their surface is whitish, smooth and soft. They gradually increase in size (up to 30 cm), become hard, woody, brown and their surface becomes wrinkled. The tumor is usually a formation made up of intertwined parenchymatous and vascular elements. Smaller tumours partially or completely break down during dormancy. In perennial host plant species, however, a new tumour often forms at the same site during the subsequent growing season. Non-specific symptoms of infestation include stunting of plants, chlorotic leaves, low productivity and eventually premature plant death. Bacterial tumours may be confused with healing callus or with tumours caused by insects, mites, fungi, viruses, genetic disorders and growth substances. Ri-plasmids give rise to more fibrous roots. Initially, when the bases of these roots are forming, it is difficult to distinguish tumorigenicity from hairiness. In woody plants, pilosity is most often visible near the base of the trunk.

Epidemiology. The pathogen overwinters mainly in tumours and soil. Agrobacterium is generally considered to be a true soil dweller. However, more recent

research has shown that virulent strains (carrying Ti- or Ri-plasmid) are only found in close proximity with tumour-bearing plants. Agrobacterium from the free soil and rhizosphere of healthy plants are avirulent. The infection is usually spread over long distances by contaminated or infected plant material, over short distances by rain or irrigation or soil water, soil animals and various machines used for soil cultivation.

5.2 *Erwinia* (*Pectobacterium*) *carotovora* subsp. carotovora (Jones) Bergey et al.; *Erwinia chrysanthemi* Burkholder, McFadden & Dimock

Characteristics of pathogens. *E. carotovora* subsp. *carotovora* has a wide host range, but some strains of this subspecies are more virulent to the host plant species from which they have been isolated than strains isolated from other host species. *E. chrysanthemi* is a species closely related to *E. carotovora* subsp. *carotovora*. In addition to the non-host-specialised strains, there are also host-specialised strains (e.g. pv. *chrysanthemi*, pv. *dianthicola*, pv. *dieffenbachiae*, pv. *zeae*). The systematics of *E. chrysanthemi* is not settled. Extensive studies carried out in the Netherlands have shown that there is no host specificity in this species. The existence of pathovars of *E. chrysanthemi* has thus been questioned. It is expected that the existing species *E. chrysanthemi* will be split into five separate species.

Host plant. Under favourable conditions, *E. carotovora* subsp. *carotovora* is capable of attacking the fleshy organs of virtually any plant species. *E. chrysanthemi* attacks a wide range of tropical and subtropical crops. Hosts include many greenhouse ornamentals such as cloves, chrysanthemum, philodendron and difenbachia, but also maize, for example.

Harmfulness and geographical distribution. *E. carotovora* subsp. *carotovora* is common in both temperate and tropical climates. It is one of the most economically important phytopathogens, especially in transported and stored products. Apart from potatoes, it is most commonly found on stored beet, carrot and celery, on the fruit of cucurbits and tomatoes. Major damage occurs in Chinese cabbage. *E. chrysanthemi* is mainly damaging in subtropical and tropical areas, then in Europe on greenhouse ornamentals.

Symptoms. *Erwinia* cause soft rot of plant weeds. However, both *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* can penetrate the xylem vasculature and cause systemic disease manifested by wilting or stunting. In potatoes, both pathogens can cause stem blackening, but *E. carotovora* subsp. *atroseptica* is considered to be the causal agent under our conditions. *E. chrysanthemi* causes: stem rot, soft rot and wilting in chrysanthemum, difenbachia, Euphorbia pulcherrima (Mexican roses), banana; leaf rot in philodendron, and tuber rot in dahlias; late wilting, stunting and general stunting in carnation.

Epidemiology. *E. carotovora* subsp. *carorovora* is commonly found in many crops as a contaminant or commensal (a harmless food plant). It can survive through the winter in plant debris, but also in the rhizosphere of cultivated and weedy plants or in insect pupae. Spread by: insects; irrigation water; aerosols from shredding; harvesting machinery; water used to wash vegetables before sale; in vegetative reproductive material. The bacteria enter the plant mostly at the site of injury. They can also be injected inside the plant by contaminated insects. The main factor that determines whether a commensal becomes a pathogen is probably the presence of free water on the surface of the weeds. This can lead to an increase in turgidity (water bloat) of the plant plexuses, oxygen depletion, disruption of cell membranes and release of

solutes from the cell into the intracellular spaces where the pathogen then finds favourable conditions for multiplication and spread. Temperatures are probably the main factor potentially causing soft rot in a particular location and the most damaging in a particular year. Protection lies mainly in prevention. For vegetatively propagated plants, use healthy reproductive material. Eliminate sources of infection. Do not leave them loose in piles with diseased plants or plant parts. Further protective measures are given for blackening of the potato stem.

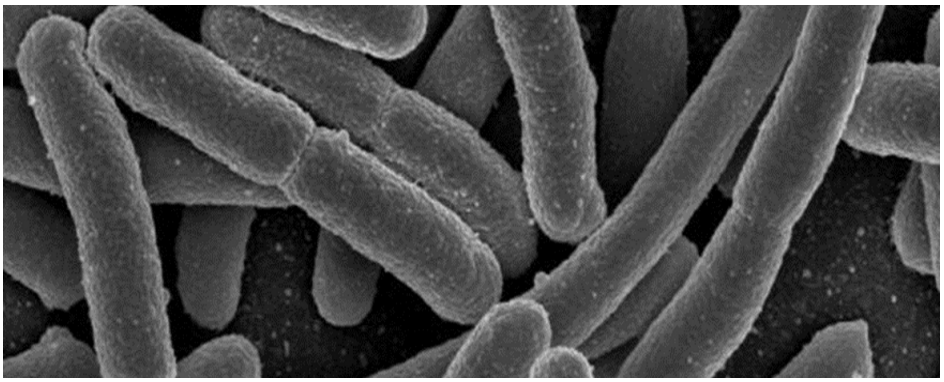

Figure 31 *Erwinia carotovora* (URL 31)

5.3 *Pseudomonas syringae* pv. *syringae* van Hall

Characteristics of the pathogen. It is a true epiphytic bacterium found on a wide range of plants worldwide. It is an occasional pathogen, causing various types of symptoms that have been described in more than 40 plant species. Isolates from different hosts have slightly different host characteristics. None of these isolates has the ability to infect all plant species that are on the host list of *P. syringae* pv. *syringae*. It is likely that within pv. *syringae* there are strains specialized to a particular host. There are also non-pathogenic strains in the pv. *syringae* population. Strains of pv. *syringae* exhibit ice-crystallising activity. An important differentiating biochemical feature of *P. syringae* pathovars is the inability to form oxidase. This feature, together with *P. viridiflava*, distinguishes them from other phytopathogenic and non-phytopathogenic pseudomonads. It is not uncommon for other pathovars of *P. syringae* to be involved in the development of identical or similar symptoms at the same time as pv. *syringae*. For example, in addition to pv. *syringae*, pv. *phaseolicola* is also involved in leaf spot in beans, pv. *pisi* in peas and pv. *tomato* in tomatoes. Sudden death of buds and flowers and necrosis of the rind in stone fruits is caused by pv. *morsprunorum* together with pv. *syringae*, in peaches also by pv. *persicae*. Scarlet fever of blossom and bark necrosis in pear and apple is caused by *Pseudomonas syringae* pv. *syringae* and is interchangeable with symptoms caused by *Erwinia amylovora*.

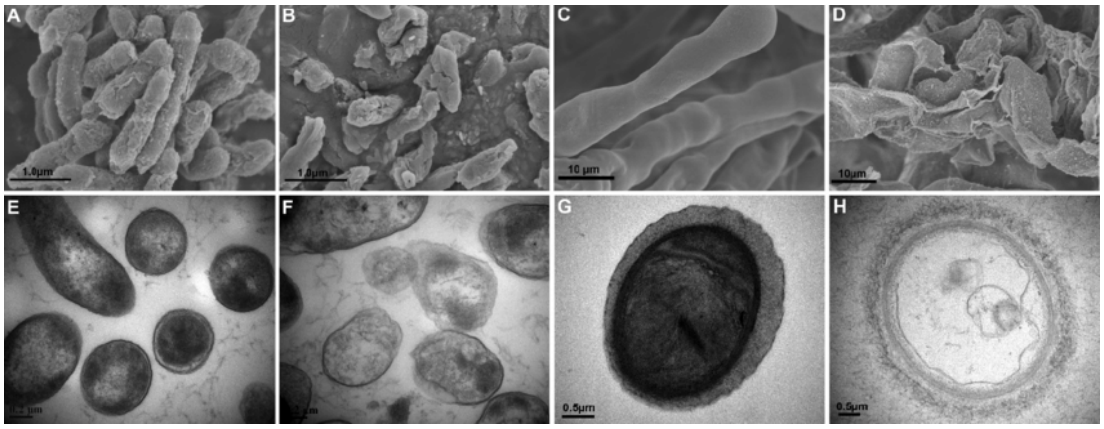

Figure 32 *Pseudomonas syringae* (URL 32)

Harmfulness and geographical distribution. The losses caused by Pathovar *syringae* are not well documented and are difficult to specify. However, under conditions favourable to the pathogen, yield losses can be high, especially in stone fruit crops.

Epidemiology. The pathogen survives in association with plant tissues (in the leaves of trees, in seeds). Easily spread by wind-driven rain. It enters the plant through vents and wounds caused by cutting, insects, hail, etc. Damage to flowers and fruit by low temperatures is a predisposing factor for scarlet scab of pear and lilac. Winter temperatures below freezing encourage the development of necrosis of the rind of stone fruit, especially in apricots. The nucleating ability of *syringae* pathovar increases the extent of frost damage in many host plants. Epiphytic survival of the pathogen is promoted by cold wet weather. The number of surviving bacteria decreases when the weather is hot and dry.

Protection. By spraying with copper preparations. The timing of spraying and the choice of the concentration of the active substance depend on the crop species. The aim is to reduce the epiphytic population of the pathogen at those times when the host plant is most susceptible to infection. The concentration of the product must be so high that it is not phytotoxic. In stone fruits, it is possible to reduce the number of bark necroses when the cut is made after the buds have budded.

5.4 *Pseudomonas marginalis* pv. *marginalis* (Brown) Stevens; *Pseudomonas viridiflava* (Burkholder) Dowson

Characteristics of pathogens. It is characteristic of these fluorescent pseudomonads that - unlike other species of this genus - they produce pectinolytic enzymes. They are considered to be weak or opportunistic pathogens. An important biochemical feature that distinguishes the two pectinolytic pseudomonads is the production of oxidase: *P. viridiflava* is oxidase-negative, whereas pv. *marginalis* is oxidase-positive. The species *P. marginalis* is referred to by some authors as *P. fluorescens* biovar II and IV. Until recently, this biovar was thought to be composed only of saprophytic strains commonly found on plant surfaces, in decaying plant debris, in soil and water. It is difficult to distinguish whether pectinolytic pseudomonads or *Erwinia* are the causal agents of the disease by the symptoms. Cucumber leaf spots can be caused not only by pectinolytic pseudomonads (pv. *marginalis*, *P. viridiflava*) but also by a specialised pathogen of cucumber, i.e. *P. syringae* pv. *lachrymans*, which is not pectinolytic. Symptoms may be modified by external conditions. For example, if lettuce or chicory is attacked under favourable conditions, large, watery, dark lesions

are produced, affecting the entire leaf. In contrast, under unfavourable conditions for pathogen development, only localised dry papery necrotic spots appear.

Harmfulness. Given that *Erwinia carotovora* has a similar host range and the same symptoms, it is difficult to estimate the losses caused by pectinolytic pseudomonads. Harmfulness can be high, especially for vegetables stored at low temperatures.

Epidemiology. Pectinolytic pseudomonads are commonly found on the plant surface (in the phylloplane and rhizosphere), *P. marginalis* pv. *marginalis* also in the soil. They enter the plant mainly through wounds, but also through stomata, provided that there is free water on the surface of the weeds. Many times the first symptoms appear on older leaves touching the soil surface, where the main source of infection is. Pectinolytic pseudomonads cause soft rot of tubers at lower temperatures than pectinolytic erwinia, i.e. mainly during late autumn and early spring. The infection is aided by frost damage when the husks are saturated with water.

Protection. We reduce the amount of inoculum in the soil by rotating crops and removing contaminated crop residues. Prevent water vapour precipitation on the surface of stored products.

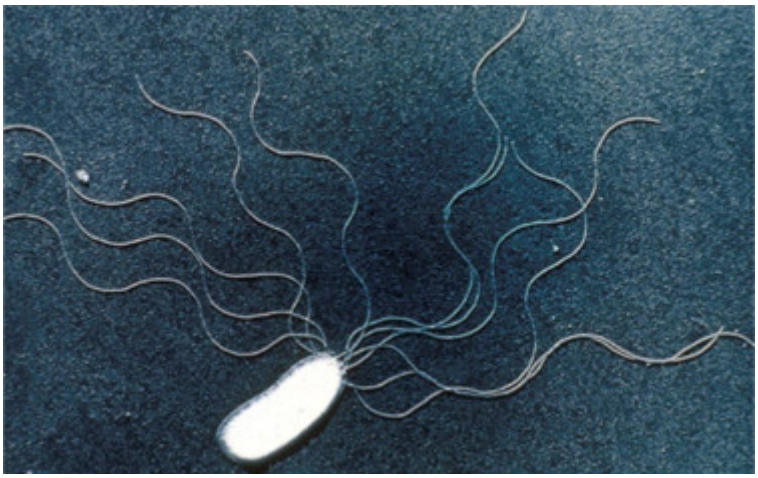

Figure 33 *Pseudomonas marginalis* (URL 33)

5.5 *Pseudomonas cichorii* (Swingle) Stapp

Characteristics of the pathogen. It is a fluorescent, oxidase-positive, and pectinolytic-negative bacterium. It is considered an opportunistic pathogen. It occurs in the epiphytic microbiota of healthy plants. The host range of the species is very wide. It includes many herbaceous plant species, of which lettuce, chicory, parsley, celery, beans, *Brassica* spp., tomato, tobacco, sunflower, coffee plant, eggplant, geranium, geranium, chrysanthemum, gerbera and dahlia are the most commonly attacked. It also probably includes 35 species of weeds from 11 families. Exceptional is the occurrence in woody plants (*Magnolia grandiflora*).

Harmfulness and geographical distribution. It causes greater losses especially in lettuce and chrysanthemums. After primary infection of the fleshy organs of host plants by *P. cichorii*, they are then sometimes secondarily infected by pectinolytic erwinia or pseudomonads.

Symptom. In lettuce, the first signs are small dark green watery patches around the stomata, epidermal trichomes or hydathodes. Under moist conditions, the spots enlarge and coalesce very rapidly, giving large, moist, dark brown lesions. When

dryness sets in, the enlargement of the lesions stops, the lesions dry out and take on a lighter colour. In other plant species the spots are similar, with some occasionally forming a narrow yellow halo around the lesions. The pathogen can also penetrate vascular plexuses. Cauliflower rosettes initially show small, off-colour spots, which quickly spread over the entire rosette and turn brown when moist. The result is soft rot, in which the bacteria *Erwinia carotovora* may also be involved. On the leaves of brassica plants, the fungus *Alternaria brassicicola* takes hold in dry weather on lesions of bacterial origin (following infection with *P. cichorii* or *P. viridiflava*). On celery, localised brown spots have been observed on the stems, especially at the base. The pathogen spreads up and down the woody tissue, not through the blood vessels. In chrysanthemum, buds, flowers, leaves and bases of stems are affected. The spots at the base are darkly coloured, sometimes covered with a coating of fusarium. Exceptions are not made when the infestation of the stems is not accompanied by the appearance of leaf spots. Gerberas develop target-like leaf spots.

Epidemiology. The pathogen survives through the winter in undecomposed plant debris. It can persist in dry soil for about 1 month. It occurs in the rhizosphere of some weedy plants. Seed transmission has been demonstrated in lettuce. It enters the plant through natural openings and wounding. A positive correlation between leaf wounding and leaf spot frequency has been observed. Temperatures around 25 °C are optimal for disease development. At temperatures below 16 °C and above 28 °C, the disease development stops. Epidemics occur after periods of frequent rainfall or spray irrigation and after strong winds. In chrysanthemum, a positive correlation between increased nitrogen and potassium rates and higher disease severity has been confirmed.

Protection. It is difficult to implement due to the common occurrence of the pathogen in the epiphytic microbiota. Modification of growing conditions (to prevent damping-off and injury to plants) and crop rotation (which is difficult in practice, e.g. for lettuce) is a consideration. Chrysanthemum varieties vary in their level of susceptibility.

Figure 34 Bacterial spotting caused by *P. cichorii* (URL 34)

6 BACTERIOSES OF FIELD CROPS

6.1 Cereals and fodder grasses

The number of known bacterial pathogens of cereals and forages is relatively high (about 30), but their economic importance is not great. Under Central European conditions, bacterioses on cereals and fodder crops occur sporadically, mostly only in association with wet weather at the beginning of the growing season. They occur most frequently in maize and forage crops, less frequently in barley, oats and wheat. On a multi-year average, their damage is low, so that no special protection is usually carried out against them. In subtropical and tropical areas, bacteroids are of considerable economic importance in rice and millet.

The lower harmfulness of bacterioses in cereals and grasses is probably related to their anatomical structure, which does not create a suitable environment for the spread of bacterial pathogens in the intercellular spaces within the host weeds. In contrast, they mostly do not colonize the cotyledon, sclerenchyma and are less frequently found in the woody parenchyma.

Surprising epidemic outbreaks of bacterioses in cereals and grasses can occur: when new crosses and new varieties are introduced into varietal trials and when new varieties are introduced into normal cultivation; when cultivation technology is changed; when the weather is abnormal.

Of the bacteria which do not normally pose a serious threat in cereal and fodder crops, necrotic mottle (in cereals), xylem vessel blockage (in fodder crops), rot (in maize), in particular, can occur unexpectedly.

Necrosis

In our oat-growing areas, bacterial blight occurs sporadically. Brown discolouration caused by *Pseudomonas syringae* pv. *atrofaciens* occurs on wheat and barley stalks, especially from the time of shedding until milky maturity when there is abundant rainfall. The susceptibility of the weeds increases markedly from the milky stage to waxy maturity. The disease is not very damaging.

In all growing areas of the world, blackening of the base of the grain occurs on barley. This disease is of economic importance, particularly in spring malting barley. Batches of malting barley containing more than 4% discoloured grains are of lower malting value or are considered unsuitable for malting until the end. Their protein content, wort colour, flavour and aroma of the beer are adversely affected. Micromycetes (in particular *Cochliobolus sativus*, *Altemaria alternata*, *Cladosporium herbarum* and others) and, among the bacteria, *Pseudomonas syringae* pv. *syringae* and perhaps also species of the genus *Bacillus* are involved in the blackening of the grains. High humidity between lactic ripening and the early waxy ripening stage is necessary for infection and disease development.

When *triticale* cultivation began to expand in the USA in the recent past, unexpected complications associated with the occurrence of black chaff and leaf spot caused by *Xanthomonas translucens* pv. *translucens* emerged. Newly bred varieties were highly susceptible to this bacteriosis. This complicated the further development of triticale cultivation. The causes of the epidemic occurrence of leaf necrosis in winter and spring wheat in some US states since the mid-1960s are not fully understood. The causal agent of this necrosis is *Pseudomonas syringae* pv. *syringae*. It is speculated that this pathovar, which is known to attack maize and sorghum among cereals, has evolved new strains virulent to wheat. The increase in the incidence of foliar necrosis caused by *Xanthomonas translucens* pv. *undulosa* (on wheat), pv. *hordei* (on barley)

and pv. *secalis* (on rye) in the USA, southern and central Africa has more recently been attributed to a shift away from the use of mercuric seed treatments.

Pseudomonas syringae can be assumed to be involved in frost damage caused in maize by early autumn frosts when temperatures drop to -5 to -7 °C.

Vascular tissue blockage

Since 1974, the harmfulness of *Xanthomonas translucens* pv. *graminis* and other related pathovars has been increasing in forage grasses. Infestation is manifested by wilting, leaf streaking, white spikes and premature death of whole plants. A potential danger is the quarantine organism *Pantoea stewartii* subsp. *stewartii*, the causal agent of maize wilt. So far, it is mainly damaging sweetcorn in North America. In other parts of the world, the pathogen can be introduced with maize seed.

Rot

In irrigated maize stands, stem rot caused by *Erwinia carotovora* subsp. *carotovora* or *Erwinia chrysanthemi* pv. *zeae* is likely to occur. Similar symptoms are caused by fungi of the genus Fusarium.

