СІЛЬСЬКОГОСПОДАРСЬКІ НАУКИ

УДК 630*436:630*17(477) **DOI:** 10.31521/2313-092X/2019-2(102)-4

PHYTOPATHOGENIC BACTERIA IN THE PATHOLOGY OF FOREST TREES OF POLYSSYA AND FOREST-STEPPE OF UKRAINE

A.F. Goychuk, Doctor of Agricultural Sciences, professor
National University of Life and Environmental Sciences of Ukraine
V. F. Drozda, Doctor of Agricultural Sciences, professor
Ukrainian laboratory of quality and safety of AIC production
I.M. Kulbanska, PhD in Biological Science, Senior Lecturer
National University of Life and Environmental Sciences of Ukraine
M.V. Shvets, PhD in Biological Science, Senior Lecturer
Zhytomyr National Agroecological University

The results of many years of experimental research of bacterial pathology of forest trees in Ukraine are presented. It is emphasized that on forest trees in the world several dozens of bacteriosis with different degrees of damage caused by bacteria of genera Pseudomonas, Xanthomonas, Enterobacter, Erwinia, Agrobacterium, Brenneria, Xylella, Rhizobium, Corynebacterium, Bacillus, Clostridium etc. are described. It is emphasized the need to deepen the research of automicrobiota, in particular its pathogenic components in the context of understanding both general biological problems of the pathology, and in order to work out forest protection measures.

Keywords: *bacterial diseases, phytopathogenic bacteria, etiology, symptomatology, pathogenesis, antagonism, systemic interaction, Pinus sylvestris, Quercus robur, Fraxinus excelsior, Betula pendula.*

Relevance. Currently, in modern scientific and educational phytopathological literature, diseases of forest trees and stands are associated with their participation mainly with an external infection. Experimental studies of recent years of epiphytic and especially endophytic auto- and microbiota (mico- and microbiota of healthy plants and their organs) including its phytopathogenic components, indicate a potential powerful endogenous vector in the emergence of pathologies, often epiphytotic, associated specifically with the so-called vital obligates [7, 9]. It is shown that pathogens, in particular, FB, in tissues of healthy plants «…6-8 orders of magnitude smaller and always less than the threshold concentration required to start the infectious process. But for bacteria in general, it's not so much the quantity as the presence: under favorable conditions (in particular, the disturbance of metabolic processes in plants, which underlie any pathological process (author's note), they can quickly colonize the ecological niche to the potential for them concentration - 1010 CFU×g-1, [7, p. 6]. We can clearly observe a similar pathology caused by endophytes of automico- and microbiota, with the mass death of forest trees.

In conditions of increasing the impact of adverse as abiotic, in particular, synoptic, and biotic, including and parasitic, factors on forest biocenosis is extremely important timely assessment (diagnosis, monitoring) of the state of forests. It is these aspects that are devoted to our research.

Analysis of recent research. Currently, bacteriosis and their pathogens are largely studied for plants of agrocenosis. As for the bacteriosis of forest trees, they have not been studied sufficiently, although in recent decades increased attention has been paid to this problem [4, 9, 13, 15, 16]. It is emphasized that on forest trees in the world several dozens of bacteriosis with different degrees of damage caused by bacteria of genera Pseudomonas, Xanthomonas, Enterobacter, Erwinia, Agrobacterium, Brenneria, Xylella, Rhizobium, Corynebacterium, Bacillus, Clostridium etc. are described [4]. Bacteriosis equally affects forest trees in natural forest stands, forest crops, field bands, urban, park and forest park stands, and so on. Currently, those or other bacteriosis are known in almost all forest trees.

The aim of the study – to study the etiology, symptomatology and pathogenesis of bacteriosis of

forest tree plants and their exciters in the stands of Polissya and Forest-steppe Ukraine in combination with harmful insects as a vector in the spread of infectious agents, in particular phytopathogenic bacteria.

Materials and methods of research. The general scheme of research on bacterial diseases of forest trees, in particular, Pinus sylvestris, Quercus robur, Fraxinus excelsior, Betula pendula, etc., included the following stages: reconnaissance and detailed forest-pathological surveys according to generally accepted forestry and phytopathological methods; selection of affected organs and tissues; isolation of myco- and microorganisms in pure culture; testing of pathogenic properties of isolated samples and their identification; research of antagonistic relations in the system "bacteria-bacteria", "bacteria-fungus" as possible factors of induction of demutation processes in forest biocenosis. In addition, the influence of meteorological (synoptic) factors as pathological catalysts and the harmful entomofauna in the context of the trophic connections between insects and phytopathogenic microorganisms and as a vector in the spread of bacteriosis were investigated.

