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BACTERIAL DISEASES OF PLANTS: EPIDEMIOLOGY, DIAGNOSTICS AND CONTROL

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Abstract

Of recognized more than 5000 bacterial species, over 100 are the causal agents of plant diseases. They constitute a very important factor limiting growth and cropping of cultivated plants. The pathogenic bacteria are divided into two major groups: eubacteria possessing cell wall and ability to grow on artificial media and bacteria without cell wall but surrounded by cell membrane only. To the last group called mollicutes, formerly known as mycoplasmalike organisms, phytoplasmas and spiroplasmas belong. The latest advances in research on plant bacterioses caused by eubacteria are presented.

Key words: plant pathogenic bacteria, characteristic, systematic, bacterioses, development, diagnosis, control.

Introduction to bacterial diseases of plants

Most of plant pathogenic bacteria are rod shaped; the only exceptions are those belonging to genus *Streptomyces* which are filamentous. The size of single bacterial cell in young cultures ranges from 0.5–1.0 to 1.0–4.0μm. The cell walls of most species are enveloped with slimy substance, which in case of forming the thicker layer is called a capsule. The capsule slimes facilitate surviving of bacteria the unfavorable conditions, e.g. dryness and protecting them against deleterious factors. Most of bacteria produce out of the cell delicate flagella, which are filamentous structures build of proteins. Flagella are significantly longer than bacterial cells; in some species only one flagellum occurs (monotrichous flagellation), in others flagella are located on both ends of cell (lophotrichous), there are also bacteria flagellated at various places on cell (peritrichous). When single cell multiplies on artificial agar media, it produces the colony. The shape and size of colonies of various bacterial species vary and constitute an important feature helping their identification. The colonies can be flat, raised or wrinkled, circular or oval, shiny or mat. The colony colour of most species is whitish or greyish, sometimes yellowish. Some species are able to produce green florescent pigment, visible especially under UV light. The cell wall of gram negative bacteria (coloured red by Gram staining) is built in somehow other way than that of gram positive (stained dark blue). This is a cause of different physiological properties of bacteria belonging to both groups. The space between cell wall and cytoplasm membrane is named periplasm. The periplasm

proteins play protective, nutritional and transportation functions. In cytoplasm nucleoid (chromosome) built of deoxyribonucleic acid (DNA) is located. Moreover, autonomic DNA structures called plasmids are often present. The size of most of plasmids ranges from less than 1% of that of chromosome to several percent of it. In some bacteria, e.g. in *Agrobacterium tumefaciens,* the exceptionally big plasmids can occur, frequently bigger than 10% of chromosome size. The plasmid genes are responsible for various traits of bacteria, like synthesis of toxins and other compounds related to their pathogenicity, as well as the resistance to unfavourable environmental conditions. In cytoplasm, the ribosomes are also present. They constitute centres of protein synthesis and play an important role in bacterial metabolism. Some of gram positive bacteria produce endospores, which help them survive unfavourable conditions / Billing, 1987; Agrios, 1997; Sobiczewski, Schollenberger, 2002; Kryczyński et al., 2002; Janse, 2005/.

Of recognized more than 5000 bacterial species, over 100 are the causal agents of plant diseases /Young et al., 2004; Staley, 2006/. Losses caused by bacterial diseases can be of great economic importance (Table). Some diseases like fire blight of apple and pear or bacterial canker of tomato can devastate the plants on considerably large areas causing production unprofitable for many years /Goto, 1992/. However, in literature there is not much detailed information on such losses. One of the reasons could be relatively low range of chemical preparations used against bacterioses, which cause impossible comparison of crop size in protected and non protected plantations. In the enclosed table an example of losses caused by some bacterial diseases is presented.

Table. Estimated US crop losses due to some bacteria in the USA in 1976 /Kennedy, Alcorn, 1980/

Disease/pathogen	Losses in milion US dolalars
Crown gall (*Agrobacterium tumefaciens*)	23.0
Bacterial ring rot (*Clavibacter michiganensis* subsp. *sepedonicus*)	1.8
Fire blight (*Erwinia amylovora*)	5.0
Bacterial soft rot (*Pectobacterium carotovorum*)	14.0
Brown rott (*Ralstonia solanacearum*)	9.0
Walnut bacterial bligth (*Xanthomonas arboricola* pv. *juglandis*)	2.2
Halo bligth or grease spot of bean (*Pseudomonas syringae* pv. *phaseolicola*)	2.0
Black rot of cabbage (*Xanthomonas campestris* pv. *campestris*)	1.0

Epidemiology of plant bacterial diseases

Plant pathogenic bacteria are heterotrophic organisms, which can develop on host plants as parasites. They are not able to penetrate directly plant tissue. Infection usually occurs through natural openings like stomata, hydatodes, nectaroides as well as injures of various origin. The important places of infection appeared to be leaf scars.