Gumming disease of wheat, and spike blight

In Europe, yellow slime mould caused by bacteria of the genus *Rathayibacter* (formerly classified in the genus *Corynebacterium* and later in the genus *Clavibacter*) is occasionally found on forage crops, and in Asia on cereals. Forage grasses are attacked by *R. rathayi*. A yellow sticky bacterial slime appears on young inflorescences, which may also cover the upper part of the stem and adjacent leaves. If the mucilage formation is large, the infected organs are stuck together, the upper internodes do not elongate, the panicles or spikelets are deformed. In dry conditions, the mucilage hardens and becomes crumbly, the infected organs dry out prematurely.

The occurrence of mucilage is linked to infestation of the plants by nematodes of the genus *Anguina*. Instead of grains, hyphae form on the infected plants, in which nematodes or bacteria are many times present. The causal agents of yellow slime mould produce a toxin that is toxic to cattle grazing the infested grassland. Toxicosis in cattle was recorded in Western and Faithful Europe in the 1960s and in Australia in the 1970s.

The pectinolytic bacterium *Erwinia rhapontici* in wheat causes grain pinking. There is a cavity under the epidermis near the germ. The occurrence of this bacteriosis is not frequent.

6.1.1 Halo Blight of Oat

Pathogen. *Pseudomonas syringae* pv. *coronafaciens* (Elliott) Young, Dye & Wilkie.

Host plant. oats, rye, barley, wheat, maize, creeping vetch.

Harmfulness. The disease occurs in all oat-growing areas, but mostly only sporadically. Yield losses are not economically significant.

Symptoms. On the leaf blade, rarely on the sheath or husks, initially light green, partly also light brown, mostly oval spots 1-2 mm in size are formed. They are sometimes surrounded by an oily rim. In the middle of the patch, around the vent (through which the pathogen has penetrated inside the plant), the mesh is sunken, later grey to brown and gradually withers. Around the perimeter of this small patch is usually a light green, later yellow yard (chlorotic halo), often bordered by a reddish-

brown border. The chlorotic halo enlarges quite rapidly to a diameter of 1 cm or more, so that many times the lesions merge into each other. When heavily infested, the entire leaf blade turns yellow and eventually dries completely. At the same time, the leaves sometimes curl at the top or at the edges. Occasionally, small silver scales of dried exudate can be seen in the centre of the lesions. Many times, the spots are also visible on the husks. The whole clump may be affected.

Unless a distinct yellow halo forms around the necrotic lesion, the symptoms of the disease can be confused with bacterioses caused by other *Pseudomonas syringae* pathovars. The chlorotic halo is formed by the action of a toxin secreted by the pathogen. The halo does not form at higher temperatures (above 22 °C).

In some cases, plants germinate from infected grains, which are necrotic, and the embryonic root is poorly developed. Infected cobs sometimes do not germinate at all and rot.

Epidemiology. The pathogen is probably caryopsis, but this has not yet been reliably demonstrated. It can survive for up to 4 years in plant residues. In vegetation, the disease is spread by raindrops and insects (but it has not been determined exactly which insects). In grasses, spread by mowing machinery during mowing is common. The pathogen enters the plant through vents or at the site of injury. Wet and warm weather in late spring and summer contributes to the development of the disease and the spread of the pathogen in the crop.

Protection. After harvesting the infested stands, we perform deep ploughing and then do not sow the land with cereals and fodder. In the past, orthognathic preparations were used to suppress the pathogen on the seed; abroad, streptomycin has been used. Among the various species of the genus *Avena*, *A. byzanthina* is one of the less susceptible. Among the highly resistant ones is the hexaploid form Ce 4146 (*A. sativa* x *A. ludoviciana*). Less susceptible varieties are represented in the world oat assortment. Resistance is reportedly monogenically inherited.

Figure 35 Halo Blight of Oat (URL 35)

6.1.2 Bacterial Wilt of Forage Grasses

Harmfulness and geographical distribution. In some areas of Switzerland, annual losses in forage grass hay yields are estimated at an average of 20%. With each successive cutting, the losses increase until they reach around 40%. The disease was first detected in Switzerland in 1974, then in Great Britain (mainly Scotland), Norway, Belgium, France, Germany and New Zealand. Extensive surveys in western Scotland and northern Belgium found the pathogen in 50 – 80% of stands.

Symptoms. They are most striking at the time of spreading. Growth of affected plants is retarded. Young leaves curl up and do not wilt without specific spots

appearing on them. Only in cold weather are longitudinal chlorotic streaks visible on young leaves. In severe early spring infestations, the plants die before the inflorescence forms. If plants are attacked at a later stage of development, they rarely form inflorescences. If inflorescences do form, they turn yellow to white, but the leaves remain symptomless. Severe infection results in stunting and many times premature death. Chlorotic streaks appear on older plants, first near the clogged vascular bundles. Chlorotic streaks often extend along the entire length of the leaf and later necrotic. Chlorosis and wilting can also be caused by herbicides and improper fertilization. Tracheobacteriosis is suspected, especially when a drop of yellow bacterial slime appears on the cross-section of severely affected stems (after a slight squeeze).

Epidemiology. There is a lack of reliable data on the mode of survival and spread of the pathogen. It is believed to be seed-borne. The pathogen spreads in stands during mowing or grazing, especially in wet weather. Once the bacteria have penetrated the inner weeds, they spread systemically in the plant through the vasculature. The influence of external conditions on the development of the disease can only be inferred indirectly. The damage caused by the disease is usually slight, but in years when there is a long dry period during the summer, the damage can be considerable. In cool, wet weather, diseased plants rarely appear.

Protection. It consists in breeding resistant and less susceptible grass species and varieties. Many of the newly bred high-yielding hybrids *Lolium multiflo rum x L. perenne* are very susceptible. Less susceptible varieties are known *Lolium multiflorum italicum, Lolium multiflorum x L. hybridum, Phleum pratensa* and *Dactylis glomerata*. To avoid heavy infestation, it is recommended to: use resistant varieties; disinfect mowing machinery when moving mowers from one stand to another; mow in dry weather; avoid mowing too low (leave taller stubble).

6.2 Legumes

Plants of the bean family are, together with the leguminous plants, among those with a relatively high number of bacterial pathogens (about 16), which cause high losses in yield and quality of the harvested products. Globally, phytopathogenic bacteria are most damaging in soybean, bean and pea, less so in bean and lupin.

The bacterioses that potentially threaten legumes most include necrosis, vascular tissue blockage and rot.

Necrosis. The symptoms of spotting and scald caused by *P. syringae* pv. *phaseolicola* in beans and *P. syringae* pv. *pisi* in peas can also be caused by *P. syringae* pv. *syringae* or *P. viridiflava* in both hosts. The causal agent of general bacterial scald of beans, *Xaxonopodis* pv. *phaseoli*, is damaging at relatively high temperatures (25-35 °C) and with high rainfall and humidity. In Europe it is mainly found in eastern and southern areas (e.g. Romania, Bulgaria, Spain). In contrast, the causal agent of bean scald, *Pseudomonas syringae* pv. *phaseolicola*, is more likely to occur at lower temperatures. However, it is not rare for both pathogens to occur together in the same stand and even on the same plant.

Vascular tissue blockage. The causal agent of bean wilt, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, is currently of minor economic importance (except perhaps in Turkey). It is listed as a quarantine organism in EPPO and EU countries, but its status is likely to be revised.

Rot. Potential pathogens of bean and lupin include the bacterium *Erwinia carotovora*, which is involved in, among other things, blackening and death of sprouts of emerging plants and stem rot of mature plants.

6.2.1 Halo Blight

Pathogen. *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young, Dye & Wilkie. By 1999, 9 races were differentiated according to the plant response of the differentiating varieties.

Host plant. Mainly common beans and scarlet runner beans, but other bean species are also natural hosts. It is possible that the host plants of the pathovar *phaseolicola* have included those species which are actually attacked by other pathovars *P. syringae*, napr. pv. *syringae*.

Harmfulness and geographical distribution. It occurs in all bean-growing areas of the world. It is one of the most economically important diseases of beans.

Symptoms. The leaves have small, irregular, dark watery spots, often surrounded by a light green or yellowish ring, up to 2.5 cm wide. This characteristic symptom, the so-called halo, is caused by a phaseolotoxin secreted by the pathogen. The initially small watery patches may become confluent, brown and dry. If the spots are numerous or if hot weather prevails at the time of infection, the halo is not evident. Phaseolotoxin formation depends on the pathogen strain and the ambient temperature; optimum is at 16 – 20 °C. At more than 22 °C its production is inhibited. Similar spots, but many times embedded, are also found on stems and pods. Sometimes, after infection of the stem, reddish, elongated stripes appear on its surface, which many times burst longitudinally. The spots on the pods are round, initially greasy and up to about 1 cm in size. In wet weather, a silvery to creamy bacterial ooze appears on the surface of the watery lesions. The spots may blend into each other. Later they dry out, turn brick red to brown in colour and are not regular in shape. The spots on the pods may also affect the vascular bundles of the dorsal and inferior suture, and elongated lesions appear in the surrounding tissue. Infected seeds have discoloured seeds (which are conspicuous on white and light-coloured seeds) or are shrivelled and shrivelled. Symptoms on leaves and pods are similar to those caused by bacteria *Xanthomonas axonopodis* pv. *phaseoli* however, the leaf spots caused by the pathovar phaseoli are never surrounded by a broad halo, but only by a narrow yellow border. In addition, the exudate formed on the lesions by *X. axonopodis* pv. *phaseoli* is yellow. If the pathogen penetrates the vascular bundles, systemic infection occurs. Affected plants are stunted and chlorotic or reversibly wilted. Leaves tend to be mosaic and misshapen. Such symptoms occur particularly in plants grown from infected seeds. A relatively common symptom in plants grown from infected seeds is a watery area at the site of the first leaf node gradually increasing in size until it envelops the entire stem. The watery mesh slowly takes on an amber colour. At this point the stem may break. Infected seeds may also produce seedlings with a damaged or dead growth apex or brownish black irregularly shaped lesions on the womb. If there is systemic invasion of vascular bundles through small leaf veins, reddish watery areas may form around the veins. If the leaf vascular bundles are colonized through the petioles, then the main vein and lateral veins are initially sort of watery and later brick red in colour.

Epidemiology. Primary disease outbreaks in the crop originate from infected seeds or infected weedy host plants. Overwintering in soil or plant debris is not common in most areas. In the post-emergence period, the disease spreads from the surface of lesions on infected plants by wind-driven rain to healthy plants over distances of up to 30 m in the direction of the wind. Under favourable conditions, one infected seed in 10 000 seeds is sufficient to cause an epidemic. However, not every infected seed will produce an infected plant. However, if the number of infected seeds drops to a ratio of 1: 20 000, an epidemic will not occur even under favourable weather

conditions. Seeds may be infected when the vascular bundles of the plant are systemically colonised. However, direct penetration of the pathogen into the seeds from lesions on the pod valves is more common. The pathogen can also be spread by segments of infected plants that remain in poorly cleaned seed.

Figure 36 Halo Blight of beans (URL 36)

Protection. The most effective and economical protection is the use of disease-free seed. This can be achieved by concentrating seed crops in arid areas. Good seedbed health and satisfactory yields are achieved here by using furrow irrigation and excluding spray irrigation. 2 – 4 sprays of copper preparations (in the USA also streptomycin) applied from flowering time to pod set have proved successful. Seed treatment with streptomycin or kasugamycin or dry heat suppresses the pathogen but is not always a reliable guarantee of protection against the emergence of an epidemic in the crop. Determination of the critical limit of infected seeds (1:10 000) in seed samples is feasible in properly equipped diagnostic laboratories. An effective long-term method of protection is to breed varieties for resistance.

6.2.2 Bacterial Blight of Peas

Pathogen. *Pseudomonas syringae* pv. *pisi* (Sackett) Young, Dye & Wilkie. There are 7 known races that can be identified by the response of the pea differentiation assortment after inoculation with the pathogen. In Europe, race 2 is predominant, races 4 and 6 are less widespread. Race 6 has been shown to occur in the Slovak Republic. The predominance of race 2 can be explained by the lack of the R2 resistance gene in many cultivated varieties. In contrast, race 6 lacks avirulence genes and is therefore pathogenic for all pea varieties.

Host plant: especially peas, but also others.

Harmfulness and geographical distribution. It is an economically important pathogen on both field and garden (i.e., wood and sugar) peas. Yield losses can be as high as 30 %. Further economic losses arise from the fact that infested garden pea pods are difficult to sell. Indirect economic losses arise from the application of quarantine measures.

Symptom. They occur on all above-ground organs but are most characteristic on stems and stipulae. The localisation and nature of the symptoms depend on the weather pattern. In dry weather with occasional frosts, symptoms usually appear on the stems close to the soil surface in the form of watery, later olive-green to purplish-brown

spots. The infection spreads upwards to the stipulae and individual bracts of the scalded leaves. The veins turn brown to black. The adjacent interveinal mesh wilts, then turns yellow and brown, and finally dries and papery. In rainy weather, the lesions on the leaves are initially small, round, oval or irregular in shape, dark green and watery. A cream-coloured bacterial slime may appear on the surface of the spots, which gives the spots a glossy appearance when dry. The petals later turn yellow and the spots themselves turn brown and papery.

Figure 37 Bacterial blight of peas (URL 37)

Infection of the flowering and fruiting peduncles results in the flowers (entire inflorescences) and pods withering and dying. On the pods, the infestation is initially manifested by watery, oily, round spots, which later merge, turn brown and finally wither. Lesions on the pods may be confined to a narrow strip around the seams. If the pod is invaded at the dorsal suture, the seeds inside may be covered with bacterial slime. Infected seeds have a watery spot near the navel or are shrivelled, brownish yellow in colour. If the infection spreads throughout the plant, it may wither and die prematurely. In warm, dry weather, the infection will stop, the upper parts of the plant will remain green and produce healthy flowers and pods. Similarly, new lateral shoots grow from the base of diseased plants.

Epidemiology. The source of infection is infected seeds. External contamination of seeds is not critical for the spread of the pathogen. Plants that grow from naturally infected seeds tend to be severely affected, especially when night temperatures drop to freezing. Diseased plant residues in the soil or the roots of weedy plants are a possible source of infection if proper crop rotation rules are not followed.

During the growing season, the pathogen is spread by: wind-driven rain (especially when plants are damaged by night frost temperatures); irrigation water; touch; cultivation and harvesting machinery; and possibly insect vectors.

In leaves. The pathogen penetrates through stomata and wounding. Inside the plant, it spreads through the intercellular spaces of the parenchymatous plexuses of bark and marrow. Vascular bundles may also be colonized, through which the bacteria can spread to stipulae, true leaves and pods.

Dry weather and high temperatures prevent the spread of infection and the development of the disease. After a period of low temperatures, the frequency of infestation increases. The disease tends to develop more rapidly on soils with higher moisture content.

Protection. Breeding for resistance is aimed at incorporating six resistance genes (R1 to R6) and a race-non-specific resistance gene that has been identified in *Pisum abyssinieum*.

The IP test is used to test seed for the presence of the causal agent of pea scorch. If the test is positive, a suspension of ground seeds is spread on nutrient agar media to isolate *P. syringae* pv. *pisi*, which cross-reacts with the antiserum for pv. *syringae* in serological tests. Even the PCR method does not yet provide reliable results.

6.3 Potato

The potato, like other leafy plants, is a pathogenophilous plant where phytopathogenic bacteria cause significant economic losses. In particular, the fleshy tubers of the potato, made up of fine parenchymatous cells of the cortex and marrow, are a very suitable substrate for the damaging action of pectinolytic pathogens.

In order of economic importance, the most important bacterioses and phytoplasmoses of the potato include rots, vascular tissue blockage and sieve blight. In contrast, it is noteworthy that potato is not attacked by typical foliar bacterial pathogens.

Rot. From the point of view of practical control, it is important that, in addition to *E. carotovora* subsp. *carotovora*, they can induce soft rot of tubers under certain conditions: *E. carotovora* subsp. *atroseptica*; *E. chrysanthemi*; pectinolytic pseudomonads – some strains *P. fluorescens, P. marginalis* pv. *marginalis, P. viridiflava* and *P. putida*; pectinolytically active species of the genus *Paenibacillus* a *Bacillus* (*P. polymyxa* a *B. subtilis*), which penetrate the plant through wounds and are damaging at high temperatures; pectinolytic clostridia (*Clostridium* spp.), which are active at very low acid levels in storage areas.

Vascular tissue blockage. Potentially dangerous potato diseases include **ring rot** caused by *Clavibacter michiganensis* subsp. *sepedonicus*.

Globally, *Ralstonia solanacearum* is one of the important pathogens of potatoes and other plants (more than 450 species from 30 families). It is divided into 5 races, of which potato is attacked by races 1 and 3. It is a vascular pathogen (similar to *Clavibacter michiganensis* subsp. *sepedonicus*) causing wilting and dieback in potato plants. Although it is a typical occludogen, it is also capable of producing some pectinolytic enzymes and causes **brown rot**. It occurs mainly in tropical, subtropical and temperate warm regions of all continents and is considered to be a major limiting factor in potato cultivation here.

The causal agent of bacterial brown rot has in the past been said to be unlikely to persist and become an economically important pathogen in our conditions, given its high heat requirements (optimum for growth is between 35 – 37 °C for race 1). This is no longer the case. In fact, in the first half of the 1990s, race 3 was found to be the causal agent of brown rot in many European countries. Strains of this race have a lower temperature optimum than race 1 (around 27 °C). They also have a narrower host range, including potatoes, tomatoes, paprikas and eggplants, and, among European weeds, some leucaena species (*Solanum dulcamara*, *S. nigrum*, but also *Urtica dioica*). *R. solarzacearum* is considered a quarantine organism in the European Union and in the Slovak Republic.

Blockage phloem. The causal agent of **stolbur**, Potato stolbur phytoplasma, is included in the list of quarantine organisms in the Slovak Republic. Plants of the family Globuliaceae, with the exception of seeds, are subject to quarantine control. The natural host range includes 45 species of the onion family, including potato, tomato, paprika and onion. However, the host range is much wider; other plant species from 6 families can be considered susceptible, including some weedy species from the starflower, dicotyledonous (e.g., evening primrose) and bean families. The disease

manifests itself in the germination of potato tubers by filamentous sprouts, later by branching of the stem, yellowing and curling of the leaves, the appearance of watery stolons with tubers and tubers in the leaf axils. The infection is spread by: insect vectors from weedy host species; infected tomato, paprika and onion seedlings. Transmission by potato tubers is unlikely.

In the 1950s, stolbur in potato, tomato and other plants was considered to be an economically important disease, especially in the southern regions of Moravia and Slovakia, as well as in other countries of central and southern Europe. Nowadays, its importance is invisible. It is confirmed that the conditions for disease epidemics in potato and tomato occur only in certain long-term cycles. The emergence and development of epidemics is dependent on conditions favourable to the insect vectors of the pathogen, which are certain species of cicadas (e.g. *Hyalesthes obsiletus*) that spread the disease from certain wild host plants.

6.3.1 Blackleg and Soft Rot

Pathogen. *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye,
Erwinia carotovora subsp. *carotovora* (Jones) Bergey et al.,
Erwinia chrysanthemi Burkholder, McFadden & Dimock.

Which of these pathogens is most frequently and intensively involved in the development of stem blackening and soft rot is determined by temperature conditions. This is related to the different optimum growth temperatures of the different bacterial species or subspecies. At this point, we present data relating to *E. carotovora* subsp. *atroseptica*.

Host plant. Although the main host of *E. carotovora* subsp. *atroseptica* is the potato, natural infection has also been found in brassicas (cauliflower, cabbage), tomatoes and various ornamentals (*Iris* sp., *Delphinium* spp.), among others.

Harmfulness and geographical distribution. The frequency of clusters with symptoms of stem blackening does not usually exceed 2% in a stand. The main damage is in the form of tuber rot during storage; the extent of total losses is 5% or more.

Symptoms. In cold weather and high soil moisture, the mother tubers can die before they grow and emerge; there are gaps in the stand. In most cases, the first symptoms appear at the beginning of the growing season, before the stand is established. Infested plants tend to be stunted. The leaves, especially at the top of the stems, are stiff and erect. Their margins curl towards the face.

The base of the stems up to about 10 cm above the soil surface is black or dark brown and rotten but firm. In dry weather this basal rot is light brown, dry and often cracked. Not all stems of a single cane need be infected; often symptoms are only on 1 – 2 stems. Localized rot lesions may also occur on the leaf blade, petioles and stems. On a cross-section through the stem, about 5 cm above the rot lesion, brown discolouration of the main xylem cords can be seen. At an advanced stage of vegetation, the leaves of infected plants turn brown, and the entire stem dies. In plants in which the disease appears late (after stand establishment) or during a period of wet weather, the basal rot is darker in colour, wet and soft. In longitudinal sections, blackish streaks are visible in the inner tissue towards the top of the stem. Such plants often wilt and die very quickly.