The number of microorganisms, depending on functional and other characteristics, was tested on their growth in special nutritional media (PA, MPA, MPB, malt extract of agar, Capek's medium and others like that). Pathogenic properties of isolates were detected by artificial infection of vegetative and generative organs of experimental and indicator plants (Phaseolus vulgaris L., Nicotiana tabacum L., Kalanchoe laciniata L.) bacterial suspension with a titre of 108–109 C×ml-1 (according to the standard of turbidity) in vitro and in vivo. Control – sterile water. The anatomic-morphological and physiological and biochemical properties of the isolates were studied using appropriate methods and techniques [1, 10].

The relationship between microorganisms has been studied for deferred antagonism. Test cultures were standard set of strains of pathogenic bacteria collection Department of phytopathogenic bacteria of the Institute of Microbiology and Virology D. K. Zabolotniy of the National Academy of Sciences of Ukraine, in particular Pseudomonas syringae 8511, Pseudomonas savastanoi 9174, Pseudomonas fluorescens 8573, Erwinia carotovora 8982, Xantomonas campestris 8003 б, as well as isolated from vegetative and generative organs of tree plants.

The names of types of bacterial and fungi isolates are given by determinants [2] and other special literature [3].

Results of the research and their discussion. The massive death of forests, both in the world and in Ukraine, has been known since the 19th century. Currently, epiphytotic dying of coniferous trees within their range, in particular pine, which causes significant environmental and economic losses, is a matter of serious concern.

The first of disease focus in pine forests were recorded in Ukraine in 2011 in the Zhytomyr region, and by 2015 the "biological fire" (Fig. 1.) has spread in the northwest direction. In 2017, pine death was detected in the central regions of Ukraine, and by spring survey in 2018 disease in the forest-steppe were also installed.

At present, the centers of massive death of pine trees only in the forests subordinated to the Forest Resources Agency, make up more than 400 thousand hectares and cover Volyn, Zhytomyr, Kyiv, Lviv, Rivne, Khmelnytsky, Cherkasy, Chernihiv regions and continue to spread [6].

Fig. 1. Disease focus in pine forests

On the Scots pine were detected bacterial diseases such as cancerous-ulcer disease (causative agent – Pseudomonas syringae), bacterial burn (causative agent – Erwinia amylovora), bacterial dropsy (causative agent – Erwinia nimipressuralis), bacterial ulcers (causative agent – Erwinia quercina pv. rubrifaciens), bacterial root cancers (causative agent – Agrobacterium tumefaciens), tumorous bacteriosis (causative agent – Agrobacterium tumefaciens), black bacteriosis (causative agent – Pseudomonas fluorescens) and vascular bacteriosis of seedlings (causative agent – Raistonia solanacearum) [9].

From organs of externally healthy Scots pine, including seeds, we isolated real FB-endophytes (P. syringae, P. carotovorum, E. nimipressuralis), which caused Pinus sylvestis disease in an artificial infection, and conditional phytopathogenic (P. fluorescens, P polymyxa, P. agglomerans) and saprotrophic (Bacillus subtilis, B. pumilus) bacteria, as well as various systematic and functional groups of mycosomes, including anamorphs of the genus Ceratocystis sp. (known as pathogens sinensis) as

components of the deep pathology of this valuable forest tree plant.

The catalytic factor in the epiphytotic dying of forest trees, in particular pine trees in Ukraine, is the so-called hydrothermal stress (there are reasons to believe that in other countries as well). For example, during the last decade (for example, Zhytomyr Polissya), in the period of vegetation in the Polissya region of Ukraine, the precipitation of 44 to 98 mm was normal for a region of an annual rainfall amounting to 491-625 mm. At the same time there is a steady decrease in the amount of rainfall during the growing season in recent years (chart 1.).