After entering the plant and adapting to new environment, bacteria start to multiply in intercellular spaces following dissemination into various plant tissues. Their further development is conditioned mainly by temperature and humidity. Optimum temperature and high relative humidity favour increase of bacteria population and appearance of disease symptoms as well as their intensity.

Most of plant pathogenic bacteria can survive on plant surfaces also as epiphytes and some as saprotrophes. However, many bacteria infecting above ground organs are not able to survive on dead plant debris. When the infected tissue dies, the number of bacteria rapidly decreases, which leads to their total decline. In such situations, infection source can be drastically reduced, which is very important from practical point of view.

The bacteria infecting above ground part of plants are disseminated by wind, rain, insects, and birds as well as infected plants. Pathogens of root system are spread mainly by soil solution and soil fauna. Important role is played by man, also through management practices. Some bacteria can be disseminated with nursery material, like seeds, scions and seedlings.

Certain bacterial pathogens like *Erwinia amylovora* can survive and multiply on living plant tissue only. On the other hand, pathogens like *Agrobacterium tumefaciens* or *Ralstonia solanacearum* are typical soil organisms. Although their main development occurs in host-plants, they can also survive for a long time in soil. The length of this period depends on abiotic factors like temperature, humidity and availability of nutrients as well as on biotic factors, especially antagonistic bacteria and fungi.

Necroses, blights, cankers, wilting, soft rots and tumours are the main symptoms of bacterioses. Thus, plant pathogenic bacteria can be divided into: necrogens, macergens and oncogens. The range of host plants of particular pathogens varies from very wide – consisting of several hundreds plant species (like *Agrobacterium tumefaciens*) to very narrow, e. g. *Xanthomonas fragariae* – the pathogen of strawberry only. Some species of bacteria are divided into pathovars, the variants differing in range of host plants /Billing, 1987; Klement et al., 1990; Agrios, 1997; Sobiczewski, Schollenberger, 2002/.

Systematics of plant pathogenic bacteria

Traditionally, the taxonomy of bacteria was based on their phenotypic characters (morphological, physiological and biochemical). The principles established earlier for plants were used. The author of the first taxonomic system for bacteria, which was commonly applied for many years, was American scientist D. H. Bergey. His system, successively improved, was presented in consecutive editions of Bergey's Manual of Determinative Bacteriology edited by new generations of bacteriologists. In the last, 9th edition of this manual /Holt et al., 1994/, which was developed mainly for bacteria identification and bacteriological diagnostics, all bacteria were divided, depending on temporary accepted criteria, into 35 groups. The groups were combined into 4 categories: I – Gram negative eubacteria possessing cell wall (Groups 1–16), II – Gram positive eubacteria possessing cell wall (Groups 7–29), III – Bacteria without cell wall (Group 30) and IV – Archebacteria (Groups 31–35). The plant pathogenic bacteria (23 genera) are classified into 7 groups:

– Group 4 (subgroup 4A): Gram-negative aerobic/microaerophilic rods and cocci (genera: *Acidovorax, Agrobacterium, Gluconobacter, Pseudomonas, Rhizobacter, Rhizomonas, Xanthomonas, Xylella* and *Xylophilus*).

– Group 5 (subgroup I): Facultatively anaerobic Gram negative rods, (genera: *Enterobacter, Erwinia, Pantoea* and *Serratia*).

– Group 18: Endospore forming Gram positive rods and cocci (genera *Bacillus* and *Clostridium*).

– Group 20: Irregular, nonsporing Gram positive rods (genera: *Arthrobacter, Clavibacter* and *Curtobacterium*).

– Group 22 (subgruop 1) Nocardioform actinomycetes (genera *Nocardia* and *Rhodococcus*).

– Group 25: Streptomyces and related bacteria (genus *Streptomyces*).

– Group 30: The Mycoplasmas (or Mollicutes): cell wall less bacteria.

The newest systematics of bacteria is worked out on the basis of DNA relatedness and reflects their phylogenic relationship. Of the existing systems, the systematics accepted by American Society of Microbiologists and presented in "Bergey's Manual of Systematic Bacteriology" is most commonly used /Boone et al., 2005/. According to this systematics, the bacteria (*Bacteria*) belong to one of two domains, which consist of all prokaryotic microorganisms (the second domain is *Archea*). The domains are divided into phyla, classes, orders, families, genera and species. Plant pathogenic bacteria are placed in 3 phyla: *Proteobacteria, Firmicutes* and *Actinobacteria*.