Figure 38 Soft rot of potato (URL 38)

During storage of tubers, the disease manifests itself as soft rot under conditions favourable to the pathogen. If dry conditions prevail during storage, 'hard rot' appears in the form of brown dry lesions, which are usually located around the lenticels, do not extend to any great depth and are sharply demarcated from the healthy tissue. It is difficult to distinguish which of the three potential pectinolytic erwinia is involved in the infestation of the aboveground organs and tubers from the symptoms.

Three types of symptoms can be distinguished on aboveground organs, the occurrence of which is influenced by the weather, the way the pathogens penetrate the plant and their spread in the plant:

- type la – blackening of the base of the stem, associated with tuber infection and cold weather during the growing season, especially in early summer. The pathogen spreads from the aboveground organs through the blood vessels to the daughter tubers;
- type lb – browning of the flesh and vascular bundles of the stem, with no external symptoms at the base of the stem. The first external symptom is wilting from the top of the stem. These symptoms are related to tuber infection and warm weather at the beginning of the growing season. Infection of the daughter tubers occurs similarly to type la;
- type 2 – stem rot associated with infection at the site of injury at any point on the aerial organs, from where the pathogen spreads through the vasculature to the daughter tubers;
- type 3 – localised rotting lesions on leaf blades, stems or petioles related to infection at the site of injury; the pathogen does not penetrate to the daughter tubers.

In Central European conditions, *E. carotovora* subsp. *atroseptica* is most frequently involved in the development of la-type symptoms, *E. carotovora* subsp. *carotovora*, and exceptionally *E. chrysanthemi* in the development of lb-type symptoms. Type 2 and 3 symptoms are generally caused by *E. carotovora* subsp. *carotovora*.

Epidemiology. The pathogen is commonly present on tubers (usually lenticels) used for planting. If it is present in the vascular system, it can penetrate from the mother tubers to the stems and then through the stolons to the daughter tubers. From one vegetation to another, the bacteria can survive in the soil in plant debris or on unharvested tubers, possibly in the pupae of some insects, but also in the rhizosphere of weedy hosts. surface or inside the seedling tubers.

During the growing season, the disease can be spread by: soil water from decomposing mother tubers to daughter tubers; rain from stems to daughter tubers; insect vectors (mainly double-winged insects) from rotting tubers in piles after pre-planting spring thinning; aerosol from mechanical shredding of the panicle before harvest. During storage, the pathogen can spread to healthy tubers by contact with rotting tubers. Inside the tubers, the bacteria enter through lenticels and wounds.

The concentration of inoculum, soil temperature and moisture, or tuber wounds determine whether contaminated or infected tubers will grow stems with blackening symptoms at the base of the stem. The influence of external factors on the development of soft rot in tubers is discussed in the article on the polyphagous phytopathogenic bacteria *Erwinia carotovora* subsp. *carotovora* and *E. chrysanthemi*.

Protection. There is no effective protection against stem blackening. There are differences in susceptibility between varieties, but targeted practical protection is not based on differences in resistance between varieties. Negative selections are made in seedling stands, but their effectiveness cannot be relied upon too much, as the daughter tubers may be contaminated with erwinia, which pass into the soil environment from the decaying mother tubers. The following measures are taken against soft rot during storage: harvest in dry weather or dry the tubers before storage, prevent high humidity and the formation of a water film on the tubers by ventilating and keeping the temperature below 6 °C.

6.3.2 Vascular Wilt and Tuber Rot of Potato; Ring Rot

Pathogen. *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kott hoff) Davis et al.

Host plant. The natural host is only the potato.

Harmfulness and geographical distribution. Yield and storage losses of 15 – 40 (50) % are recorded in areas of pathogen distribution. The losses are due to non-harvesting, fewer tubers per cluster, smaller size of tubers and, in particular, rotting of tubers in the soil before harvest and during storage. In the case of seed potatoes, the presence of a single diseased cluster is a reason for rejecting the crop. For many years, ring rot was the main disease of potatoes in the USA and Canada. As a result of the great efforts of local phytopathologists and growers, the disease has clearly declined in recent years. The damage is economically significant especially in countries where sliced tubers are used as planting material. In European countries, the disease occurs rather sporadically and to a lesser extent. This is probably because the slicing of seedlings and the use of spike planters are not common in Europe. Indirect economic losses are linked to the application of phytoquarantine measures (banning potato exports from infested areas, destroying infected seed lots or banning sales for planting purposes, etc.).

However, the presence of the pathogen in our territory cannot be ruled out, as bacterial ringspot undoubtedly occurred in neighbouring Germany until the 1930s. It is assumed that the pathogen was reintroduced to the Czech Republic in the 1990s (possibly earlier) by seedlings imported from Western Europe. As the optimum growth temperature for the pathogen is 21 °C, favourable conditions for development are found in northern, north-western and central Europe.

Symptoms. On the above-ground parts of infected plants, the first symptoms usually appear at the end of August or even later. Diseased clusters are faintly yellowish, the leaves curl upwards in a spoon-like manner around the veins. Eventually they necrotise and wither from the edge. Mostly, however, external symptoms on the

aerial organs of diseased plants are not visible at all. Only rarely is there also a browning of the blood vessels inside the stalk, but when the base of the stalk is compressed, a cream-colored exudate oozes from the vascular bundles. In years when the spring is cool and the summer is warm, one or more stunted bullae may appear in the cluster, while the remaining ones are normally tall.

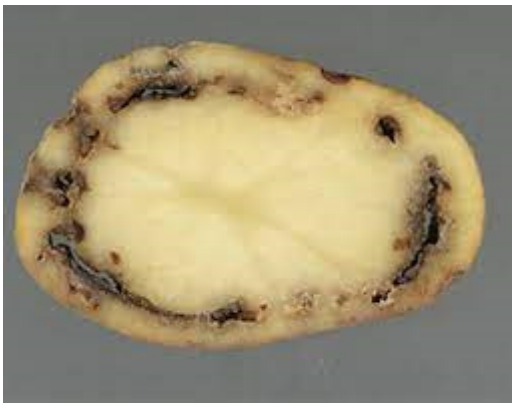

Figure 39 Potato ring rot (URL 39)

Characteristics are the symptoms visible on longitudinal section through the tubers. They appear on the tubers either before or after harvesting. They may occur only on some tubers of an infected plant. A creamy yellow to slightly brown discolouration is evident where the vascular bundles are located. When the split tuber is lightly squeezed, a mushy mass can be extruded from this yellowish to brownish zone (at the site of the vascular bundles, the tissues are spread out). In some cases, the local decomposition of the tissue and the formation of cavities at the site of the vascular bundles are visible on the top of the tubers in the form of shallow pits and cracks. Secondarily, pectinolytic erwinia penetrate the tubers and many times complete decomposition of the tubers ensues.

Epidemiology. The source of infection is mainly infected or contaminated tubers, through which the pathogen spreads over long distances. From infected to healthy tubers, the pathogen spreads easily when the seed tubers are cut with a knife. After cutting one infected tuber, 20 – 30 healthy tubers may subsequently be infected. To a lesser extent, the spread of bacteria by contaminated harvesting machinery, sacking and transport vehicles is also a possibility.

Transmission of the pathogen from contaminated soil to a healthy plant cannot be ruled out, but the soil as a source of infection is of minor importance, since bacteria in free soil are unable to overwinter. From the soil, the bacteria can enter the vascular system of the plant through wounds. Cases of spread of the pathogen during the growing season from diseased to healthy plants are usually very sparse. Transmission of the pathogen by insects, including potato caterpillars, cicadas and aphids, has been successful in trials. From infected mother tubers, the bacteria are spread by blood vessels into the vascular system of the plant and, via stolons, into the daughter tubers.

Disease development is favoured by soil temperatures above 18 °C. Symptoms on above-ground organs only become more pronounced at temperatures around 24 °C. By contrast, in cold weather, infected plants may not show any symptoms. Protection. This consists almost exclusively of producing and planting healthy seedlings. Diseased clusters are not tolerated in seed potato stands. Potatoes should not be planted on plots where the pathogen has occurred for at least 2 years. In countries where the pathogen is absent or only locally present, strict quarantine measures are applied. Tubers are

subject to quarantine inspection. Several tolerant varieties have been bred for resistance, but their cultivation is not widespread.

6.3.3 Potato Scab

In the past, actinomycete scab of potato was considered a single disease. In recent years, however, evidence has been presented that there are several diseases with different aetiology, symptomatology and severity, their causal agents have different demands on the environment, and thus protection against them has its own specificities. In Europe, general scab and net scab are of greatest importance.

Harmfulness and geographical distribution. Both common and net scab reduce the market value of affected ware potatoes, radishes and carrots. Tuberous net scab is associated with necrosis on the roots, resulting in a decrease in tuber yield. Common scab occurs worldwide. Net scab is most commonly reported from western and northern Europe (the Netherlands, Denmark, Sweden and also Switzerland). Both common and reticulated scab can occur on the same plot.

Symptoms. Scab symptoms are a manifestation of a defensive response to the penetration of pathogen hyphae into the surface layers of the immature tuber at the site of lenticels. Brown lesions of about 1 mm in size initially appear at the site of penetration and gradually enlarge as the tuber grows. Later, the appearance of the lesions varies depending on the species and strain of the pathogen, host variety, soil moisture and temperature.

Ordinary scabies. Depending on the virulence of the pathogen strains and the susceptibility of potato varieties, lesions may be superficial, raised or sunken. Most lesions have a raised, rough, corky appearance. There is or is not a raised margin around sunken lesions. Scabby lesions may be confluent and cover more or less of the tuber surface.

In the mild form of common scab, only the skin is damaged. A strong reaction to infestation is manifested by the formation of cork layers and the accretion (hyperplasia) of the surface plexuses. The depressions result from the collapse of the host tissues by the action of a toxin (called thaxtoxin) secreted by the pathogen.

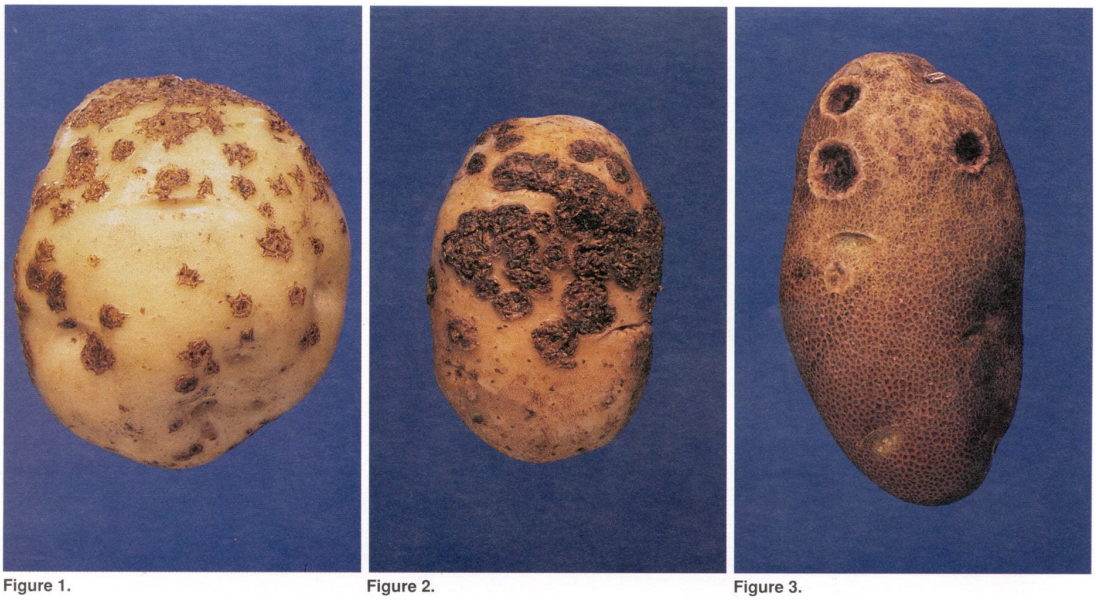

Figure 40 Potato scab (URL 40)

Reticular scab. Lesions on tubers are only superficial. The skin (periderm) is brown and thick, fissured in a reticulate pattern. In addition to the tubers, other organs, i.e. the roots and the unthickened stems, may also be affected in the form of necrotic lesions.

Epidemiology. Phytopathogenic streptomycetes are common inhabitants of soil where they are able to live outside the host plant's skin. Scabby mother tubers are probably of minor importance as a source of inoculum. However, it has been shown in some experiments that the use of seed tubers infected with the causal agent of reticulate scab resulted in delayed emergence, a reduction in the number of stems per cluster and a decrease in yield. Infestation of daughter tubers, however, depends primarily on the source of infection in the soil. High soil moisture inhibits the growth of *S. scabies*.

Streptomycetes penetrate the plant only through young lenticels. Penetrating hyphae of the pathogen stimulate the tuber tissue to form defensive cork barriers just below the surface. barrier, a second and sometimes a third barrier is formed until pathogen penetration is stopped, more barriers and deeper penetration, coupled with continued tuber enlargement, results in the development of severe forms of 'normal' scab. If the growth of the tuber stops, the lesions also stop enlarging. The main sites of mycelial penetration are the intercellular spaces and the middle lamella. Inside the cells, mycelium can only be seen at the periphery of the lesions, where streptomycetes can sporulate abundantly.

The secondary infection cycle is of no practical significance, not because conditions are suitable for infection only a few days after the start of tuber formation.

Influence of external conditions. The optimum growing temperature is 30 °C. Growth is possible in a pH range of 4,8-8,5. *S. acidiscabies* has been found in the USA growing at pH 4,5. Many of the literature references on the influence of external conditions on the development of scab must be taken with caution, as it is usually not clear whether they refer to general scab or reticulate scab.

Infestation of tubers tends to be stronger on permeable gravelly or alkaline and calcareous soils. In some rainy years and with irrigation, the incidence is less. General scab appears to be insignificant when the soil is moist, but the damage increases when the soil is dry. The disease develops at soil temperatures between 13 and 25 °C and most lesions form at 20 °C. The frequency of the pathogen population in the soil, and thus the frequency and intensity of tuber infestation, can be influenced by: the previous crop; liming for barley; supplementary lazy fertilisation; grazing (*S. scabies* can commonly colonise grass roots); the abundance of susceptible host crops and the susceptibility of the varieties grown.

Infection can occur when the lenticels are young, dry weather persists. Infection will not occur in moist soil, even when the pathogen is present in large numbers. It is believed that in moist soil, antagonism of the bacteria against actinomycetes is exerted in the vicinity of the lenticels.

Protection. It is aimed both at reducing the amount of inoculum on the seed tubers in the soil and at preventing infection. The fact that varieties differ in their susceptibility to the pathogen can also be exploited. Tuber pickling (in the past carried out with mercuric preparations, formalin, quintocene) has been of doubtful value because, with exceptions (e.g. on ashes), the pathogen is present in all agricultural soils.

Much effort has been concentrated on reducing the population of the pathogen in the soil, but none of the recommended methods is effective in all soils. The most commonly recommended chemicals to inject into the soil prior to planting are formaldehyde, manganese sulphate, chloropicrin, and pentachloronitrobenzene (the latter, however, is suspected to be carcinogenic). Sulphur was also used to acidify the

soil to about pH 5,2. Scab has been reduced, but at the same time the yield of potatoes and other crops sensitive to higher acidity, such as barley, has been reduced. Selection for acid-tolerant strains of *S. scabies* cannot be ruled out.

To reduce soil populations of the pathogen, it is sometimes recommended to use green manures (e.g., green rye) or to apply canola seed, soybean meal, or soybean plants to the soil to increase soil acidity and moisture or after antagonists have proliferated. However, if the pathogen population is too large, these measures are usually uneconomical and usually ineffective. In contrast, where irrigation is possible, irrigation of the crop at the critical period at the onset of tuber formation (usually 5 – 6 weeks after planting) is an effective measure. Cultivars vary in their level of susceptibility to the causal agents of scab; none of the genera is highly resistant. Resistance to net scab is not correlated with resistance to general scab.

6.4 Beet

Beet is one of the crops where bacterioses do not cause economically significant damage in Central Europe. The data in the older literature on the whole range of beet bacteraemia are, according to the present state of knowledge, doubtful.

The causal agents of beet bacterioses are weak pathogens. No special protection against them is practised in Central Europe. Contrary to earlier data, phytopathogenic bacteria are not now thought to be involved in infecting emerging beet.

Bacterial blight and vascular blackening caused by *P. syringae* pv. *aptata* may occasionally occur, especially on seedlings. The pathogen is seed-borne. In normal beet stands, the disease appears after hailstorms and in cold, wet weather. Symptoms are mild on both aboveground organs and on the eyeballs. On the leaves there are watery at first, later parchment-like spots. The veins inside the spots are blackened, the mesh on the periphery of the spots is watery. There are black elongated spots on the petiole, which later burst. Blackened vascular bundles can be seen on the transverse section of the eyeball. This is a non-specific symptom which is also associated with other diseases (bacterial, viral, fungal) and nutritional disorders.

Figure 41 Beet bacterial blight (URL 41)

Silvering disease causes greater losses in England and Scotland in beet seedlings. One or more leaves on the plant have a silvery appearance, initially mainly along the veins. The epidermis cracks and the leaf has a roughened appearance.

Eventually the plant wilts and dies within a few days. The pathogen is seed-borne. One of the most striking bacterioses of beet is the canker disease caused by *Agrobacterium tumefaciens*. Only individual plants in a stand are affected. More severe infestations sometimes occur on alkaline soils. Beet scab is not of economic importance. However, in red beet for pickling or canning, scabby root bulbs are unacceptable. Some types of rot of bacterial origin interfere with sugar production, e.g. as little as 2% of tubers infected with slime rot cause problems in filtering beet juices are, according to the present state of knowledge, doubtful.

Rot is often found in the accumulation dumps of sugar mills. Bacteria are usually involved in these rots as secondary pathogens.

6.5 Oilseeds

In oilseeds, which include mainly winter rape, mustard, sunflower and poppy, bacterioses are of economic importance in sunflower and poppy. Special protection against them is generally not carried out.

Leaf spot of bacterial origin occurs only sporadically on sunflowers. The spots caused by *P.* s. pv. *helianthi* are angular, dark brown, surrounded by a chlorotic halo. Colonisation of vascular bundles may also occur; the leaves are then shrivelled due to uneven growth of vascular and parenchymatous plexuses.

Stem and stem rot caused by pectinolytic erwinia appears towards the end of the growing season. It is usually related to the ageing of the weeds or plant stress. Rot at the base of the stem is manifested by blackening. The inner woody tissue is inky black, watery, often more or less digested. The stems break. Sometimes the cuttings are also affected by rot. The incidence of rot is more frequent after a hailstorm.

Leaf spot caused by *X. c.* pv. *papavericola* is often mistakenly attributed to fungal disease agents (especially *Helminthosporium papaveris*). Spots of bacterial origin are initially watery, later turn yellow, dry and translucent. Lesions also occur on the stems. If they encircle the stem, the apical part dies. All parts of the flower and the pistil may also be affected. Humid and warm weather contributes to the development of the disease (optimum temperature is 25 – 30 °C). Premature wilting of the leaves results in a reduction in seed yield and quality. Extensive leaf necrosis of poppy is also caused by the polyphage *Pseudomonas cichorii*.

Stem rot, caused by *E.* c. subsp. *carotovora*, appears on poppies already at the beginning of the development of the first buds. Affected plants lose their turgor in green from the top and wilt completely within 2 – 3 days. Watery lesions are visible in the upper half of the stem. The netting softens at this point and the stem breaks. The stems are brownish to blackish, mostly digested. If older plants are attacked, the inner wood of the stem decomposes, the plants wither and die prematurely. The occurrence of stem rot is often related to plant injury caused by *Timaspis papaveris*, Hartig, *Calocoris norvegicus*, *Ceutorhynchus macula*-alba.

Figure 42 Sunflower stem rot (URL 42)

6.6 Lucerne and clover

Like other leguminous plants, alfalfa and clover are attacked by several different bacterial pathogens, of which alfalfa wilt is the most economically important.

Spotting on the aerial organs of alfalfa and clover caused by bacteria of the genera *Burkholderia* and *Xanthomonas* occur at high humidity and high temperatures. Spotting caused by *P. syringae* pv. *syringae* tends to be visible mostly in the first cut on young leaves. The development of the disease stops with the onset of dry and warm weather. However, the activity of the pathogen may resume in cool wet weather at any time during the growing season.

Clover and lucerne **root and crown rot complex** is traditionally attributed to fungal pathogens (*Fusarium*, *Rhizoctonia*, *Phoma*, *Pythium* and others). The active involvement of bacteria in this complex disease has only recently been demonstrated.