Table 1

Synoptic indicators of the region of research

Year	Average annual temperature / during the growing season, °C	Annual amount of precipitation / during the growing season, mm	Humidity Index / during the growing season
2007	7,8 / 20,8	552 / 65	3,1/ 2,1
2008	7,1 / 19,3	568 / 77	3,4 / 2,6
2009	7,4 / 20,1	550 / 79	3,2 / 2,6
2010	6,8 / 18,9	625 / 92	3,7 / 3,2
2011	7,1 / 19,0	587 / 73	3,4 / 2,5
2012	7,0 / 21,1	560 / 59	3,3 / 1,9
2013	7,6 / 19,4	562 / 51	3,2 / 1,7
2014	8,3 / 20,5	510 / 46	2,8 / 1,5
2015	8,9 / 21,2	491 / 44	2,6 / 1,4
2016	8,7/ 20,9	524 / 38	2,8/ 1,2

It should be noted that in the given region, the birch began to drying first (2009), and then pine (2011), which closely correlates with the index of water supply, whose average varied within the range of 2.6-3.4, and for the growing season from 2012 to 2016 – within the range of 1.2-1.9. It was during this period that there was a rapid increase in focus of the disease of both pine and birch stands.

Due to the extraordinary intensity of the pathological process, we have reason to assert that the epiphytotic death of the pine, causing the two most harmful bacteriosis on the pine – bacterial burns (usually spreading to the upper part of the crown and leading to its rapid dying) and bacterial dropsy (in the lower part of the trunk) associated with the activation of FB-endophytes under the influence of adverse synoptic (as catalysts) factors.

In all cases, harmful insects massively populate the weakened trees, accelerating their death and acting as vectors of the spread of infection in such trees, but are not factors of primary pathology. In any case, the presence of xylotrophic insects in the pine tree trunk, as well as other tree plants with systemic disorders of metabolic processes under the influence of various adverse abiotic and biotic factors, indicates a profound non-inverse pathology of the tree, which always ends with their death.

In recent decades there has been epiphytotic dieback of the birch associated with vascular parenchymal bacteriosis (Fig. 2.), which has different names (bacterial dropsy, bacterial wet cancer, brown slime, watermark disease, "crying" of birch, "wetwood", "Slime flux", "alcoholic flux", etc. (Fig. 3.), but a common etiology and athogenesis.

Fig. 2. Disease focus in birch forests

The causative agent of the bacterial dropsy of the birch in Ukraine is the polybiotroph Enterobacter nimipressuralis, the pathogenicity of which is proved in the experiment both during spring and autumn inoculation of experimental plants.

In the pathology of bacterial dropsy, in addition to E. nimipressuralis, have been identified Xanthomonas campestris, Pantoea agglomerans associated with disease (showed variable pathogenic properties indicating a possible extension of the range of nutritional plants for the mentioned bacteria), Bacillus subtilis and micromycetes from

the Zygomycota, Ascomicota, Deuteromycota divisions.

Fig. 3. Diagnostic sign of the disease "bacterial dropsy"

The mass death of Fraxinus excelsior was first registered in the early 1990's in northeastern Poland and Lithuania (according to recent data, more than 30 thousand hectares, or 60 % of the total area of the forest stands, are now affected of disease). Then the disease spread to the north to Latvia and Estonia. Currently, death Fraxinus excelsior occurs in 30 European countries, in particular Germany, Sweden, the Czech Republic, Slovakia, Finland, Denmark, France, Italy, and others [5]. Programs of countries where signs of ash dying are revealed, aimed at identifying the origin of the pathogen, assessing its impact on forests, developing methods for diagnostics and forest management in affected disease forests, including in the direction of selection of ash for resistance to pathogens.

Currently, in European countries, mass death of ash is associated with the anamorphic fungus Chalara fraxinea, an anamorphous Hymenosyphus pseudoalbidus [11], causing the pathology of the so-called «ash dieback». We have isolated several species of anamorphic fungi and bacteria from such lesions in the conditions of Podillya of Ukraine, in particular Pseudomonas syringae pv. savastanoi, Erwinia horticola and Xanthomonas sp. It is worth knowing that the E. horticola bacteria was first isolated from the symptoms similar to the "ash dieback" of the black bacteriosis Fagus sylvatica L. [8]. Artificial infection of ash with micromycetes did not lead to symptoms like "ash dieback", and infection with bacteria caused pathological processes, similar to tuberculosis Fraxinus excelsior (Fig. 4).