- In phylum *Proteobacteria,* plant pathogens are grouped in 3 classes: *Alfa, Beta* and *Gamma*. In class *Alfa,* 4 orders contain pathogens: *Rhizobiales* with genera *Agrobacterium* and *Rhizobium*, *Rhodospirillales* with *Gluconobacter* and *Acetobacter, Sphingmonadales* with genus *Rhizomonas* and *Rickettiales* with genus *Rikettsia*.
  In class *Beta,* only order *Burkholderiales* contains plant pathogens of genera: *Acidovorax, Ralstonia, Burkholderia* and *Xylophilus*.
  In class *Gamma* there are 3 orders of bacteria-inducing diseases: *Pseudomonadales* with genera *Pseudomonas* and *Rhizobacter, Xanthomonadales* with genera *Xanthomonas* and *Xylella* and *Enterobacteriales* with: *Erwinia, Pantoea, Enterobacter, Pectobacterium, Serratia* and *Brenneria*.
- In phylum *Firmicutes,* class *Mollicutes* is placed with orders *Entomoplasmatales* with genus *Spiroplasma* and *Acholeplasmatales* with genus *Candidatus* Phytoplasma, as well classes: *Bacilli* with genus *Bacillus* and *Clostridia* with *Clostridium*.
- In phylum *Actinobacteria*, class *Actinobacteria,* in order *Actinomycetales* suborders are distinguished: *Actinomycineae* with genus *Actinomyces*, *Micrococcineae* with genera: *Clavibacter, Curtobacterium*, *Arthrobacter* and *Rathayibacter* and *Streptomycineae* with genus *Streptomyces* and *Corynebacterineae* with genera *Rhodococcus* and *Nocardia*.

Diagnosis of plant bacterial diseases

Determination of the nature and cause of the diseases can be based on the presence of typical symptoms associated with etiological signs. However, very often the symptoms can be similar to those caused by other factors, both biotic and even abiotic. Therefore, etiological studies must be performed. They consist of application of conventional and/or serological as well as molecular methods. In most cases, the isolation of suspected causal agent is necessary. This can be done using selective, semiselective or standard bacteriological media. Isolated bacteria are then subjected to various tests allowing their identification. The pathogenicity of studied isolates should be proved using rules proposed by Robert Koch.

For identification of many plant pathogenic bacteria, serological methods like agglutination and precipitation tests, immunofluorescence (IF) and enzyme-linked immunosorbent assay (ELISA) can be very useful. In recent years, methods based on DNA analyses, including DNA hybridization and polymerase chain reaction (PCR), have become increasingly important. The main advantages of these methods are high sensitivity and selectivity as well as relatively short time of performance /Schollenberger, 1984; Klement et al., 1990; Schaad et al., 2001; Pulawska, Sobiczewski, 2002; Janse, 2005; Pulawska, Sobiczewski, 2005/.

Prevention and control of bacterial pathogens and diseases

The control of bacterial diseases is one of the most difficult problems of plant growing. It consists of various integrated activities aiming at the elimination of disease sources, protection of plants against infection and decreasing of plants susceptibility. Of chemical preparations, only copper compounds and antibiotics are listed in the assortment. However, in the EU the use of the mentioned group is prohibited. Therefore, other methods including biological, based on beneficial bacteria and yeasts are more common.

Basic principles

- The selection of method against particular disease should be based on wide knowledge of specific characters of causal agent, particularly of its pathogenic abilities and possibilities of survival on plant surfaces, in plant tissues and out of host plants.
- The protective activities should be concentrated on the localization and elimination of primary inoculum source.
- If possible, the eradication (destroying or removing the pathogen or eliminating the plant carrying the pathogen) should be applied.
- The seed and nursery material production should be concentrated on areas free of quarantine diseases (monitoring, inspections, seed and nursery material indexation is necessary).
- In regions where economically important diseases are endemic the cultivars resistant or tolerant to diseases should be planted.

- For plant protection the chemical preparations (besides copper-based, in some countries antibiotics are allowed in very special regulations) as well as biopreparations should be applied.
- To reduce plant susceptibility, proper soil and plant management (plant rotation, proper fertilisation, irrigation etc.) should be applied.

One of the best methods to protect plants against bacterioses is growing of resistant cultivars. In various research centres of the world, programs directed for breeding of cultivars with increased resistance to diseases are conducted. The conventional and genetic engineering methods are used in such programs. There are some examples where resistance to pathogenic bacteria was successfully incorporated into commercial crops (*Clavibacter michiganensis* subsp. *nebrascensis* and corn, *Pseudomonas syringae* pv. *glycinea* and soybean, *Pseudomonas syringae* pv. *pisi* and peas, *Pseudomonas syringae* pv. *tabaci* and tobacco, *Erwinia amylovora* and apple, *Xanthomonas vesicatoria* and tomato. *Xanthomonas campestris* pv. *campestris* and cabbage). Another strategy to enhance resistance in plants is using the chemical substances that activate systemic acquired resistance (SAR) /Janse, 2005/.