In Europe, the strain of phytoplasma from the aster yellows group, subgroup 16Srl-C, is most commonly implicated as the cause of **clover greening.**

Clover stunting, manifested by abnormally small leaves, shortened internodes and general stunting, appears to have a complex aetiology. Phytoplasmas of the X-disease or aster yellows group are involved in the disease.

6.6.1 Bacterial Wilt of Lucerne

Pathogen. *Clavibacter michiganensis* subsp. *insidiosus* (McCulloch) Davis et al.

Host plant. *Medicago sativa*, *Melilotus albus* and other

Harmfulness and geographical distribution. In the USA, bacterial wilt has been considered a major cause of premature thinning of alfalfa stands since the 1920s. Annual yield losses were around 20%. After the introduction of more resistant varieties, the harmfulness of the disease decreased. Since the mid-1960s, the disease has also occurred in lucerne in some European countries, together with verticillium wilt and fusarium wilt.

Symptoms. As a rule, it is only in the second year and subsequent years of life that the presence of the pathogen in the xylem vasculature becomes apparent by stunting of the plants, increased number of stems and small leaves. The leaves turn yellow from the edges, wither and curl. The xylem part of the vascular bundles is yellowed to brown at the root cut and at the base of the stem. Plants die prematurely, the growth thins.

Epidemiology. The source of infection is infected seeds, through which the pathogen spreads over long distances. It survives in the soil only if undecomposed infected plant debris persists in the soil. Inside the stand, the pathogen spreads from diseased to healthy plants, mainly during mowing. To a lesser extent, the nematode *Ditylenchus dipsaci* may also be involved in the spread of the bacteria. The penetration of the bacteria from the soil environment into the plant is possible through various root wounds caused by frost, nematodes and insects.

Protection. Breeding for resistance, started in the 1950s in the USA and in the 1970s in Europe, provided varieties that allowed the most susceptible old varieties to be eliminated from cultivation. Seeds are subject to quarantine inspection.

7 BACTERIOSES OF VEGETABLES

7.1 Tuber vegetables

Tuberous vegetables belong to the brassica family (Brassicaceae). All plant organs can be attacked by the bacteria. The greatest damage occurs when the productive parts are affected, i.e. the head (in cabbage, kale, brussels sprouts), the leaf rosette (in kale, curly kale), the inflorescence (cauliflower, broccoli) or the stem tuber (in kohlrabi). In total, about 10 bacterial pathogens of brassicas have been described.

Leaf spot. In cauliflower and other brassicas, leaf spot is caused by *Pseudomonas syringae* pv. *maculicola*, especially in association with frost damage. Sometimes *P. viridiflava* and *P. syringae* pv. *syringae* also cause similar spots. Among the xanthomonads, *Xanthomonas campestris* pv. *aberrans* may be involved in the development of cabbage blight. The angular spots, bordered by a narrow margin, are formed around the stomata through which the pathogen penetrates into the weeds. Horseradish and other brassicas also sometimes have a spotting disease caused by *X. campestris* pv. *armoraciae*.

Soft rot. Bacterial agents of soft rot mainly attack vegetables where the head (cabbage) or the inflorescence (cauliflower, broccoli) is the productive part. A less vulnerable species is the kohlrabi, the productive part of which is the stem tuber. During storage, cabbage sometimes suffers a 35 – 44% loss of origin from the soft rot *Erwinia carotovora* subsp. *carotovora*. This pathogen often also attacks the cauliflower florets. *P. marginalis* and *P. viridiflava* are similarly damaging.

Vascular tissue blockage. In humid and warm growing areas, *Xanthomonas campestris* pv. *campestris* is one of the most common and feared pathogens, causing black vein spot in cabbage and other brassicas.

7.1.1 Black Rot

Pathogen. *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson. Existing variability in pathogenicity of *X. campestris* pv. *campestris* was explained by differences in aggressiveness until 1992. Later, however, the existence of six races was demonstrated, of which race 1 (about 62% of the isolates tested) and race 4 (32%) predominate.

Harmfulness and geographical distribution. Occurs wherever host plants are grown. It is considered to be the most important disease of brassica plants, especially in tropical, subtropical areas and conditions of humid continental climate. In contrast, in the cool maritime areas of northern Europe and North America it rarely causes major damage, as infestations here do not usually result in plant death. Damage increases

with spray irrigation and repeated cultivation of host plants. Cabbage and cauliflower show the greatest losses. Seed crops tend to be affected.

Symptoms. The disease manifests itself both as leaf spotting and as tracheobacteriosis. If the plants are infected at the seedling stage, they may either die in the beds before planting in the field or remain stunted.

Leaf spot begins on the underside of the leaf as a faint small watery lesion, which soon becomes conspicuous as necrotic spots with distinct margins on both sides. The surrounding tissue is pale and slightly chlorotic. Infection through hydathodes, injured leaf veins or roots results in chlorotic to necrotic brown 'V' shaped lesions with indistinct margins and blackening of the veins on the leaf margin. Affected leaves rarely drop. The external symptoms sometimes disappear for several weeks or are masked by the brassica fungus (*Peronospora parasitica*). During this time, however, the pathogen develops systemically throughout the plant, the external symptoms then reappear on the leaf margin only in summer with the onset of warmer temperatures.

Tracheobacteriosis is manifested by browning to blackening of the vascular system. Cabbage plants infected during the growing season rarely remain symptomless until the flowering stage, when the pathogen becomes established in the xylem of stems, petioles, and fruits. The invasion of the fleshy petioles and leaves is then followed, under the right conditions, by the invasion of rotting bacteria, namely *Erwinia carotovora* subsp. *carotovora* or *Pseudomonas marginalis* (hence the name of the disease in English: black rot). In cauliflower, invasion of the vascular system can result in taste changes in the florets, making them unmarketable.

Epidemiology. The pathogen persists latently in the vascular system. Infected but asymptomatic plants may therefore produce infected seeds. Another source of infection may be overwintering weedy brassica species or undecomposed plant debris in the soil. The pathogen is spread from plant to plant by rain (especially wind-driven rain), aerosols and insects. A large number of small primary outbreaks are required to infect the entire stand, as rain can spread the bacteria from infected plants over distances of 3 – 5 m.

The pathogen enters the plant both through the hydathodes of the uterine and right lower leaves and at the site of injury to the leaf veins and roots. Once inside the plant, the bacteria colonise the vascular system. If the bacteria penetrate into the flower axes, the risk of seed infection increases. The emergence and development of the disease is favoured by warm, humid weather. At temperatures above 27 – 28 °C, the bacteria spread rapidly through the xylem and produce the extracellular polysaccharide xanthan. This, together with various substances of host origin (degraded cell wall products), clogs the blood vessels. At less favourable temperatures, the pathogen persists in the vascular plexus without visible symptoms. The frequency and intensity of the disease in the stand is usually correlated with the number of rainy days.

Protection. It is necessary to use healthy seed from recognised stands. In threatened areas, it is recommended to check seed samples for the presence of the pathogen (30 000 seeds per lot are needed for analysis). In the past, hot water pickling of seeds (50 °C for 25 – 30 minutes for cabbage, 15 minutes for other brassica species) was used. Germination is rarely impaired. Such combined pickling has been tried by immersing the seeds in a solution of antibiotics (aureomycin, terramycin or streptomycin) at a concentration of 500 $\mu g.1^{-1}$ ml for 2 hours and after rinsing in a 0,5% sodium hypochlorite solution for 30 minutes. Sprays of copper preparations were recommended for treating the stand, but not at the time when the stand is wetted. If irrigation is necessary, sub-irrigation is preferable to spray irrigation. Cabbages are bred for resistance.

7.2 Root vegetables

The most important root vegetables are celery, carrots, parsley and parsnips of the carrot family (Apiaceae) and radishes, turnips and swedes of the brassica family (Brassicaceae).

Leaf spot. The occurrence of leaf spot in carrot vegetables is only sporadic and the harmfulness is insignificant. Leaf spot resulting in defoliation of leaves could result in greater losses in seed crops. Carrot leaf spot is caused by *Xanthomonas hortorum* pv. *carotae*. This disease is more damaging at higher humidity and at temperatures around 25 – 30 °C than at temperatures around 20 °C and in wet conditions. In addition to the leaves, stems, stems and flower parts, and even roots (where it produces symptoms similar to scab) can be affected).

The pathogen is seed-borne. The bacteria are found on the surface of the achenes and penetrate through the stomata into the cotyledons during germination. Seed disinfection can limit one of the important sources of primary infection.

Spotting and canker. On light alkaline soils in dry years, scab, caused by *Streptomyces* spp., appears on the surface of carrot roots. Only rarely does carrot blight caused by *Agrobacterium tumefaciens* occur.

Rot. Soft rot causes the greatest losses in transported and stored root vegetables. Rot starts before harvest, either from the base of the stems or from the top of the root. Carrot roots are an ideal breeding ground for the soft rot pathogen *Erwinia carotovora* subsp. *carotovora*. Stored roots can be completely decomposed within a few days under conditions favourable to the pathogen. On average, storage losses are 3 – 5%, but can rise to 40%. Soft rot also affects celery, parsley and parsnips.

Cavity spot is the name given to a disease of carrots that is manifested by black, round, oval-shaped, sunken spots on the surface of the roots. The periderm is blackened but otherwise undisturbed. In contrast, below the insertion patch, the mesh is spread, and a cavity is formed. As the root grows, the lesions enlarge, the depression deepens, and the periderm breaks. Various bacteria and fungi (e.g., *Pythium* spp.) penetrate the site of the lesion. The ethology of the disease is complex in nature. Poor aeration of the soil, which allows the activity of opportunistic phytopathogenic anaerobic bacteria of the genus *Clostridium* to develop, is involved in the development of the disease.

7.3 Leafy vegetables

Among the many species of leafy vegetables, the most important are: species of the Asteraceae family, such as lettuce, endive and chicory; spinach of the Chenopodiaceae family; Beijing cabbage of the Brassicaceae family; celery and parsley of the Apiaceae family. The useful parts are: leaves (leaf lettuces, endive, spinach, parsley, celeriac, etc.); stems (stalked celery); heads (lettuce, Peking cabbage). Most of the reports on the harmfulness of bacterioses of leafy vegetables concern lettuce. Symptoms of mottling, wilting and rotting of leaves and stems, and corkiness on the root, have been reported.

Spotting, wilting and rotting. In regularly irrigated lettuce stands, dark brown, firm, shiny (as if varnished) and necrotic spots appear on the blades of the inner leaves (symptoms are visible when the outer leaves are removed) and on the stems. The causal agent of the spot is *Pseudomonas cichorii*.

In lettuce, endive and salad chicory, symptoms of wilting and browning of the edges of older leaves (heart leaves remain healthy) are common in humid conditions. Whole blades may be affected. Browning of the vascular bundles can be seen on the

cross-section. The causal agents are *Pseudomonas marginalis* pv. *marginalis* or *Erwinia carotovora* subsp. *carotovora*. Soft rot of spinach leaves is caused by *P. marginalis* pv. *marginalis*.

Xanthomonas axonopodis pv. *vitians* is also a potential danger to lettuce. The disease caused by this pathogen manifests itself in various forms, namely leaf spot, wilting and rotting of leaves and stems. Infected plants often break off near the soil surface. The inside of the stem is initially blue green, later turning brown. Leaf spot is characterised by dark brown to black or olive-coloured watery lesions 1 – 2 mm in size, usually located on the margins of the lower leaves. From there, the lesions spread along the leaf veins, resulting in 'V' shaped spots. Individual black spots also appear, scattered over the entire blade of the paprika.

The pathogen survives on infected plant debris and on the seeds (and probably also inside the seeds), through which the bacteria spread over greater distances. Protection is mainly aimed at selecting resistant or tolerant varieties and disinfecting the seeds (with 0,5% sodium hypochlorite solution for 5 minutes or 1% sodium hypochlorite solution for 5 – 20 minutes) and treating the seedlings by spraying with copper preparations alone or in combination with mancozeb (fungicide).

Corky root becomes a problem when lettuce is grown repeatedly in the same location, with low oxygen content and high soil moisture. The above-ground parts are stunted, the outer leaves turn yellow and wilt, and the heads are poorly formed. The aetiology of the disease has recently been elucidated. The causal agent is the soil bacterium *Sphingomonas suberifaciens*, originally described as *Rhizomonas suberifaciens*.

7.4 Fruiting vegetables

Fruiting vegetables are mainly tomatoes, paprikas (in the cucurbit family) and cucumber, melon and pumpkin (in the squash family). Not only the fleshy fruits are attacked, as might be expected, but also other organs, leaves and stems. The scale of losses caused by bacterioses is considerable, so growers cannot do without protective measures. Also, the total number of known pathogens of bacterial origin is quite high; for cucurbit plants about 12, for squash about 6. Many phytopathogenic bacteria attacking cucurbits are classified as quarantine organisms.

Spotting. On tomato and paprika leaves and fruits, *Xanthomonas vesicatoria* causes blight. Since the 1970s, the formerly insignificant spot caused by *Pseudomonas syringae* pv. *tomato* has been gaining in severity. In European conditions, *Pseudomonas syringae* pv. *lachrymans* and some other pseudomonads are damaging the leaves and fruit of cucumber (*P. marginalis* pv. *marginalis*).

Vascular tissue blockage. In tomatoes, *Clavibacter michiganensis* subsp. *michiganensis* is one of the most feared pathogens. It is a vascular pathogen that causes wilting and leaf dieback, but also causes fruit mottling. *Pseudomonas corrugata*, the causal agent of stem necrosis, causes similar symptoms to *Clavibacter michiganensis* subsp. *michiganensis*, i.e. browning of vascular bundles. Under greenhouse conditions, it is possible that *Ralstonia solanacearum*, which is more commonly found on aubergine plants in tropical and subtropical areas, could be involved in tomato wilt. Unlike in North America, *Erwinia tracheiphila* does not cause cucumber wilt in Europe.

Blockage phloem. The quarantine organism Potato stolbur phytoplasma is found on tomato plants. In the 1950s, stolbur was considered to be an economically important disease in potatoes and tomatoes in southern Moravia and Slovakia, but

nowadays its harmfulness is low. On tomato, stolbur manifests itself by striking changes in all the aerial organs of the infected plant. Leaves that formed before infection begin to yellow from the margin and curl upwards. Leaves produced after infection are smaller and yellowish. The stems are thin and top growth stops. At the point of infection, the stem enlarges due to increased phloem formation. Numerous lateral shoots form, giving the infected plants a bushy appearance. The flower buds are abnormally erect. The sepals remain fully united, and the entire calyx is enlarged, as if inflated (this symptom is referred to as 'big bud' or bigstemmed). If the flowers are already formed at the time of infection, they are also erect, they may be sterile, and the corollas are greenish rather than yellow. The corolla petals of young flowers are completely stunted and green. Fruit development is arrested after infection. The already formed green fruits do not reach normal size, are woody, sour and not normally coloured. The seeds are stunted and do not ripen.

Soft rot of tomato, paprika and pumpkin fruits is caused by *Erwinia carotovora* subsp. *carotovora*, in tomato also by *Pseudomonas marginalis* pv. *marginalis* and *P. aeruginosa*.

Cucumber root mat nematode is an uncommon disease of cucumber that was first recorded in the UK in the 1970s, where it occurred when grown in greenhouse and in water-wave culture on rockwool. The characteristic symptom is the multiplication and recruitment of roots growing upwards. A dense carpet-like tangle of multiplying roots is conspicuous on the surface of the soil (in greenhouse) or rock wool (in hydroponic culture). Occasionally, reduced root production and a greater abundance of curved fruits were observed. The causal agent of the disease is *Agrobacterium tumefaciens* with Ri-plasmid.

7.4.1 Bacterial Speck

Pathogen. *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie. **Host plant.** Only tomato has been cited as a natural host, but artificial infections have also succeeded in inducing blight in potato.

Harmfulness and geographical distribution. Although the disease was described as early as 1933, it was not considered significant until the 1970s. Since the 1970s, reports of its occurrence and increasing harmfulness have been increasing. It occurs wherever tomato is grown, probably because the pathogen is seed-borne. It is particularly damaging to young plants, which may die. Infected plants with symptoms of leaf spot give a lower fruit yield. If fruits are infected, they become unmarketable. The increase in the incidence of the disease since the early 1970s is explained by the introduction of new varieties (hybrids), which are much more susceptible than previously cultivated varieties.

Symptoms. The pathogen attacks all organs of the tomato plant. 1 – 3 mm large, dark to black spots appear on the leaves, which are usually surrounded by a distinctive yellow yard (halo). The spots on the leaf blade may merge to form larger, dark brown necrotic continuous areas, mainly on the margin, or necrotic streaks along the main and secondary veins. Similar symptoms are caused by *P. syringae* pv. *syringae*. Watery, dark brown, irregularly shaped lesions form on stems, flower stalks and leaf stalks. These lesions coalesce to form large necroses involving the epidermal tissues, but do not involve the cambial and vascular tissues (reminiscent of the spots caused by *Alternaria solani* or *Phytophthora infestans*). On the fruit there are small, dark brown to black, slightly raised lesions, round or irregular in shape. They are only superficial; they affect only the epidermal plexuses. The pathogen has the ability to colonise the

vascular plexus, resulting in a slight discolouration (yellowing to browning) of the xylem vessels and severe stunting of the plants. There are indications that the pathogen can infect the roots, resulting in discoloration.

Figure 43 Bacterial speck of tomato (URL 43)

Epidemiology. The pathogen is seed-borne and can survive for up to 20 years. The pv. *tomato* bacteria are found in the rhizosphere and on the leaves of tomato, but also on various non-host plants (wheat, beet, chamomile, stonecrop, starflower, etc.) without causing disease in them. It is believed that pv. *tomato* is primarily a resident of the phyllosphere and rhizosphere of both host and non-host plants, and the pathogenic phase in the life cycle is not necessary for the survival of the bacteria. From the rhizosphere, the bacteria reach the above-ground organs where they survive for a longer period of time in the so-called resident phase without causing any symptoms of damage on the leaves. The population is strongly reduced by temperatures around 32 °C, even when the leaf is covered with a water film. Nevertheless, a certain proportion of the bacterial population persists (especially on trichomes) even during prolonged periods of heat and drought. It is only in cool, wet weather, when the leaves have been wet for 24 hours and are also wounded, that the bacteria multiply, penetrate the host tissues and produce the first symptoms of the disease within 3 – 5 days.

The pathogen penetrates the leaf sheaths through the stomata and holes at the site of the broken trichomes. It enters immature fruits through wounds at the site of the fallen trichomes, originally developed on the testis. The bacteria attack only green, but not blushing and red fruits, which is related to the increase in hydrogen ion concentration (higher acidity) in ripening fruits. The frequency of leaf spots is correlated with the number of spots on the fruit. Optimum temperatures for successful infection are 18 – 25 °C and 100% relative humidity (heavy fog). Drought and cold or hot and dry conditions prevent the emergence and development of an epidemic. Disease development is suppressed when average daily temperatures rise above 21 °C.

Protection. The basic prerequisite for successful protection is to obtain healthy (non-contaminated, non-infected) seed from stands in arid areas not irrigated by spraying. The seeds are soaked in hot water (at 50 °C for 25 min or at 48 °C for 1 hour or at 56 °C for 30 min). This treatment also suppresses the causal agent of bacterial wilt, *Clavibacter michiganensis* subsp. *michiganensis*. However, some varieties are sensitive to hot water seed treatment. Regular sprays of bactericides are recommended during the growing season (copper preparations in combination with maneb, streptomycin abroad), but the efficacy is not always satisfactory, especially if the treatment is delayed. An alternative or complementary measure is the cultivation of resistant varieties.

7.4.2 Bacterial Spot of Tomato and Paprika; Stem Canker

Pathogen. *Xanthomonas vesicatoria* (ex Doidge) Vauterin et al., *X. axonopodis* pv. *vesicatoria* Vauterin et al.; syn. *X. campestris* pv. *vesicatoria* (Doidge) Dye.

Until the 1990s, it was thought that bacterial blight of tomato and paprika was caused by a single pathogen. During the 1990s, it was shown that there are 4 distinct phenotypic groups among the pathogenic xanthomonads for tomato and paprika, of which of practical importance are group A, constituting the distinct pathovar *X. axonopodis* pv. *vesicatoria*, and group B, constituting the species *X. vesicatoria*.

Within groups A and B there are races that are pathogenic to tomato but not to paprika, races that are pathogenic to paprika but not to tomato, and races that are pathogenic to both tomato and paprika. Races are distinguished on the basis of virulence to specific genotypes of paprika and tomato. So far, 9 races on paprika and 3 races on tomato have been described. It can be assumed that their number will increase.

Host plant. paprika, tomato and other bulbous plants, especially weeds.