Fig. 4. Tuberculosis of ash: natural (on the left) and artificial (on the right) damage

From the lesions of tuberculosis, we have identified 7 types of bacteria – Pseudomonas syringae pv. savastanoi, Pseudomonas fluorescens, Pseudomonas syringae, Pseudomonas sp., Erwinia herbicola, Erwinia horticola, Xanthomonas sp. Also, in samples of diseased tissues revealed sporonic bacteria, mainly the genus Bacillus, which in further studies revealed some antagonistic properties to P. syringae pv. savastanoi and associated and conditionally pathogenic bacteria associated with it in the F. excelsior tuberculosis pathology.

An attempt was made to investigate the systemic relationships between myco- and microorganisms associated with pathogens of bacterial diseases of forest tree plants in the context of both understanding of general biological problems and in terms of theoretical substantiation and practical application of antagonistic properties in limiting bacterial pathology. In the "bacterium-bacterium" system, Bacillus subtilis exhibits a partial toxic effect on bacterial pathogens. Unlike bacteria isolated from bacterial pathology, micromycetes have a higher antagonistic activity with regard to phytopathogenic bacteria. Regarding the reciprocal effect of FB-bacteria on micromycetes, in most cases, their antimylic activity was practically zero. We can assume that in nature phytopathogenic bacteria outside the pathological process do not affect the growth of micromycetes.

In order to confirm our opinion, in the natural (field) conditions, an artificial defeat of the Fraxinus excelsior shoots was made by a mixture of pure cultures of collection (Pseudomonas savastanoi 9174) and isolated by us from the vegetative and generative organs of F. excelsior Pseudomonas syringae pv. savastanoi (N1, 9k1 – isolated according to tuberculous lesion of seeds and from lesions of type "ash dieback"), Pseudomonas sp. (Kr4) and

preparations P27ant, Victant and Bacillus sp. (K1-4ant) working solution with a titre of 1 × 107 CFU × ml-1. Control – injection of preparations and pure culture of Bacillus sp. In this experiment, we obtained variable results. (Fig. 5.).

Fig. 5. The result of the injection of the mixture is Victant-N1 (P. syringae pv. savastanoi) (on the left) and P27ant– N1 (P. syringae pv. savastanoi) (on the right)

At present, the following bacterial diseases are found in oak: soft rot of acorns (pathogen – Pectobacterium carotovorum), bacterial dropsy (causative agent –Enterobacter nimipressuralis), droplet disease of acorns (causative agent – Erwinia quercina), dry rot of branches and trunks (causative agent – Erwinia rhapontici), cancer-ulcer disease (pathogens – Pseudomonas fluorescens and Pseudomonas sp.) and associated other systematic and functional groups of mycosis and microorganisms.

In recent years there have been reports of single deaths of oak. In the last century in Ukraine there were three waves of mass destruction, the most powerful in the 70's. There is currently a certain activation of the bacterial dropsy. The analysis of model trees indicates the systemic nature of the disease, the causative agent of which affects the plumbing system of trunks and branches of various orders, and externally manifested by the release of a dark, almost black exudate. (Fig.6.).

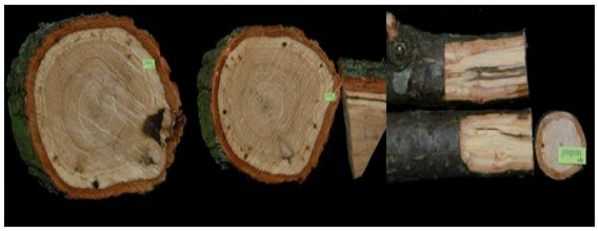

Fig. 6. Bacterial dropsy on an oak: section of the trunk in the place of excretion of the exudate (on the left), 2 meters above the place of defeat (in the center), skeletal branches in the crown (on the right)