In recent years, there are also examples of biopreparations developed against some infective diseases. For control of crown gall (*Agrobacterium tumefaciens*) in commercial products Galltrol, Norbac 84 C, Polagrocyna the *Agrobacterium radiobacter* strain K84 or its genetically engineered form K1026 (Nogall) are used. Also, against fire blight (*Erwinia amylovora*) some bioproducts are registered: Sonata (*Bacillus pumilus* QST2808), Biopro (*B. subtilis* BD170), Serenade (*B. subtilis* QST713), BS-F4 (*B. subtilis* BS-F3), Blightban C9-1 (*Pantoea agglomerans* C9-1), Bloomtime (*P. agglomerans* D325), BlossomBless (*P. agglomerans* P10c), PomaVita (*P. agglomerans* P 10c), Blightban (*Pseudomonas fluroescens* A506) /Sobiczewski, Schollenberger, 2002; Sobiczewski et al., 2007/.

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REFERENCES

1. Agrios G. N. Plant Pathology. – Academic Press, 1997. – 635 p.

2. Billing E. Bacteria as Plant Pathogens. Aspects of Microbiology 14. / ed. Van Nostrand Reinhold. – Wokingham (UK), 1987. – 79 p.

3. Boone D. R., Castenholz R. W., Brenner D. J. et al. Bergey's Manual of Systematic Bacteriology: The Proteobacteria. – Springer, New York, 2005, vol. 2. – 1106 p.

4. Goto M. Fundamentals of bacterial plant pathology. – San Diego: Academic Press, 1992. – 342 p.

5. Holt J. G., Krieg N. R., Sneath P. H. A. et al. Bergey's manual of determinative bacteriology / 9th ed. – Baltimore: Williams and Wilkins, 1994. – 787 p.

6. Janse J. D. Phytobacteriology, principles and practice. – Wallingford: CABI Publishing, 2005. – 360 p.

7. Kennedy B. W., Alcorn S. M. Estimates of US crop losses to prokaryote plant pathogens // Plant Disease. – 1980, vol. 64, p. 674–676

8. Klement Z., Rudolph K., Sands D. C. Methods in phytobacteriology. – Akademiai Kiado, Budapest, 1990. – 568 p.

9. Kryczyński S., Mańka M., Sobiczewski P. Słownik fitopatologiczny (Phytopathological Dictionary, *in Polish*). – Warszawa: Hortpress, 2002. – 179 p.

10. Puławska J., Sobiczewski P. Detection of *Erwinia amylovora* in and on apple tissue using PCR // Acta Hort. – 2002, vol. 590, p. 163–166

11. Puławska J., Sobiczewski P. Development of semi-nested PCR based method for detection of tumorigenic *Agrobacterium* in soil // Journal of Applied Microbiology. – 2005, vol. 98, p. 710–721

12. Schaad N. W., Jones J. B., Chun W. Laboratory guide for identification of plant pathogenic bacteria. – St. Paul: APS Press, 2001. – 373 p.

13. Schollenberger M. Materiały do zajęć specjalizacyjnych z fitopatologii, część II: Zasady identyfikacji bakterii patogenicznych dla roślin. (The principles of plant pathogenic bacteria identification – manual for students, *in Polish*). – Wydawnictwo SGGW-AR, 1984. – 64 p.

14. Sobiczewski P., Schollenberger M. Bakteryjne choroby roślin ogrodniczych. Podręcznik dla studentów. (Bacterial diseases of horticultural plants - manual for students, *in Polish*). – PWRiL, Warszawa, 2002, p. 156-187

15. Sobiczewski P., Mikiciński A., Berczyński S. Biologiczna ochrona jabłoni i grusz przed zarazą ogniową *Erwinia amylovora* – szanse i ograniczenia. (Biological control of fire blight ona apple and pear – chance and limitations, *In Polish, English summary*) // Progress in Plant Protection (Postępy w Ochronie Roślin). – 2007, vol.47 (2), p. 330–337

16. Staley J. T. The bacterial species dilema and the genomic-phylogenetic species concept // Phil.Trans. R. Soc. B. – 2006, vol. 361, p. 1899–1909

17. Young J. M., Bull C. T., DeBoer S. H. et al. Names of plant pathogenic bacteria published since 1995 // Report of the Taxonomy of Bacterial Plant Pathogens Committee of the International Society of Plant Pathology, 2004. Available from: http://www.isppweb.org/names_bacterial_new2004.asp.