Harmfulness and geographical distribution. The pathogen can cause considerable damage by attacking leaves and herbs, especially in young seedlings. The most striking damage is to the infected fruits, which cannot be marketed because they are disfigured by corky scarring. Bacterial blight is one of the major diseases of tomato and paprika in the USA, India, Argentina, Sudan, Nigeria, Egypt, and Australia. In our country it damages tomato and paprika, especially in southern Moravia.

Figure 44 Bacterial spot of paprika (URL 44)

Symptoms. The pathogen attacks leaves, herbs and fruits. The leaves have initially watery, later purple-grey spots with a black centre, irregularly shaped, about 3 mm in size. The periphery of the spots is a narrow yellow areola. When heavily infested, the leaves are ragged and often fall off. Infection of the flowering parts usually results in heavy flower drop. When green fruits are infected, initially watery, slightly raised, 3 – 6 mm large spots with a pale green halo appear on the fruit. Later, the halo disappears, and the spots turn brown to black and slightly creep. Their surface is rough, scabrous, the epidermis peels off and curls around the periphery of the spots. Soft rot agents penetrate the fruit through the cracks at the site of the spots. In this case, the fruit rots within a few days.

Epidemiology. The pathogen overwinters on seeds that are contaminated with bacteria during extraction. Of lesser importance is the survival of the bacteria in plant

debris in the soil and in the rhizosphere of non-host plants. It is spread during the growing season by rain, wind and direct contact with plant organs. The bacteria may be present on the surface of tomato leaves during the so-called resident phase, during which they multiply on the leaves but do not produce any disease symptoms. The pathogen penetrates into the leaf sheaths through stomata and wounds, and into the fruit through wounds. A prolonged period of heavy winds, high relative humidity and temperatures of around 24 – 27 °C are necessary for the epidemic to develop. It is important that night-time temperatures are also relatively high. Heavy infestations tend to occur in irrigated plots.

Protection. Tomato seeds are obtained from healthy stands. During the growing season, spray with copper preparations, especially in combination with zineb, maneb or mancozeb (also streptomycin in the USA) at intervals of 2 – 10 days. Rotate host plants in the same plot at intervals of 3 – 4 years. Seeds and seedlings are subject to quarantine inspection.

7.4.3 Tomato Bacterial Canker; Bacterial Canker and Wilt of Tomato

Pathogen. *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al.

Host plant. tomato and wild plants of the genus Solanum, e.g. *Solanum nigrum*.

Harmfulness and geographical distribution. Revenue losses range between 10 – 30%, exceptionally reaching up to 70%. Field crops, but especially greenhouse crops, tend to be affected.

Symptoms. The first noticeable symptom is wilting of individual leaves on one side of the oddly curled leaves, which curl, dry and turn brown (unilateral wilting). The manifestation of the first signs of the disease may differ somewhat between plants grown under field and greenhouse conditions. In field crops, the first symptom is wilting of the leaf tips on the lower leaves, which gradually spreads to the higher leaves. The whole plant gradually withers without any signs of wilting. In greenhouse stands, the first symptom is usually reversible wilting on hot days. White, later brown interveinal lesions can be observed on the leaves. The wilting soon becomes irreversible and the whole plant eventually dries out.

The discoloration (yellowing to browning) of the vascular bundles and surrounding parenchyma is evident on the leaf petiole and bull sections. When the infected byssus is squeezed, yellowish brown bacterial mucilage oozes from the browned tissue. Sometimes there are cavities at the site of the vascular bundles and the accompanying tissue, which extend to the surface of the bullae. The discolouration of the vascular bundles is not visible in the lower part of the bulla and in the roots.

At an advanced stage of the disease, light-coloured longitudinal stripes, later cracks, are sometimes present on the main veins of leaves, leaf stems and byssus, through which bacterial mucilage (exudate) oozes out onto the surface of the plant in wet and warm weather. The pathogen penetrates through the stalk by vascular bundles into the seeds and flesh of the fruit. Small brown cavities sometimes form inside these fruits.

Figure 45 Tomato Bacterial Canker (URL 45)

If the bacterium gets from the conductive weeds through longitudinal cracks on the surface of the plant (not very often), it can secondarily infect the leaves, herbs and fruits and cause mottling. On the surface of the fruit, initially watery whitish, later brown, slightly raised spots with a rough surface appear, surrounded by a light-coloured yard (bird's-eye spots), which often merge flatly. The spots on the fruit are not, however, common in infested stands. Leaf spot occurs in wet weather.

Initially, blister-like spots appear, which turn brown and merge with age. Infected seeds are many times deformed and black. Plants grown from infected seeds may die prematurely. Only a relatively small percentage of infected plants will grow from infected seeds. If systemically infected plants do not die and flower, the flowers and fruit drop. If they do not drop, they are either symptomless or whitish in colour or show a whitish reticulate pattern.

Epidemiology. At greater distances, the pathogen is spread by seeds or latently infected seedlings. The frequency of infected seeds does not generally exceed 1%. The bacteria are localised on the surface of the seed or between the seed and the endosperm. The pathogen may also overwinter in infected plant debris. It survives in free soil for a maximum of 3 – 6 weeks. It spreads over a shorter distance when lateral shoots are grafted, also by nutrient solution in hydroponic cultivation. Secondary dissemination of the pathogen; which enters from the blood vessels to the surface of the plant, is possible by raindrops, especially in strong winds.

The pathogen enters the plant through wounds on the roots and stems, but also through the stomata, lenticels and holes created by breakage of trichomes. The bacterium multiplies in the blood vessels and spreads throughout the plant. The walls of the blood vessels are disturbed, and the pathogen often reaches the accompanying parenchyma. Eventually it also penetrates the phloem. The incubation period of the disease is quite long (2 – 3 weeks or more). The further development of the disease is also slow. From the appearance of the first obvious symptoms until the death of the whole plant, 8 weeks or more may elapse.

High humidity is necessary for the success of the infection. The optimum temperature for disease development is around 25 °C.

Protection. The best protection is the use of disease-free seed. It is recommended to obtain seeds by fermenting the fruits. This procedure, combined with subsequent treatment of the seeds with 0.6% hydrochloric or acetic acid for 1 – 2 hours, kills most of the surface adhering bacteria; a 3% tartaric acid solution eliminates them

completely. Immersion of the seeds in a 0.5% sodium hypochlorite solution for 10 minutes has also proved effective. Warm water treatment is also possible. Disinfect tools and hands (e.g. at the end of each row) when pinching the shoots. If the disease occurs in field crops, the cultivation of host plants should be excluded for 4 – 5 years. If occurring in a greenhouse, the soil substrate should be sterilised, plant debris removed and destroyed. Seeds and seedlings are subject to quarantine inspection.

7.4.4 Angular Leaf Spot and Fruit Spotting of Cucurbits

Pathogen. *Pseudomonas syringae* pv. *lachrymans* (Smith & Bryan) Young, Dye & Wilkie.

Host plant. cucumber, but also pumpkin and melon. Harmfulness and geographical distribution. Commonly found in all growing regions in field crops, less commonly in greenhouses. Early infection results in a demonstrable decrease in yield and fruit quality. It causes the greatest damage in pickling plants.

Symptoms. Watery spots of a round or irregular shape first appear on the uterine petals. On the true leaves there are angular, watery, later brown spots, which subsequently dry up and sometimes fall out; the leaf-mesh is as if torn. When wet, the surface of the spots is covered with droplets of mucilage, when dry, with scales.

The stems and petioles sometimes have watery spots covered with whitish exudate. On the fruit there are initially small, round, watery areas, from which a whitish mucilage exudate when wet, which dries to form a whitish coating (crust) when dry. If the pathogen itself or secondary rotting bacteria penetrate deeper into the flesh, the fruit may rot. The pathogen can also colonise the vascular tissue of the stem and fruit.

Epidemiology. The pathogen is seed-borne and can also overwinter in the plant debris of host plants. During the growing season, it is spread by raindrops, probably also by insects, workers' hands, and tools, especially if the leaves are wetted.

Figure 46 Bacterial leaf spot of cucumber (URL 46)

Protection. We use healthy seeds. In field crops, sometimes satisfactory protection has been achieved by spraying copper preparations. After three years we rotate the host crops, destroying plant residues. In the greenhouse it is necessary to prevent the appearance of a water film on the surface of the plants. In practical breeding

of cucumber for resistance, no significant successes have yet been achieved because of the lack of suitable sources of resistance.

7.5 Onion vegetables

All onion vegetables belong to the Liliaceae family. The spectrum of pathogens for each crop is therefore similar. The focus of attention is on bacteria attacking the useful parts, which are the true (retracting) bulbs (as in the case of kitchen onions, shallots and garlic), the non-retracting false bulbs (in the case of leeks) or the panicle (in the case of chives). The occurrence of bacterioses is most common in kitchen onions, especially under unsuitable conditions during transport and storage (high temperature and humidity).

Scarlet fever is a disease occurring most frequently on leeks, but also on other bulbs, both in seedlings and in seed crops. Young leaves develop watery, later yellow to orange elongated lesions. The infected leaves bend and curl. On the flowering stems there are watery dark green areas which enlarge until they envelop the whole stem. *P. syringae* has been identified in the past as the causal agent of scab. More recently, however, it has been shown to be a host-specialised pathogen, *P. syringae* pv. *porri*. The bacteria are seed-borne, so protection is oriented towards eradication of the pathogen in the seed.

Bacterial rots occur occasionally, especially in onions. Among the spectrum of potential pathogens, bacteria of the genus *Burkholderia* are able to attack onions not only in storage but also in the field, whereas *E. carotovora* subsp. *caratovora* and *P. aeruginosa* are more likely to attack onions only during storage in unsuitable conditions. *B. gladioli* pv. *alliicola* is characterised by the fact that affected onions initially appear healthy on the surface. It is only on cross-cutting that watery and softened bulbs are visible, which later turn brown. In contrast, when infected with *Burkholderia cepacia*, the outer skirts are affected by rotting. Leaf streakiness and onion rot are caused by the polyphagous bacterium *P. viridiflava*. The disease occurs with great intensity, especially after a prolonged period of rainy weather in the first half of the growing season. The source of infection may be weedy species on which the pathogen can epiphytically survive.

8 BACTERIOSES OF AROMATIC CARROT PLANT

Among the group of spice vegetable plants, bacterioses are of economic importance in dill, cumin, fennel and coriander, which are cultivated for their fruits (achenes) containing aromatic essential oils.

On dill, cumin, fennel and coriander plants, bacterial scarlet fever occurs on the leaves and surroundings. The causal bacteria are *Pseudomonas fluorescens*, *Erwinia carotovora* subsp. *carotovora* and *Xanthomonas campestris* pv. *carotae*. They enter the plant at the site of injury by insects or hail. The damage caused by scab is relatively high in years favourable for the spread, development and spread of the disease, as it usually results in a severe reduction in yield and fruit quality.

9 BACTERIOSIS OF STRAWBERRIES

A cultivated species of strawberry (*Fragaria ananassa*), grown in many cultivars for its sweet fruits, hosts two quarantine organisms of bacterial origin.

Bacterial leaf spot, of which *X. fragariae* is the causal agent, causes some reduction in yield, but generally the damage is not great. An exception to this is the losses that can occur when a strawberry plantation is irrigated by spraying. It occurs worldwide, including Europe (France, Greece, Italy, Portugal, Romania, Spain, but more recently also in Switzerland).

Figure 47 Bacterial leaf spot of strawberries (URL 47)

Leaf spots are initially 1 – 4 mm large, watery, angular, bordered by the smallest veins. In the early stage the spots are visible only from the underside of the leaves; they appear translucent when viewed against the light. They gradually enlarge, merge and turn reddish brown in about 2 weeks after infection. They have a glossy appearance and are usually covered with bacterial slime, which turns brown and forms scales when dry. The spots merge most commonly along the primary and secondary veins. The dead mesh is torn, giving the leaves a ragged appearance. In addition to the leaves, the root collar may be infected. In severe infestation, circumscribed watery zones localised to one side of the root collar can be seen on a transverse section through the root collar. Plants wilt or die suddenly, as in the case of *Phytophthora cactorum* oomycete infestation.

The source of infection is bacteria found in the remains of infected leaves and inside the root collar. During the growing season, the bacteria are spread to healthy plants by rain. Inside the leaves, the pathogen penetrates by currents. In addition to the leaves, the flowers may also be infected, but not the fruit. Under favourable conditions for pathogen development, systemic infection and the development of bush lesions may occur. Infection is favoured by high humidity, daytime temperatures around 20 °C and low night-time temperatures.

It is assumed that in central and northern Europe the pathogen would find suitable conditions for development if introduced here. It is spread by infected and contaminated seedlings. Therefore, strawberry plants intended for cultivation, with the exception of seeds, are subject to quarantine inspection.

Witches'-broom occurs only in North America and Japan. Affected plants are stunted, have a bushy appearance, neck branched. The erect erect stems bear small leaves. Fruits usually do not form. Since the affected plants are very conspicuous, they can be easily removed from the stand, keeping the percentage of infected plants low.

The economic importance of the disease in North America is relatively low. Concerns about the possible higher susceptibility of European strawberry cultivars to the pathogen and the possibility of the existence of efficient vectors were crucial for the inclusion of phytoplasma witches'-broom as a quarantine organism in European countries. Strawberry plants are subject to quarantine inspection.

9.1 Minor bacterioses and phytoplasmose

Sporadically, cauliflower strawberry stunting occurs in spring as a variant of stunting caused by *Rhodococcus fascians* with the participation of nematodes. Strawberry has been an infrequently listed host plant of the causal agent of rose scab, *Erwinia amylovora*, in the past. According to the current assessment, strawberry is not one of the hosts of the causal agent of scarlet fever that are of epidemiological or economic importance. There is no evidence of scarlet fever on the strawberry *Fragaria ananassa* x Duch, ev. *Senga Sengana* was found to be affected by phytoplasma greening. The occurrence of the disease is quite rare. Infected clusters are conspicuous by smaller flowers with green crown petals and testes. Leaves are asymmetrical, light green with marginal chlorosis and shorter petioles.

10 BACTERIOSES OF FRUIT TREES

10.1 Pome fruits

Among fruit trees, bacterioses and phytoplasmoses are of relatively high importance in pome and stone fruits, and of less importance in peel fruits.

Crown gall and hairy root. Of the large number of hosts of the causal agents of bacterial canker and hairy root, apple and pear trees grown for fruit production and as ornamentals are among the most susceptible. The disease causes the greatest damage in nurseries, where up to 80% of infected cuttings have been recorded.

Bacterial blister bark of apple-tree. It is a disease resembling scarlet fever caused by the bacterium *Erwinia amylovora*. In blister necrosis, however, the causal agent is *Pseudomonas syringae* pv. *syringae*.

Necrotic symptoms can occur on tree rings, brachyblasts, leaves, shoots, branches and trunk. Pseudomonads necrotic lesions of the bark tissue on branches and trunk differ from *Erwinia* symptoms in that the outer bark blisters, later cracks and detaches. The boundaries between healthy and necrotic tissue are sharp and there is no bacterial slime on the surface of the affected tissue.

Unlike scarlet fever, the development of which is favoured by warmer temperatures, blister bark necrosis can be expected to occur in years when temperatures fall below freezing in the spring. The resulting frost damage to the tissues allows epiphytic pseudomonads to enter the plant.

Bacterial blister spot caused by *P. syringae* pv. *papulans* is considered an economically important disease of Mutsa apple trees in the USA and Canada. The pathogen was recorded in Italy in 1983 and in France in 1997 and 1999. Symptoms are most striking on the fruit. Small, greenish, watery, raised blisters appear at the site of lenticels. Later, the lesions enlarge to 4 – 5 mm and darken. They rarely extend more than 1 – 2 mm into the flesh of the fruit. Up to 80% of the fruit in the orchard may be affected. Only rarely may leaves be infected in addition to the fruit. Necrosis occurs on the underside of the leaves on the midvein; the blade is deformed. It is thought that the pathogen may spread to new areas in the vegetative propagating material.

Bacterial blossom blast of flowers, caused by *P. syringae* pv. *syringae*, can occur especially in pears if night temperatures drop to freezing at flowering time.

Fire blight of rose plants caused by *E. amylovora*. In 1999, data were published on a new pathogen, *Erwinia pyrifolia* Kim et al. 1999, which is closely related to *E. amylovora* and occurs in South Korea. It causes necrotic symptoms on leaves and branches of the Asian pear *Pyrus pyrifolia*. Further data on the host species of the newly described pathogen are lacking so far.

Apple proliferation is one of the most important phytoplasmoses in our country, as it affects almost all varieties and causes a severe reduction in size, weight (up to 63-74 %) and fruit quality. Infected trees tend to be more severely affected by apple powdery mildew than healthy trees.

Figure 48 Bacteria blister spot of apple (URL 48)

Symptoms are visible on the bark (longitudinal striations, reddish-blue colour), leaves (irregularly serrated and smaller), stipulae (abnormally long and up to four per leaf), flowers (flowering is delayed) and fruits (about one-quarter the size). Symptoms on shoots are the most characteristic. The narrow (axillar) buds in the upper third of the shoots grow prematurely. Lateral shoots are thin, erect at an acute angle to the main axis.

Protection against proliferation focuses on preventing the spread of the pathogen through reproductive material (scions, rootstocks) and the use of resistant rootstocks. Research is looking into the possibilities of breeding varieties for resistance. Quarantine inspection focuses on apple plants, excluding seeds.

Pear decline is a disease of phytoplasma origin, causing large economic losses. Affected trees either do not bear fruit or bear a small number of small fruits. Infection may result in slow or rapid premature death of the trees. After removal of the bark at the grafting or grafting site, it is possible to see both a brown line on the inside of the bark at or below the junction and longitudinal grooves and ridges.

Protection is focused on prevention through the selection of non-infected re-production material. Quarantine inspection is aimed at apple and quince plants with the exception of seeds.

10.1.1 Fire blight of rosaceous plants

Pathogen. *Erwinia amylovora* (Burrill) Winslow et al.

Host plant. They include 146 species from 20 genera of the rose family, subfamily Appleaceae. The so-called main hosts are considered to be the following species: *Pyrus*, *Malus*, *Cydonia*, *Eriobotrya*, *Crataegus*, *Cotoneaster*, *Sorbus*, *Pyracantha*, *Chaenomeles*, *Mespilus*, *Amelanchier* and *Stranvaesia*.

Harmfulness and geographical distribution. Scarlet fever is one of the most destructive plant diseases. If the flowers and fruit are infected, the current year's yield is destroyed. Infestation of lethal growth and older shoots reduces yield in subsequent years after infection. If the infection spreads to the skeletal branches, trunk and roots, the life of the whole tree is compromised. Affected plants die prematurely and plantings thin out. Geographical distribution: Great Britain, Poland, the Netherlands, Denmark, Germany, France, Italy, Belgium, Sweden, Norway, Ireland, Greece, Czech Republic, Switzerland, Austria, Hungary, Bulgaria, Serbia, Bosnia and Herzegovina, Croatia, USA, Canada, New Zealand, Egypt, Israel, Cyprus, Turkey, Armenia, Iran.

Symptoms. Infected flowers become watery, then wilt, dry and turn brown to black. The leaves suddenly turn brown to black and curl in a few hours. Infected flowers and leaves are left hanging on the tree. The leaflets become watery, later turn brownish-black, wither and shrivel. Their tops droop and bend hook-like.

Scarlet fever bacteria pass from the flowers, lethal growth and leaves to the older shoots. The bark of the shoots becomes watery, necrotic and reddish-brown, which is best seen in longitudinal sections. The necrotic mesh drops slightly in autumn. The periphery of these necrotic deposits are evident by fine cracks. The pathogen enters the fruit either from the infected fruit-bearers through the pedicels or directly through the lenticels and wounds. A watery spot develops at the site of infection, which later turns brown to black.

A specific symptom of scald is the formation of bacterial slime, which appears on the surface of infected organs in wet and warm weather in the form of sticky, whitish and later browning and solidifying drops, coatings or fine fibrous formations.

Figure 49 Scarlet fever rosaceous plants (URL 49)

Epidemiology. The pathogen overwinters in bark tissues at the edges of necrotic lesions on branches or trunk. In spring, it rises to the surface of plants in the form of slime. At shorter distances (up to 100 m), the dormant bacteria are transported mainly by atmospheric water (especially wind-driven rains), insects, mites and spiders; at medium distances (100 – 5 000 m) by pollinating insects; at long distances (over 5 000 m) by birds, air currents and the activities of man, who is the intermediary for the transfer of infected or contaminated reproductive material (grafts, scions, fruits and whole plants).

Bacteria enter the plant through natural openings, glandular trichomes, hydathodes, nectartoids), non-cutinized stigma and anther webs, bulging anther vesicles, cracks caused by injury (insects, cutting, wind, hail, etc.). Flowers, leaves and non-woody leaf litter are the most common sites of pathogen penetration.

In general, warm and humid weather with heavy rainfall promotes the development of the disease, cool and sunny weather slows it down and very dry weather stops it. The risk of scarlet fever is greatest when temperatures exceed 18 °C (ranging between 18 and 28 – 33 °C) and when it rains. Infection of flowers is very likely on warm sunny days (21 – 30 °C) when insect activity – a potential vector of scarlet fever bacteria – is high.

Protection. Quarantine measures are applied to prevent the introduction of the pathogen into new territories. In areas regularly threatened by scarlet fever epidemics, chemical protection with copper preparations or antibiotics, which are more effective, is carried out. In the USA, streptomycin and oxytetracycline are used. Streptomycin-resistant strains of *E. amylovora* were first reported in 1971. Resistance of the pathogen to tetracycline has not yet been detected. In most European countries, the use of antibiotics in plant protection is not allowed. Biological control uses bacterial strains that are antagonistic to *E. amylovora*, such as *Erwinia herbicola* C9-lS or *Pseudomonas fluorescens* A506 (contained in the US-registered BlightBan A506), which does not produce any antibiotics and acts on the principle of nutrient competition. The effectiveness of biological control is variable, which is probably related to the variability of external conditions, which are not always suitable for the establishment of the bioagent and its secondary dissemination on the surface of plant organs threatened by the pathogen. Pears, apple trees as well as rock and hawthorn trees are bred for resistance. All plant parts, including fruits, except seeds, are subject to quarantine inspection.

10.2 Stone fruit

The economic importance of bacterioses and phytoplasmoses in stone fruits, i.e. peaches, apricots, cherries and sour cherries, is comparable to that of pome fruits or even higher, with the difference that the scale of stone fruit cultivation in the Czech Republic is considerably smaller.

Bacterial crown gall of stone fruits is of economic importance especially in fruit nurseries.

Bacterial cancer is the most serious bacterial disease in stone fruit. Both *Pseudomonas* and *Xanthomonas* pathogens can be the causative agent. The range of symptoms caused by these pathogens is very wide: buds do not grow in spring and, if they do, wilt and die, leaves and fruit are mottled, bark tissue is necrotised, shoots and branches die. Bacterial cancer, resulting in the gradual (chronic) death of branches and whole trees, is of the greatest importance for the life of trees. In addition to bacteria, low temperatures during dormancy contribute to this.

Figure 50 Bacterial crown gall of stone fruits (URL 50)

The presence of phytopathogenic pseudomonads in stone fruit canopies and low temperatures during the growing season are also associated with apoplectic (corpse) dieback of stone fruits. In spring, the whole tree does not flower at all, even though in previous years it looked apparently healthy. If the tree does flower, the flowers, leaves and leaflets suddenly wilt, turn brown, dry and remain hanging on the tree within 4 – 6 weeks after flowering. The sudden wilting and death is a sign of the tree's inability to form new xylem in spring due to damage to the cambial plexuses at the base of the trunk and skeletal branches. The cambium is browned, and the bark can often be easily peeled away from the trunk. The root system appears healthy, but the hair roots are poorly developed and disfigured.

Xylella fastidiosa, the causal agent of peach stunting, is a potential danger, as are phytoplasmas causing peach rosette, X-chorosis and yellows. These pathogens are on the quarantine list.

Phony disease of peach, caused by *Xylella fastidiosa*, is so far mainly known from North America. It is not known to occur in Europe. The disease is manifested by shortening of the internode of young shoots. The leaves are piled on top of each other and are greener than on healthy trees. Lateral branches grow horizontally or are drooping, so that the crown appears solid, compact and round. Leaves and flowers appear earlier in spring and remain on the tree longer than on healthy trees. Infested trees produce progressively fewer fruits and smaller size until they are economically worthless after 3 – 5 years. The strain of *X. fastidiosa* that infects peach is economically less important than the strain found in grapevine.

European stone fruit yellows is a major contributor to the death of stone fruit trees. The causal agent is widespread in a number of European countries, including the Slovak Republic and Slovakia. The most economically important occurrence of the pathogen is on apricots, where it has been shown that affected trees usually die within 1-2 years after the first symptoms appear. It also occurs in peach plantations and sporadically in cherry, cherry and plum orchards.

The first non-specific symptoms can be observed in apricots at flowering time, when infected tree rings show a smaller flower set. Leaf curling and yellowing are typical symptoms. The fruits are generally smaller, ripen prematurely and drop or dry on the tree. The disease is spread by infected reproductive material. Among the insect vectors, *Fieberiella florii* (which, however, has a low incidence in our area) and perhaps also *Cacopsylla pruni*. Protection consists in producing healthy reproductive material, planting healthy nursery cuttings and applying phytoquarantine measures.

Peach rosette disease of peach has been safely demonstrated in the southwestern states of the USA (Texas and others). It occurs sporadically and is currently of little importance. Similar symptoms have been recorded in peaches in Italy. Symptoms are reminiscent of the virulence caused by Peach rosette mosaic virus. Symptoms are most common in peaches but are also seen in almonds and plums. But their shoots have very short internodes. The leaves of the older shoots drop prematurely in autumn, leaving only a tuft of young leaves at the top, while the rest of the shoot is bare. Infected plum trees are severely stunted. The insect vector is unknown, although in nature the disease is spread from wild *Prunus* species (*P. angustifolia*) to peach and plum plantations.

Peach X-disease is so far only found in North America and is of much greater economic importance here (e.g. in cherry and peach) than phytoplasma yellows and phytoplasma rosette disease. The first symptoms on peaches are yellow mottling and leaf curling. Shortly afterwards the whole tree becomes chlorotic, and the leaves drop. Only a tuft of leaves at the top of the shoots remains. Young trees die in 1 – 3 years from the first signs of the disease. Chronically infected older trees may survive for a few years but produce little or no fruit. Infected cherry trees growing on the rootstock die very quickly. The rootstock is resistant, and a hypersensitive reaction occurs at the grafting site. On other rootstocks, cherry dieback is slower. The leaves are smaller and have a red tinge, sometimes with enlarged palisades. The fruit ripens more slowly, has shortened stems and watery, tasteless flesh. The pathogen is spread by several insect species such as *Fieberiella florii*, *Colladonus montatus*, *C. geminatus*, *Scaphytopius acutus*, *Paraphlepsius irrotatus* and other species of this genus and some species of the genus *Osbornellus*.

Peach yellows is known only in North America (USA and Canada). It has not yet occurred in Europe and Asia. In fact, all the cases described so far in Europe and Asia are caused by other phytoplasmas or have a different aetiology. In the USA, it caused enormous damage in the 19th and early 20th centuries. It has now almost disappeared. It is considered to be an effective conservation measure, but it is possible that its occurrence is subject to long-term cyclical changes. The causative agent of phytoplasma yellows is on the quarantine list of most European countries, but the risk of transmission is considered to be much less than that of the causative agent of phytoplasma X-disease. The pathogen is transmitted by the leafhopper *Macropsis trimaculata*.

10.2.1 Bacterial Canker of Stone Fruits

Harmfulness and geographical distribution. Under favourable conditions when the disease develops, dieback of young trees, especially plum trees, often occurs. In other hosts, infection at a young age can strongly affect the shape of the trees. Infection in older trees results in the death of smaller branches, which often does not escape attention. Infection resulting in the death of thicker branches becomes more noticeable. While pv. *syringae* and pv. *morsprunorum* are widespread throughout the world, pv. *persicae* is so far known only in France and New Zealand.

Symptoms. They are visible in the bark tissue of branches and trunk, on leaves and fruits. **Bacterial canker of stone fruits** in cherry, sour cherry, and cherry plum are usually localized on the branches around the fruit-bearing area or in the fork of the tree branches. The necroses are first visible in spring as slightly sunken spots in the bark together with drops of amber-coloured gum on the surface of the branches (especially cherry). Gum formation increases during the growing season. On thicker

branches, the presence of gum is usually the first, more obvious sign of infection.

Dieback and dieback can occur at any time during the growing season, depending on when the necrosis envelops the perimeter of the branch or trunk: buds do not grow in spring; leaves, flowers or young fruit wilt and wither.

The plum, unlike the cherry, is most susceptible to infection of the trunk. Necrosis can spread along the length of the trunk in the form of sunken streaks in the bark. If the entire trunk is affected by necrosis, tree death follows by the end of July at the latest.

The first infection of the lateral leaves appears in cherry after the crown petals have fallen off, in plum a little later. Leaves developing on the leaflets may be infected during the summer. As soon as the leaves mature, they are resistant to further infection. The leaf spots are dark brown, round or almost square, about 2 mm in size. The spots sometimes merge, especially at the edges and apex, to form larger areas of dead foliage. There is sometimes a yellow halo around the spots, especially on plum trees. During the summer, these spots fall out and the leaves become perforated. In warm temperate regions, *Xanthomonas campestris* pv. *pruni*, which attacks plum, cherry and peach trees in particular, can also cause symptoms similar to those of *Pseudomonas syringae* pathovars.

Figure 51 Bacterial canker of stone fruits (URL 51)

Epidemiology. During vegetation, the pathogen is part of the epiphytic population. It overwinters either saprophytically in buds or parasitically inside cortical plexuses. It is spread by wind-driven rain. Leaf spot occurs after penetration of the pathogen through stomata. In autumn, the inoculum is washed by rain onto the surface of fresh leaf scars and soaked inside the vascular plexus by negative pressure in the vascular plexus. The penetration of bacteria into the plant is particularly intense when strong winds in autumn cause premature defoliation. Branches and trunks can be infected through wounds (e.g. mechanical damage and abrasion, animals, frost cracks) or through natural openings such as lenticels.

Similar to other bacterioses, bacterial cortical necrosis is promoted by wet weather. Rain, especially when associated with strong winds, allows the pathogen to spread. The foliar population of the pathogen increases in cool wet weather, in contrast, it is reduced in hot, dry weather. The greatest necrosis occurs during dormancy and in spring when frost temperatures are present. Vigorously growing shoots and branches on trees grown on fertile deep soils appear to be more susceptible than those growing on trees grown on poor or nitrogen-deficient soils. According to some evidence, low potassium levels seem to favour the development of the disease.

Protection. Due to the high cost of antibiotics and the ban on their use in most European countries, only copper preparations (e.g. based on copper oxychloride) are available for protection. They are applied weekly against leaf spot, if necessary, but the risk of phytotoxicity cannot be excluded. Autumn infection through leaf scars can be reduced by applying copper preparations at three-weekly daily intervals throughout leaf fall. Chemical control is particularly useful in cherry trees. Tree trunks should be protected from injury to prevent the pathogen from entering the weeds. Pruning should be avoided during dormancy and should be carried out during the growing season (after buds have grown). In many countries, rootstocks and varieties are being bred for resistance.

10.3 Nut trees

The nuts include the royal walnut (*Juglandaceae*) and the hazelnut (*Betulaceae*). It is common for all the bacterial pathogens of walnut and hazel to attack the buds, leaves, fruits, shoots, branches and trunk. If the bark is infected and necrosis envelops the shoots and branches, the upper parts die.

Bacterial blight of hazel caused by *X. arboricola* pv. *corylina* causes damage in nurseries, young and older plantations, usually in association with abiotic stresses such as frost damage, sun scarlet fever, pruning wounds, etc. To reduce tree mortality in new hazel plantations, adequate irrigation is recommended for the first 2 – 3 years after planting.

Bacterial cancer of hazel can be caused by *P. avellanae* and in walnut by the polyphage *P. syringae* pv. *syringae*. Economically significant losses caused by *P. avellanae* have been reported from Greece and Italy, where thousands of hazel trees die each year.

10.3.1 Bacterial Blight of Walnut

Pathogen. *Xanthomonas arboricola* pv. *juglandis* (Pierce) Vauterin et al.

Host plant. *Juglans regia*, but also other species of the genus *Juglans*.

Symptoms. Numerous necrotic spots on leaves, leaflets, and young fruits. Many fruits drop prematurely. Fruits that reach normal size have more or less blackened skin, shell and core. Necrosis of the cortical tissue sometimes appears on the shoots and branches. Similar symptoms to *X. arboricola* pv. *juglandis* can also be caused by *P. syringae* pv. *syringae* in walnut.

Epidemiology. The pathogen overwinters as an epiphyte on buds and lambs quarters, from which it passes to leaves in spring. From the leaf lesions, the infection spreads to fruits and developing buds and lambs quarters. The bacteria are spread to neighbouring trees by raindrops, insects (e.g. tree nut borer). Another possible source of inoculum for foliar infection is contaminated pollen from infected lambs. Disease development is favoured by rainy weather, especially during and after flowering.

Figure 52 Bacterial Blight of Walnut (URL 52)

Protection. Sprays with copper preparations have good efficacy. In wet years, up to three sprays are needed: before flowering (to reduce the risk of toxicity to very young leaves, an oil emulsion is added); during flowering; three weeks after flowering, and possibly during fruit development. Some varieties are resistant.

11 BACTERIOSES OF GRAPEVINE

Grapevines have several potentially harmful bacterial and phytoplasma pathogens, but in Central European conditions growers are not forced to carry out direct protection against them. In contrast, nurserymen have to pay attention to protection against bacterial blight because its occurrence is a barrier to the sale of reproductive material. The Phytoquarantine Inspectorate is obliged to protect the territory of the Slovak Republic against the introduction of three potentially dangerous agents of grapevine diseases.

11.1 Crown Gall

The causal agent of grapevine crown gall, *Agrobacterium vitis*, was previously reported as *A. tumefaciens* biovar 3. It differs from other species of the genus *Agrobacterium* not only in biochemical but also in pathogenic properties and distinct host species. In contrast to *A. tumefaciens* strains, which are characterised by both tumorigenic and rhizogenic activity, *A. vitis* strains show only tumorigenic activity. While *A. tumefaciens* strains have a very wide host range, including vines, *A. vitis* strains primarily attack vines. Although *A. vitis* is predominant on the vine, *A. tumefaciens* can still be isolated from vines here and there. A striking symptom is tumours on both above and below ground organs of the vine.

Significant damage can be caused by the disease in fruit nurseries. Tumours form at the base of rooted seedlings, at the point of grafting and lateral bud break. Seedlings with tumours are unsaleable. Infestation of symptomless seedlings is also serious. On older shrubs, tumours appear on the stem, but also on annual shoots. Symptoms may not be present in the vineyard for several years unless the conditions are right for the disease to develop. Tumours appear to a greater extent in years following severe winter frosts. The consequences of infestation are not catastrophic and grape production is not compromised. Typical tumours do not occur on the roots of the vines. A less conspicuous and therefore often overlooked symptom is the watery decomposition of the fine hair roots and necrotic lesions on the roots. Systemically infected vine seedlings generally grow less well and the disease may in some cases result in partial or complete death of the bushes.

Figure 53 Crown gall of grapevine (URL 53)

Epidemiology. The survival of the pathogen in the xylem of the vascular bundles of the grapevine is essential in the infectious cycle. The pathogen is thought to overwinter mainly in the root xylem. In spring, the bacteria are then pushed by root pressure into the aerial organs. The bacterium can be detected in the sap flowing at the site of spring pruning of annual shoots. Infected plants often remain symptomless until frost or other mechanical injury gives rise to disease development. Bacteria of the genus *Agrobacterium* are considered to be common soil organisms, but most strains that have been detected in soil have been non-tumorigenic. Strains of *A. vitis* remain tumorigenic in the undecomposed remains of vine weeds. Root infection occurs with the participation of nematodes (*Meloidogyne hapla*). Lesions caused by nematodes on roots are an entry point for agrobacteria.

Protection. Indexing of grapevine plants for the presence of the pathogen is carried out in order to produce disease-free reproductive material. Biological control with the non-tumorigenic *A. radiobacter* strain K84 is not effective against *A. vitis*. However, promising results have been obtained using a non-tumorigenic strain of *A. vitis*. Preventing stamen injury. It is recommended to carry out control of soil nematodes in nurseries. Before grafting, we treat the variety and rootstock material with oxyquinoline sulphate.

11.2 Leaf Spot and Grape Rot

Not only *Pseudomonas syringae* pv. *syringae*, but also *P. viridiflava* can be the causative agent. In addition to small necrotic spots on the leaf blade, *P. syringae* pv. *syringae* can produce longitudinal necrotic lesions on leaf veins, leaf petioles, leaf blades, flower tendrils and flower stalks, which often do not develop into a crack. *P. viridiflava* is involved in grape rot.

11.3 Bacterial Blight/Bacterial Necrosis

The causal agent of grapevine blight, *Xylophilus ampelinus*, was formerly known as *Xanthomonas ampelina*. The pathogen is mainly found in the wine-growing areas around the Mediterranean (Greece, France, Spain, Italy and Turkey) and also in southern Africa, where it causes up to 50% yield loss and bush dieback in susceptible

varieties. Effective protection does not yet exist, and attention is therefore focused on preventing further spread of the disease by applying phytoquarantine measures. Symptoms occur on tree rings, leaves, flowers, and roots. The buds do not bud or are delayed in budding. The tree rings are stunted, weak, chlorotic, with reddish brown stripes or longitudinal scars. Eventually the tree ring wilt and dry. Necrosis and cracks are on the flower stalks or berries. The flowers turn black and die. Root infestation results in stunted growth of above-ground organs. Seedlings and vegetative parts of the vine used for propagation are subject to quarantine inspection, not fruit and seeds.

11.4 Pierce's Disease

The pathogen *Xylella fastidiosa* is found on grapevine (*Vitis vinifera*) and also on the American species *V. labrusca* and *V. riparia*. *X. fastidiosa* is currently associated with many bacteria colonising xylem vasculature in various host plant species. However, strains of *X. fastidiosa* from grapevine are unable to infect peach. Likewise, peach strains do not induce disease in grape vines.

In the USA, in areas of natural outbreak of *X. fastidiosa* (on the Gulf Coast), its harmfulness is so great that susceptible varieties of *V. vinifera* and *V. labrusca* cannot be grown here at all. However, not all vine-growing areas are infested, which is probably related to the presence of vectors and climatic conditions. Potential vectors are all mammalian insects, but cicadas (*Empoaska vitis*) are the most common.

Figure 54 Pierce's Disease (URL 54)

The most characteristic symptom of primary infection is leaf blight, i.e. sudden drying and browning of the marginal part of the leaf blade, while the adjacent leaf blade mesh turns yellow or red. The desiccation spreads to the entire leaf, which curls and falls off, but the petiole remains. The leaflets mature irregularly, and large areas of brown and greenish tissue are visible. In subsequent years after infection, the bush develops stunted chlorotic tree rings. Infected shrubs rarely survive for one or two years.

The vine strain of *Xylella fastidiosa* is on the quarantine list in many European countries, as it is known to have the potential to kill large numbers of bushes and make vine cultivation unprofitable. In Europe, the best conditions for the pathogen to become

established seem to be in the warm Mediterranean area. In 1998, the first report of Pierce's disease of grapevine in Europe was published in Kosovo.

11.5 Grapevine Flavescence Dorée

Several diseases of phytoplasma origin have been described in grapevine, but until their aetiology is clarified, it is more likely to be a complex of phytoplasmas. Symptoms are visible on leaflets, leaves and fruit. The leaflets on susceptible infected shrubs are thin, rubbery (non-woody) and bent downwards. Later, the tree rings become brittle and the apical and lateral buds often necrotic. During the winter the unwoody shoots turn black and die. In the more resistant varieties, the bark at the base of heavily infected shoots is cracked lengthwise. The leaves show colour changes and their edges curl downwards. The parts of the blade that are exposed to the sun turn yellow and the leaf surface has a metallic sheen. Towards the end of vegetation, distinct cream-yellow to reddish spots appear along the veins, several millimetres in size, which later enlarge and form continuous yellow stripes along the veins and gradually spread along the leaf blade. If infection occurs early in the growing season, the inflorescence dries out and falls off. If infected later, the grapes turn brown and shrivel as their pedicels dry out.

The disease is randomly distributed in the vineyards, which is probably related to the way the disease spreads. The vector of the pathogen is the monophagous insect *Scaphoideus titanus*. It was introduced into Europe from North America. The pathogen is on the quarantine list of many countries because there is a risk of spread from outbreaks from France, Italy, Spain, Germany and other.

12 BACTERIOSES OF RASBERRY, BLACKBERRIES, CURRANTS AND GOOSEBERRIES

Red raspberry (*Rubus idaeus*) and European blackberry (*Rubus fruticosus*) of the Rosaceae family, as well as currants and gooseberries (*Ribes* spp.) of the Grossulariaceae family, belonging to the small fruit family, are host to a few that do not usually cause major economic losses.

Cane gall. Both the polyphagous species *A. tumefaciens* and the highly specialised species *A. rubi* may be involved in the development of malignancy in raspberry. Symptoms caused by *A. rubi* appear on fruiting shoots as round or elongated whitish protuberances. These growths enlarge rapidly and may completely cover part of the shoot surface. The growths are most numerous at the base of the shoots but may also appear at the top. Affected shoots crack and dry out. Use of healthy reproductive material is a safeguard.

***Pseudomonas* blight**, caused by the polyphage *P. syringae* pv. *syringae*, occurs in early spring on blackberries in the form of brown watery spots on leaves, leaf petioles and internodes of young shoots. In wet weather the young leaves may turn completely black. On older leaves, the spots increase more slowly, and dieback of the entire leaf does not occur. If bark necrosis affects the entire circumference of the shoots, death usually follows.

Rubus stunt. Infected shrubs produce a greater number of thin and short shoots. Various floral anomalies are frequent; calyx petals are enlarged. Infected plants of susceptible varieties may die prematurely within one year. The disease is spread by rats. Protection is based on the selection of healthy seedlings.

Full blossom was first recorded in 1971 in the former Czechoslovakia on the red currant variety Hougton Castle. The affected bushes were smaller in size and gave a low yield of small berries. In 1998 – 1999, the disease was detected in several places in Bohemia and Moravia on red and white currant varieties, but not on blackcurrants. There are no data on the occurrence of the disease outside Bohemia, Moravia and Slovakia. A phytoplasma aetiology of the disease and spread of the pathogen by reproductive material are assumed.

13 BACTERIOSES OF ORNAMENTAL PLANTS

Due to the large number of ornamental plants, both herbaceous and woody, belonging to a wide variety of families, only the most serious bacterioses in the most commonly cultivated species will be mentioned.

13.1 Herbaceous ornamental plants

Crown gall caused by *Agrobacterium tumefaciens* can occur in most ornamental plants, especially outdoor plants, but the damage caused is usually slight.

Stem **fasciations** and cauliflower tumours on aerial organs in a number of ornamental plants, e.g. in hybrids of *Pelargonium zonale* and *P. grandiflorum* or *chrysanthemum*, *carnation* and *gladiolus*, are caused by *Rhodococcus fascians*.

Leaf spot and wilt, caused by *Xanthomonas hortorwn* pv. *pelargonii* in geraniums and *X. axonopodis* pv. *begoniae* in begonias, are serious diseases in these plants against which special protection is needed.

Bacterial leaf spot of Tagetes species, caused by *Pseudomonas syringae* pv. *tagetis*, occurs only sporadically in years with above-normal rainfall and below-normal temperatures in early summer.

Soft rot and wilt caused by *Erwinia chrysanthemi* occurs in carnations. Similar symptoms are caused by *Burkholderia caryophylli*. The wilting caused by *B. caryophylli* occurs at temperatures of 20 – 25 °C. The longitudinal section of the stem shows brownish-yellow streaks at the vascular bundles and the transverse section shows irregular brownish spots with a watery margin. At lower temperatures, longitudinal cracks at the base of the stems and yellowing of the leaves are more frequent. A brownish-yellow slime appears on the cracks, which is often overgrown by the saprophytic fungus *Cladosporium herbarum*. The presence of bacterial slime is a diagnostic feature which makes it possible to distinguish this bacteriosis from stem cracking of abiotic origin, which occurs at temperatures below 17 °C.

Bacterial blight of *Aracea* plants caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* occurs mainly in tropical and subtropical areas. From European countries it has been found in Italy and the Netherlands on plants of the genus *Anthurium* grown in greenhouses. The pathogen poses the greatest threat to *A. andraenum*, which is the most important cut flower crop worldwide (and the tenth most important agricultural commodity by volume of international trade). It is on the EPPO (European and Mediterranean Plant Protection Organisation) list of quarantine organisms. On plants of the genus *Aglaonema* and *Anthurium*, the disease manifests itself both in the form of leaf spot and in the form of systemic infection. The systemic infection is characterised by yellowing of the vascular bundles, visible on a cut through the petiole. The leaves and flowers are easily broken, with dark brown streaks at the base of the leaves, which gradually enlarge. Eventually the whole plant dies back.

The causal agents of **soft rot and wilt**, *Erwinia chrysanthemi* and *E. carotovora* subsp. *carotovora*, are capable of causing economically serious damage in hyacinth, iris, dahlia, dahlia, chrysanthemum, but also in species of *Aloe*, *Cactus*, *Calla*, *Dieffenbachia*, *Saintpaulia* and others. In carnation, it causes late wilting and stunting. In hyacinths, losses to yellow rot, caused by *Xanthomonas hyacinthi*, have been reduced compared with the past. Hyacinth bulb rot is also caused by *Erwinia rhapontici.*

The most well-known bacteriosis of gladioli is the so-called ***Gladiolus* scab**, which is caused by *Burkholderia gladioli* pv. *gladioli*, but its harmfulness is not high. In areas where tulips are intensively grown outside greenhouses (the Netherlands and the UK), the typical pathogen *Curtobacterium flaccumfaciens* subsp. *oortii*, which causes yellow blister rust, has been found to occur. The pathogen causes major losses here in years with low temperatures, as infected plants do not form flowers.

13.1.1 Bacterial Leaf Spot of Pelargonium

Pathogen. *Xanthomonas hortorum* pv. *pelargonii* (Brown) Vauterin et al.

Host plant. a cross between *Pelargoniwn zonale*, *P. peltatum*, *P. grandiflorum* and probably *Geranium*.

Harmfulness and geographical distribution. Harmful wherever geraniums are grown. The average annual losses caused by the causal agent of spotting and wilting of muscadines, especially in the production of cuttings, are estimated at 10-15 %. The reputation of horticultural enterprises specialising in the production of seedling geraniums, and even their very existence, is rarely independent of the successful management of control of this disease.

Symptoms. The disease occurs both in the form of leaf and stem spot and in the form of vascular wilting and rotting of the base of the stem.

Spots on leaves and stems are initially visible as small, dark green lesions 1 – 2 mm in size, resembling watery blisters (oedema). They gradually develop into brown or brownish-red spots, round or irregular in shape, 2 – 3 mm in diameter. A dried yellowish-grey bacterial ooze is sometimes visible at the site of the spots. The edges of the individual dark brown dry patches are clearly demarcated and rarely merge. Often individual necrotic spots are surrounded by yellow haloes or large sections of yellowed tissue contain several necrotic spots. However, if the plants are in a cooler environment (up to 18 – 21 °C), the development of the disease remains in the watery blister stage for a prolonged period of time (up to 4 months) and only develops further with the arrival of warmer temperatures. Localised leaf spots are formed at the site of the stomata through which the pathogen penetrates inside the plant. They occur when irrigating after the spray, whereas they may not appear at all when irrigating from below. In addition to the round leaf spots distributed all over the blade, yellow, later necrotic 'V' shaped areas appear on the edge of the blade, which are thought to be related to the penetration of the pathogen through the hydathodes. Infection of *P. peltatum* plants is characterised by yellowing, death and dropping of entire leaves without other symptoms common in *P. zonale*.

If bacteria from the leaves or roots penetrate the vascular system, this results in wilting at high temperatures, starting from the edge of the leaves. Affected leaves curl and die, but do not drop. Infestation is sometimes manifested by a change in the shape of the leaf blades, which take the form of umbrellas. The colonised conductive tissue tends to turn brown, but this is not always clearly visible. At an advanced stage of bacterial invasion into the vascular bundles of the infected stem, they turn brown to

black, but usually remain firm and dry. These external stem symptoms do not appear until the bacteria have penetrated from the blood vessels into the parenchymatous cortical tissue of the stem. If secondary phytopathogenic micromycetes, including species of the genera *Pythium* and *Fusarium*, as well as other bacteria, penetrate the plant before the stem dies, the rot then takes on a blackish, moist appearance. It is worth noting that three other bacteria are common causal agents of leaf spot in geraniums (*P. syringae, P. cichori* a *Acidovorax* spp.).

Epidemiology. The main source of infection is latently infected symptomless plants from which cuttings are taken for propagation. If the bacterium is present in vascular bundles, the infection is spread by a knife used to take cuttings from the parent plants. However, there is also evidence of epiphytic survival of the pathogen for 2 months. Rain or spraying spreads the bacteria from leaf spots to hitherto healthy organs of the plant and to neighbouring plants. Transmission of the pathogen by moths (*Trialeurodes* spp.), although possible, is probably not important. The pathogen is able to survive for several months in dry plant debris, 3 months in moist soil, and several months longer in dry soil.

The pathogen penetrates the leaves through stomata and hydathodes, provided that there is a water film on the leaf surface for several hours. The bacteria mainly enter the vascular bundles when the cuttings are taken. Plant invasion can also occur through the roots. The emergence and development of the disease is favoured by higher temperatures (above 21 °C and especially around 27 °C) and slowed down at lower temperatures (10 – 15 °C).

Protection. An effective measure is to prevent transmission of the pathogen during vegetative propagation from mother plants to cuttings. Take cuttings only from mother plants free of infection (developed by several procedures, both simple and more complex, to detect the presence of the pathogen in the plants, called indexing). Preventive protection is by spraying with copper preparations (or streptomycin). However, sprays provide only incomplete protection when pelargoniums are grown outdoors. In greenhouses, leaf and stem blight can be prevented by avoiding the formation of a water film on the surface of the plants. This can be achieved by drip irrigation, temperature control and ventilation to prevent water vapour condensation on leaves and stems.

13.1.2 Yellow Rot of Hyacinth

Pathogen. *Xanthomonas hyacinthi* (ex Wakker) Vauterin et al.

Host plant. *Hyacinthus orientalis, Scilla tubergeniana*.

Harmfulness and geographical distribution. Yellow rot is the most serious disease of hyacinths. The occurrence of the pathogen threatens the prosperity of exporters of mother bulbs in particular. Losses of 20-50 % have been recorded in the Netherlands. In recent years, however, improved protection has succeeded in preventing epidemic outbreaks of the disease.

Symptoms. Plants emerge from weakly infected bulbs, which tend to be free of obvious disease symptoms until flowers develop. At that time the leaf tips begin to turn yellow and yellow stripes appear along the veins on the blades. Likewise, the vascular bundles on the flower stalks turn yellowish. The infection is systemic, but sometimes can affect only one half of the plant. At a more advanced stage of the disease, stunting, wilting, shriveling, or flower drop is evident. A yellow bacterial slime may appear on the leafless and unbranched flowering stem (stem). If spontaneous infections of stems and leaves occur during the growing season (through wounds or hydathodes),

longitudinal, initially watery, later brown streaks appear on the stems and leaves, spreading to the base of the leaf and sometimes extending to the bulb. Symptoms are characteristic on longitudinal sections through the bulb. There are yellow spots in the hypocotyl (shortened axis) and fleshy scales. On the individual scales there are yellow-coloured bulges, inside which are cavities filled with yellow bacterial slime. In the presence of other bacteria, the bulbs rot and smell unpleasantly pungent.

Epidemiology. The source of primary infection is infected bulbs. During the growing season, the bacteria are spread by rain, cultivation tools and knives. They enter the plant through wounds or hydathodes. Humid warm weather favours the development of the disease, dry weather inhibits it.

Protection. Use healthy bulbs for cultivation. Onions suspected of being infected are recommended to be treated with warm air.

13.2 Ornamental tree

Bacterioses in ornamental trees do not usually pose a serious threat to their health under Central European conditions. Data on the prevalence of phytoplasmoses in ornamental trees are scarce. For the purpose of differential diagnosis (differentiation from diseases of other origins), knowledge of the symptoms caused by the bacteria is useful, especially in oleander (*Nerium oleander*), ivy (*Hedera helix*), rose (*Rosa*), lilac (*Syringa*) and forsythia (*Forsythia*). From an epidemiological point of view, ornamental woody plants that host the causal agent of scarlet fever deserve attention.

Agrobacterium tumefaciens-induced **crown gall** occur in ornamental nurseries during propagation of roses and rhododendrons, particularly following wounding. For example, large-scale cankers have been found in rhododendron cuttings wounded on the roots by larvae of *Otiorhynchus sulcatus*.

It turns out that cases of latent infection with the causal agent in rose are relatively common. It has also been shown that a small number of bacteria are able to migrate both basipetally and acropetally, at least up to 25 cm from the site of inoculation. All rose cultivars tested so far were susceptible to infection but differed in the size of the tumours produced.

A new strain, named *A. larrymoorei*, was isolated from tumours on branches of ficus (*Ficus benjamina*).

In greenhouse roses, a so-called **spongy gall** caused by the strain *Erwinia herbicola* pv. *gypsophilae* was detected. Infected plants are stunted.

Olive not, oleander bacterioses on young branches and shoots caused by *Pseudomonas savastanoi* pv. *nerii* is most common in oleander. Infestation of other plants cannot be ruled out (*Forsythia*, *Jasminum*, *Ligustrum*, *Ligustrum*). Tumours up to 2 cm in size usually appear at the mouths of new leaf budding or on leaf scars, or at the site of cutting or rubbing. In oleander, tumours can also be seen on the leaf blade or in the flowers. Disease on the shoots of *Forsythia* is caused by *Rhodococcus fascians*.

Leaf spot and shoot tip dieback in ivy is caused by *Xanthomonas hortorum* pv. *hederae*. The lesions on the leaves are initially oily, later drying out, turning brown and cracking. Symptoms of bacterial spotting are interchangeable with necroses of fungal origin (*Colletotrichum hedericola*, *Phyllosticta hedericola* and *Vermicularis trichella*).

The polyphagous pathogen *Pseudomonas syringae* pv. *syringae* causes both flower and leaf bacterial blossom blight and canker in ornamental species of the genera *Syringa*, *Pyrus*, *Prunus* and *Forsythia*. Damage to the flowers, leaves and leaflets of

lilacs by this pathogen occurs in spring in association with a drop in temperatures to freezing point and rainy weather. If warm weather follows, the development of the disease stops. In outdoor conditions, greater damage can be prevented by preventive spraying with copper preparations with the addition of a wetting agent.

Necrosis of rose leaves and entire leaflets caused by *Pseudomonas syringae* pv. *syringae* is not infrequently confused with symptoms of rose scarlet fever.

Fire blight of rosaceous plants caused by *Erwinia amylovora* is more damaging in ornamental and wild woody plants (mainly hawthorn, to a lesser extent in rock apple) than in commercial species (pears and apple trees). Wild and ornamental host plants are the main source of infection for pear and apple plantations. The following susceptible ornamental trees have not yet been confirmed to have scab: *Amelanchier ovalis*, *Matus floribunda*, *Prunus serrulata* „Kanzan", *Pyrus salicifolius* and *Stranvaesia davidiana*. Resistant host plants include species of the genus *Rosa*, *Matus baccata*, *M. sieboldii*, *M. sub lobota*, *Potentilla* spp., *Spirea* spp., *Aronia melanocarpa* and others.

14 BACTERIOSES OF HOPS

Hops (*Humulus lupulus*), belonging to the Cannabaceae family, is one of the few cultivated plant species in which bacterioses are not of economic importance. Hops are one of the host plants of *Agrobacterium tumefaciens*. Due to the perennial nature of the crop and the vegetative multiplication, the occurrence of phytoplasmoses can be assumed, but no evidence of this has been found so far.

15 BACTERIOSES OF FOREST TREES

Bacterial diseases do not pose a serious threat to forest tree species. In fact, the hosts of phytopathogenic bacteria are predominantly cryptogams. Relatively few hosts are found among conifers, which make up the majority of our forest stands. Among deciduous forest trees, the most frequently attacked species are those of the willow family (Salicaceae), especially poplar. The health of deciduous forest and ornamental trees appears to be compromised by phytoplasma phloem blockage and *Xylella fastidiosa* vascular tissue blockage to a much greater extent than has been observed to date.

Neither **bacterial not and crown gall** causes economically significant damage to forest trees. An exception is the harmfulness of *A. tumefaciens* to poplar hybrids belonging to the Leucaena section (*Populus albumu*, *P. tremula* and *P. tremuloides*). These hybrids are suitable for large-scale planting on hydromorphic forest soils. And it is the progeny of this section that have been shown to be severely damaged by **crown gall** in forest nurseries and poplar plantations. The frequency of crown gall ranged from 3 to 67 %. Since the isolated strains of *A. tumefaciens* proved to be sensitive to *A. radiobacter* K84, the incidence of crown gall in nurseries can be suppressed by biological control.

Bacterial blight, caused by *Erwinia amylovora*, affects, among others, rowan (*Sorbus* spp.). Their susceptibility is much lower than that of hawthorn, cotoneaster, and pome fruit. There is considerable variation in resistance among species of the genus *Sorbus*. *S. intermedia* is the most resistant species and is therefore not subject to the export, transport and import restrictions applied to other species of rowan and other host plants.

Bacterial cancer of poplar caused by *Xanthomonas populi* is considered a serious disease because necrosis of the bark sheaths of the trunk and branches impairs

the quality of the wood, and necrosis surrounding the shoots can lead to premature death of the trees. Typical symptoms are longitudinal cracks with irregular margins from which a whitish sticky slime oozes out in spring. Some authors distinguish two pathovars within *X. populi*: pv. *populi*, infectious to falling species of the genus Populus (poplar); pv. *salicis*, infectious to species of the genus Salix (willow). Based on testing 19 strains of *X. populi* (originating from Belgium, France and the UK) on a set of five clones of poplar and one clone of willow, five races were distinguished that were not reduced in their ability to cause different sizes of bark necrosis on the host clones tested.

The Slovak Republic is one of the countries where the pathogen occurs. Protection is oriented towards the selection of resistant species, varieties and clones.

Leaf scorch disease caused by *Xylella fastidiosa* has only been the subject of research since the late 1980s, particularly in North America. There are indications that the presence of the pathogen in the xylem vasculature (e.g. in maples, elms, oaks and plane trees) is quite abundant and may result in reduced lethal growth and dieback of trees in addition to marginal and interveinal leaf necrosis, which in oaks is associated with the fungus *Armillaria tabescens*, in elms with tracheomycosis caused by *Ceratocystis ulmi*. However, a synergistic effect of bacterial and fungal pathogens on the reduction of water transport through the vasculature cannot be ruled out. Symptoms on leaves may occur on individual branches or throughout the crown. Symptomatological diagnosis is not practically useful because marginal necrosis of leaves is a common symptom of moisture bone stress in deciduous trees.

Elm phloem necrosis is manifested by phloem necrosis only in the highly susceptible species *Ulmus americana*. The most characteristic symptom is yellows. The pathogen is known from North America. The occurrence of the disease is linked to insect vectors of the Cicadellidae family, in particular *Scaphoides luteolus*. Trees infected with phytoplasma are more likely to be affected by the causal agent of elm dieback, the fungus *Ceratocystis ulmi*.

16 BACTERIOSES OF MUSHROOMS

In the case of mushrooms, various colour changes are evident on the cap and head during cultivation and after harvesting, which reduce their market value. The warm and humid environment creates favourable conditions for the development of several mycopathogenic bacteria, which are the causative agents of various spots and slime moulds. It is not uncommon for infestations to appear on mushrooms after they have been harvested, washed and packed. *Fluorescent pseudomonads* are involved in the development of most of the more serious bacterial diseases of mushrooms. Of these, *P. tolaazii* is the most important and is commonly found on mushrooms and in nutrient media. It is phenotypically identical to *P. fluorescens*, a biovar of V. It causes widespread chocolate **brown blotch** on the cap and the mushroom head. The spots are sunken, the surface is sticky to the touch. If high humidity and constant wetting of the hats is avoided, the damage is not great.

The ginger blotch is caused by strains of '*Pseudomonas gingeri*', which phenotypically correspond to *P. fluorescens*, biovar III and V. The disease resembles brown spot but is not as severe. The lesions on the cap are initially pale, yellowish to reddish brown. The spots are not raised. Similar but milder symptoms are caused by strains of *'Pseudomonas reactans'* which are phenotypically identical to *P. fluorescens*, biovar III and I.

A widespread but rarely economically important pathogen is *Pseudomonas agarici*. The disease it causes, drippy gill, is characterised by droplets of exudate secreted from small dark brown watery lesions on both sides and the lower end of the petals. The lesions enlarge and a bacterial ooze usually appears in their centre. In severe infection, the underside of the cap is entirely mucilaginous, and the petals are spread out.

Burkholderia gladioli pv. *agaricicola*, but also other pathovars of *B. gladioli*, are involved in the development of soft rot in *Agaricus bitorquis*, especially at temperatures above 25 °C.

17 BACTERIOSES OF TROPICAL AND SUBTROPICAL PLANTS

The tropical humid climate with little daily and annual temperature variation (with an average daily temperature of 20-28 °C) is particularly favourable for the emergence and development of bacterial plant diseases. The same is true in the humid and monsoon subtropics, where, however, short-term drops in air temperature below freezing are not excluded.

Of the large variety of tropical and subtropical plants, bacterioses will occur in only a few selected economically important crops, namely sugarcane (the most important sugar crop), cotton (the most important textile crop) and banana trees (providing the popular fruit or vegetable banana). A brief mention will also be made of the bacterioses of the avocado. Avocados, stone fruits which are easily perishable after harvesting, are rather exotic fruits in Europe. Their presence on non-European markets has only been made possible by rapid air transport or special shipping (at 5 °C) of special low-oil varieties which are cold-resistant but of inferior quality.

The interest of plant health officials in temperate countries in the health status of tropical and subtropical plants is justified by the fact that plant products imported from the tropics and subtropics may carry a variety of harmful organisms that are capable of degrading or destroying imported plant products during storage, thereby causing economically significant losses. Some of the introduced harmful organisms may also pose a potential hazard to temperate plants.

17.1 Sugarcane

Sugarcane is a monocotyledonous plant of the lichen family (Poaceae), phylum Andropogoneae, genus *Saccharum*. Commercially grown varieties (clones) are complex hybrids of two or more species of the genus *Saccharum*. The cultivated species are: *S. officinarwn* L., *S. barber* Jesewiet, *S. sinense* Roxb., *S. edule* Hassk. These species are relatively high in sugars and have a certain amount of fibre. However, they are susceptible to various diseases and their vitality is less than that of wild species. The species *S. officinarum* is called the noble sugar cane for its succulent stalk with a high sugar content. *S. edule* is not cultivated for its sugar but for its edible inflorescence.

Of the five major bacteria organisms that attack sugarcane, three are specialized pathogens of the xylem portion of the vascular bundles (*X. axonopodis* pv. *vasculorum*, *X. albilineans*, *Clavibacter xyli* subsp. *xyli*), the fourth colonizes the sieve cells (phytoplasma) and the fifth *(Acidovorax avenae* subsp. *avenae)* is primarily a pathogen of leaf parenchyma, but eventually also colonizes conductive tissues. The vegetative reproduction of sugarcane allows the easy spread of bacterial infection and goes some

way to explaining the special position of Sugarcane among monocotyledonous plants in that bacterioses are economically important diseases in this crop. Two bacterioses (gumming disease; gummosis and leaf scald and white stripe) will be discussed in more detail in the following, while the others will only be briefly described.

Ratoon stunting disease. The causal agent of stunting, *Clavibacter xyli* subsp. *xyli*, was described in 1984. Previously, a rickettsia-like organism had been identified as the causal agent. Infected plants show no obvious external symptoms except stunting and stunted growth, which may be confused with disorders caused by low soil moisture or nutrient deficiency. On a longitudinal section of the stem, orange-coloured vascular bundles are visible at the nodes and in the apical part. Under natural conditions, sugarcane is probably the only host.

The disease occurs in all major sugarcane growing areas. In protecting against stunting, the emphasis is on using healthy reproductive material for cultivation. Of secondary importance are resistant clones, although several are available (CP 29-116, C 52-68, CP 63-588, L 60-25, Q 50, Q, 61, Q 95 and especially H60-6909). Resistant are also *S. robustrum* a *S. barberi*.

Red stripe and top rot. The disease agent *Acidovorax avenae* subsp. *avenae* attacks, in addition to sugarcane, the species *Euchlaena mexicana* (teosinte) and the grasses *Paspalum nutans* and *P. paniculatum* (in Mauritius) and also *P. americanum* (in Nigeria) under natural conditions. The disease occurs in almost all growing areas worldwide but causes only sporadic and serious damage. Many sugarcane varieties are susceptible to the pathogen, but many resistant varieties are now available.

In Queensland (Australia), it is recommended to plant sugarcane in the autumn (March and April) rather than in the spring months (August and September). As the disease agent is not a true vascular pathogen, the spread of infection by the tools used for cutting does not pose a serious problem.

17.1.1 Gumming Disease (Gummosis)

Pathogen. *Xanthomonas axonopodis* pv. *vasculorum* (Cobb) Vauterin et al.

Host plant. corn, palm (*Dictyosperma album, Roystonia regia, Areca catechu*), *Thysanolaena maxima* and Guatemalan grass (*Tripsacum fasciculatum*). Other plant species may also be infected under field conditions, but they do not harbour the pathogen for long periods of time and are therefore not important sources of infection for sugarcane.

Harmfulness and geographical distribution. Bacterial gummosis is a serious problem only in the Mascarenes, islands in the Indian Ocean where frequent occurrence of the disease is aided by high winds and rains during cyclones. However, it could also be a serious disease in other areas where weather conditions are conducive to the spread of the pathogen.

Symptoms. The disease occurs both in the form of leaf streakiness and in the form of systemic stem disease. The initial stage of the disease is leaf streak. **Leaf stripe** form along the veins and are 3 – 6 mm wide. Initially, the stripes are yellow orange with reddish areas, which later necrotise and turn grey. They usually start from the edge of the leaf and move towards the base of the leaf. If they start at the wound site in the middle of the leaf, they may spread to both sides of the blade. In developed leaves, the stripes may be almost entirely covered with gum. In resistant varieties, the stripes tend to be less numerous and short. In plants of susceptible varieties, the stripes may be more numerous and longer; systemic infection may occur later in the plant.

Systemic infection is manifested by reddish vascular bundles of the stems, especially at the site of nodules. Inside or within the stem, cavities appear filled with a mass made up of bacteria and their polysaccharide mucilage. The stem grows irregularly, and bacterial slime can be seen on the surface. Bacteria can also penetrate the leaf mesophyll. Dieback of the growth apex of the stem also occurs. Not infrequently, the systemic phase of the disease is manifested by chlorosis of the leaves. However, the pathogen cannot be isolated from chlorotic leaves because chlorosis is caused by a toxin that interferes with chlorophyll synthesis.

Epidemiology. The pathogen is spread mainly by infected stem cuttings, wind-driven rain and tools used for cutting. Less important modes of spread are agricultural implements (shorter distances) and means of transport (longer distances). Leaves tend to become infected in high winds and wind-driven rains. Systemic infection usually follows the cutting of planting material with the use of an un-disinfected tool. Frequent occurrence of cyclones contributes to the spread of the disease and infection. High humidity and higher temperatures are required for disease development. Protection. The most important protection is the use of resistant varieties. Cuttings for new plantings should not be taken from stands where systemic infection occurs. Disinfection of the tools used for cutting is important; this reduces the incidence of systemic infection. It is recommended that planting material of a new important variety which is suspected of being contaminated with the pathogen be treated with hot water (52 °C for 20 minutes) and then treated again with hot water (52 °C for 2 hours) after 24 hours. Using this procedure, although the elimination of the pathogen is not completely guaranteed, the presence of the pathogen is reduced.

17.1.2 Leaf Scald and White Stripe

Pathogen. *Xanthomonas albilineans* (Ashby) Dowson.

Host plant. sugarcane and several species of grasses, namely *Paspalum dilatatum* (on the island of Mauritius), *Brachiaria peligera, Imperata cylindrica* var. *major*, *Paspalum conjugatum* (in QueenslanQueenslandde, Australia), *Panicum maximum, Paspalum* spp., *Pennisetum purpureum, Rottboellia cochinchinensis, Zea mays* and other grasses (in Cameroon).

Harmfulness and geographical distribution. Sporadic outbreaks have been recorded in Australia, India, Java, Fiji, the Philippines, Mauritius, Hawaii, Cameroon, Burkina Faso, Kenya, British Ghana (where the disease appeared in catastrophic form in 1950), Brazil, the Caribbean, including Mexico.

Symptoms. Systemic disease occurs in two forms, chronic and acute. Typical symptoms of the chronic form are white, 1-2 mm wide stripes along the leaves. The chronic form is often visible after the young benefits have emerged. As the leaves age, the stripes broaden to the leaf apices and margins. The infested foliage turns red, withers and the leaves have a scorched appearance. Reddening of the vascular bundles is evident on the fallen stems, especially at the nodes. The acute form is manifested by sudden wilting and death of individual stems or whole plants. It usually occurs after dry weather or after dry weather followed by rain.

Epidemiology. The main source of infection is bacteria-contaminated or latently infected cuttings, possibly also grass host plants. Pathogen is spread by imperfectly disinfected tools used for cutting. Potential vectors include insects and perhaps rodents. During the growing season, wind-driven rain aids the spread of the bacterium and penetration into the plant, and during harvesting, the mowing mechanisms of harvesting machinery also aids the spread of the bacterium. In

Australia, the emergence of the acute form of the disease has been noted to be favoured by dry weather in autumn and low temperatures in winter (below -5 °C).

Protection. We use pathogen-free plant material for cultivation. To eliminate the bacterium in cuttings, it is recommended to let the cuttings soak in water at ambient temperature for 24 hours and then treat with hot water (50 °C) for 3 hours. However, hot water treatment alone is not sufficient to eliminate the pathogen. Disinfect the cutting tools and the cutting mechanisms of the harvesting machines. The source of the inoculum can be significantly reduced if the sugarcane residues are ploughed back in time. We destroy weedy host plants and volunteer sugarcane plants.

17.2 Gossypium

Plants of the cotton plant genus *Gossypium*, Malvaceae, are the most important producers of natural fibre. The four main species cultivated are: *Gossypium hirsutum*, *G. barbadense*, *G. arboretum*, *G. herbaceum*. They are cultivated in tropical and subtropical areas in both herbaceous and arboreal forms, with annual or perennial stems.

The fruit of the cotton plant is a capsule. When the cotton plant is mature, the capsules dry out, burst and a tuft of cotton-like fibres appears. The fibers are formed from the epidermal cell of the cotyledon by the outgrowth of some of the cells into long trichomes, which are almost pure cellulose. Cotton is used to make fabrics and related products. The seeds provide good quality vegetable oils, and the pomace is a protein feed.

Two phytoplasmas have been included among the pathogens of cotton in tropical Africa, but no further information is available.

Crown gall has occurred in Israel in plants of the species *G. hirsutum*, variety Acala SJ2. It was caused by a bacterium *Agrobacterium tumefaciens*, biotype 2, in combination with nematodes of the species *Meloidogyne incognita*. Damage was estimated at 60%.

Boll rot has also been recorded in the USA, but without the participation of nematodes. Capsule rot is caused in California (USA) by the polyphagous bacterium *Erwinia carotovora* subsp. *carotovora*. The disease occurs in humid and warm weather, especially with spray irrigation and windy rains.

17.2.1 Angular Leaf Spot (Black Arm, Boll Ro, Bacterial Blight, Gummosis)

Pathogen. *Xanthomonas axonopodis* pv. *malvacearum* (Smith) Vauterin et al. Using eight differentiating host varieties, 18 races are distinguished. Some races are difficult to define because differences in pathogenicity are not discontinuous.

Host plant. Natural hosts are: cultural species of the genus *Gossypium* (*G. barbadensa, G. herbaceum, G. hirsutum*, *G. populifolium*), weedy plants in Indian cotton fields (*Hibiscus vitifolius* of the family Malvaceae; *Lochnera pusilla* of the family Apocynaceae; *Ceiba pentandra*.

Harmfulness and geographical distribution. The disease occurs in all growing areas, where susceptible varieties suffer considerable damage in years favourable for the development of the disease.

Symptoms. The pathogen can attack plants at any growth stage after the time of black lesions. Necrotic lesions develop on the petals, leaves, stems and capsules. On the wombs, the lesions are round to oblong, initially watery or oily with a dark margin. The affected tissues turn brown and subsequently dry out. Lesions on hypocotyls are

dark and may result in death of seedlings. The leaf stage of the disease has been given the name **angular spot** because the lesions are angular, watery and dark, bordered by leaf veins. However, the lesions may spread along the main veins. Infected leaves usually drop prematurely. On the shoots it causes the pathogen **fire blight**. On the stems of older plants, the lesions take the form of sunken **bacterial cancer** that can girdle the stem and kill the plant. Bark necroses on older stems are called 'cankers' (black arms). The lesions on the capsules are round, watery, dark and woody. If young capsules are affected, they usually fall off. If the fruiting body is infested with bacteria, a **black rot** appears. The fibres in the capsules are moist, brown and rot. A dried whitish bacterial slime can sometimes be seen on the surface of infected leaves, stems and capsules. Of the plant organs, capsules and seeds are the most susceptible.

Epidemiology. From one vegetation to another, the pathogen can survive in infected leaves, stems, branches, capsules and seeds. Seeds are the most important source of infection and serve to transmit it over long and short distances. During the growing season, the bacteria are easily spread by wind, rain, irrigation water and dew, less easily by insects, other animals and tools. The pathogen enters the plant through vents, hydathodes, flowers, but also through wounded netting.

The penetration of the pathogen into the plant is aided by hail and wind carrying grains of sand. Temperatures between 30 and 36 °C and relative humidity above 85% are favourable for the development of the disease. Lower night-time temperatures (20 °C) and high daytime temperatures (36 °C) are conducive to the development of scorch symptoms. The intensity of the disease is greater with smaller plants than with larger plants. In irrigated plots and with higher nitrate fertilisation, especially when ammonia nitrogen is used, the infestation tends to be more severe.

Protection. Many commercial varieties of *G. hirsutum* and *G. barbadens* have low levels of resistance to scarlet fever. In contrast, *G. arboreum* and *G. hierbaceum* species are resistant to scarlet fever to varying degrees. High levels of vertical resistance to scarlet fever are conferred by the resistance genes B3, B4, BS, B7 and BN. Horizontal resistance is conferred by the B2 and B6 genes. The combination of B2 and B3 genes with B6 and B7 genes and other types of vertical resistance and other polygenic modifier genes provides a high level of stable resistance. Currently, resistant varieties are widely used in North America, South America, Africa, and Pakistan.

Crushing the stems and ploughing them into the soil to a depth of 30 cm contributes to reducing the sources of infection inside and on the surface of cotton plant residues. The transmission of bacteria on the surface of the seeds can be minimised by 'acid-ripening' the seeds (removing the fibres using acids such as hydrochloric acid).

17.3 Banana tree

The banana tree (*Musa*) is a vigorous perennial monocotyledonous herb of the banana family (Musaceae), cultivated in almost all tropical and some subtropical humid regions. The sheaths of the giant leaves are rolled into a cylindrical shape and together form an irregular ('apparent') trunk that is 2-6 metres or more tall. Leaves grow from the rhizome with numerous lateral buds from which rhizomes form. Each rhizome fertilises once and dies after the fruit ripens.

The various organs of the banana tree have a variety of uses. The fruits of the vegetable banana (*M. paradisiaca*), fleshy oblong berries, are boiled or baked (vegetable banana – plantain). The fruit of the fruit banana (*M. sapientum*) is mostly consumed as fresh fruit (figo). Since fruit banana trees produce seedless fruit, the plant reproduces vegetatively from rhizomes. Contributing to the greater vulnerability of the

banana tree to bacterial diseases is the fact that commercially grown banana trees are generally irrigated to achieve high yields.

Rhizome rot, caused by *Erwinia carotovora*, is found in all banana-growing regions, but is most damaging in Central America. In Honduras, the incidence of the disease has ranged from 10 – 20% in flooded plots, but in some places has been as high as 50% in the Gross Michel variety. The damage is reflected in the fact that newly planted rhizomes may die without producing shoots. Young plants may be stunted and yellow. Older plants with fruit may be cut down. Dark brown or yellowish watery lesions are visible in the rhizome bark layer. The presence of the insect Cosmopolites sordidus is sometimes associated with the appearance of rot. As a protective measure, it is advisable not to plant the rhizomes during the rainy season. Cavendish varieties are less susceptible than Gross Michel. *Musa* cultivars AAB and ABB are more resistant than AAA cultivars.

Soft rot of pseudostem caused by *Erwinia chrysanthemi* has so far been detected in Colombia and apparently also occurs in Mexico. Infestation of the pseudostem results in its doubling or felling of the plant. There is no information on protection against soft rot of the pseudostem. The bacterium *Pseudomonas* spp. causes relatively small losses, especially in dry weather. The pathogen is usually attached to the flowers and apex of the fruit. Sometimes it penetrates the edible flesh and causes darkening (reddening to blackening). In practice, protection is not carried out. Symptomatic fruits are excreted directly in the field or at packing stations.

17.3.1 Brown rot of banana tree (Moko Disease)

Pathogen. *Ralstonia solanacearum* (Smith) Yabuuchi et al., rasa 2, kmene D, B, SFR a H.

Phylum D attacks *Heliconia* plants, causing stunting and deformation of young plants. It is poorly virulent for banana plants, is characterized by low invasiveness through the flowering bracts and poor survival in soil (less than 6 months). **Phylum B** probably arose from a mutation from strain D. It is highly virulent for banana tree, producing no or very little mucilage on male flower buds. It is characterized by moderate invasiveness to flowering bracts. It survives in the soil for 12-18 months. **Phylum SFR** originates from either *Heliconia* plants or from phylum B. It produces mucilage on male flower buds and is highly virulent to plants of the genus *Bluggoe* (vegetable banana). It is characterized by high invasiveness to flowering bracts. It survives in the soil for 3 – 6 months. **Phylum H** probably originated in Costa Rica by mutation from phylum B. It produces mucilage on the flower buds. It is less virulent to plants of the genus *Bluggoe* than strain SFR. It is avirulent for banana trees. It shows invasiveness to flowering bracts. Survival in soil has not been accurately determined but is apparently only short-lived. On the basis of DNA restriction fragment length polymorphism analysis, it has been suggested that *R. solanacearum* from the banana tree should be considered as a separate pathovar "*musacearuni* ".

Host plants. Race 2 attacks triploid banana tree and species of the genus *Heliconia*.

Harmfulness and geographical distribution. Moko is one of the major diseases of commercially grown banana trees in Central America. In Trinidad, the vegetable banana variety Moko was almost wiped out by this disease and the entire banana industry was destroyed. Over the years, however, effective methods of protection have been developed and less than 1% of the plants in Central America are now dying from the disease. More recently, the disease has been increasing in Peru, in the Amazon basin.

Symptoms. Moko is a systemic disease. If the rhizomes of the banana tree are attacked, the pathogen gradually spreads to all other organs. Infestation may result in the plant not flowering at all and the first four leaves from the apex towards the base turning yellow and brown. If the infection is transmitted by insect vectors to the male flowers, these dry out, rot and turn brown. A black rot is visible on a longitudinal section of the partially ripe fruit. On a longitudinal section of a pseudostem, brown discolouration appears to start in the centre and spread towards the periphery. The outer skin is not affected. A whitish sticky bacterial slime can be seen on the sections through the infected tissue. Browning of the vascular plexuses and wilting is first visible on the youngest leaves. There are also cases of general plant dieback with dieback of the rhizomes at the base of the plant.

Epidemiology. The main source of infection is infected plants from which rhizomes are taken for reproduction. For several months the pathogen survives in the soil, from where it enters the plant through the rhizomes at sites of injury, which may be caused by tools or animals. The bacterium is introduced into male flowers by insects (bees, wasps, various propeller species and others). Movement of bacteria within the plant can be both acropetal and basipetal. The entry of the bacterium into the plant and its spread is favoured by flooding and wet weather.

Protection. If a disease is found in a stand, the possibility of spreading the disease by tools should be reduced. Infected plants and apparently healthy plants within a 5-10 m radius should be destroyed. Eradicate apparently healthy plants first and then the infected plants. Machetes used for fruit harvesting should be disinfected. The land on which the disease has occurred should be left fallow for 18 – 24 months. Host plants of the genus *Heliconia* located close to the plot should be destroyed. Check the affected area every 2 weeks. For field diagnosis, it is a good idea to place pieces of the chaff in a glass of clean water; if the chaff is infected with the pathogen, a string of bacterial slime will ooze out of the chaff.

Figure 55 Moko Disease (URL 55)

In the vegetable banana tree *Bluggoe* (ABB), removal of male inflorescences is proving successful. There are no known sources of resistance in fruit banana trees. However, a single resistant diploid has been found. Resistance has also been found in Pelipita-type vegetable banana, together with resistance to fusarium wilt. The Pelipita type is resistant because the male flowers do not drop and so there is no opportunity for infection. The use of resistant and tolerant materials is expected in the future.

17.4 Avocado

Species *Persea americana* Mill. is cultivated for its large pear-shaped stone fruit, avocado, with a delicious taste, which are valuable for their content of fatty acids, vitamins, and microelements. The leaves and seeds are used medicinally against various parasites and bacteria. The seeds are used in cosmetic products, soaps, creams, and shampoos. Avocado trees normally begin fruiting at the age of six years, sometimes earlier. The largest producers of avocados are Mexico, Indonesia, the USA, the Dominican Republic, Brazil, and Israel.

Bacterial blast, caused by *Pseudomonas syringae*, is the most important bacteriosis of avocados, but does not pose a serious threat to the avocado industry. It is possible that this is a new pathovar of *P. syringae*. The disease is manifested by darkening of the fruit surface and soft rotting of the pericarp (mesocarp). The pathogen is also capable of causing necrosis of the cortical tissues of the trunk.

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