Conclusions and prospects of use. The conducted experimental research in conjunction with the critical analysis of literature points to the need to expand the work on the clarification of bacterial pathology of the mentioned, and other forest tree plants. In particular, conceptual approaches to the incubation period (the period from infection penetration to the manifestation of the primary signs of the disease), which is currently associated exclusively with the external infection, are noteworthy. Studies of recent years, including and in Ukraine, experimentally confirmed the presence in healthy trees in the minor quantities of fungi and bacteria (as endophytes), the ecological niche of which are living cells. In a healthy plant, pathogenic endophytes, in particular bacteria, are in a depressed state. Their number is on orders of magnitude less than endophytic saprotrophs and is always less than the threshold concentration required to start the infectious process. This is not due to the lack of nutrients, but due to other factors, in particular the antagonistic activity of endophytic micromycetes and spore bacteria in them in the systemic interaction of the components of the endophytic automico- and microbiota of the plant. Pathogenic endophytes (the so-called vital obligates) are selected by the plant and perform a wide range of biocontrol, regulatory and protective functions through direct antagonism to pathogens or the induction of the resistance system by limiting its activity. Some endophytes prevent the introduction pathogenic species in plants, protect it from nematodes and harmful insects. In a healthy plant, pathogenic endophytes are usually in a depressed state. Their number is on an order of magnitude lower than endophytic saprotroph, and always less than the threshold concentration necessary to start the infectious process. In case of violation of systemic interactions in the plant and, in the first instance, in violation of metabolic processes under the influence of various, often not fully elucidated factors, including and abiotic, pathogenic endophytic myco- and microorganisms can cause infectious pathology of plants without the participation of external infectious agents. Therefore, it is extremely important to study the ways and methods of induction of demutation processes in forest biocenosis as regulatory factors of automico- and microbiota.

Список використаних джерел:

1. Beltiukova K.Y., Matyshevskaia M.S., Kulykovskaia M.D., & Sydorenko S. S. (1968) Methods for investigating pathogens of bacterial plant diseases. Kiev : Nauk. dumka, 316 (in Russian).
2. Bergey's manual of determination of Bacteriology. 8 th ed. (1974) Baltimore : Williams and Wilkins, 1268.
3. Bylai V.Y. (1982) Methods of experimental mycology: a reference book. Kiev : Nauk. dumka, 552 (in Russian).
4. Cherpakov, V.V. (2012) Bacterial diseases of forest species in pathology of forest. SPb. : SPb GLTU, 200, 292-303 (in Russian).
5. Davydenko, K., Vasaitis, R., Stenlid, J., & Menkis, A. (2013) Fungi in foliage and shoots of Fraxinus excelsior in eastern Ukraine: a first report on Hymenoscyphus pseudoalbidus. For. Path., 43, 462–467.
6. Desiccation of coniferous forests - the problem has become threatening scales [Electronic resource] – Resource access mode: http://khersonlis.org.ua/images/stories/buklet%20vs.pdf (in Ukrainian).
7. Gvozdyak, R.I. (2005) Perspective directions of research of phytopathogenic bacteria, Zhitomir, 3-8 (in Ukrainian).
8. Gvozdyak, R.I., & Yakovleva, L.M. (1979) Bacterial diseases of forest tree species. Kiev : Nauk. dumka, 244 (in Russian).
9. Gvozdyak, R.I., Goychuk, A.F., Rozenfeld, V.V. & Pasichnik, L.A., (2011) Bacterial diseases of pine (Pinus sylvestris L.) and its floral microflora: Monograph. Zhitomir : Polissya 224 (in Ukrainian).
10. Klement Z., Rudolf K., Sands D. S. (1990) Methods in phytobacteriology. Budapest : Academial Knido, 568.
11. Kowalski, T., & Holdenrieder, O. (2009) Chalara fraxinea causes dieback of ash (Fraxinus excelsior) in Europe report. For. Pathol., 39, 1-7.
12. Krasnov V.P., Orlov O.O., Buzun V.A. et al. (2008) Applied radio ecology of the forest. Ukrayinskyy botanichnyy zhurnal, №5, 781-786. (in Ukrainian).
13. Kulbanska, I.M. (2015) Pathogenesis of tuberculosis of an ash tree in the conditions of Western Podillya of Ukraine. Forest Journal : 6, 75–84 (in Russian).
14. Losytskyi, K.B. (1975) The phenomenon of depression in hardwood forests. Lesnoe khoziaistvo, 3, 40-44 (in Russian).
15. Sheluho, V.P., & Sidorov, V.A. (2008) Diagnosis and ways to reduce the economic importance of birch bacterial dropsy. Lesnoy hozyaystvo, 4, 48-52 (in Russian).
16. Shvets, M.V. (2016) Bacterial diseases of birch plantings in Ukraine and in the world (theoretical and applied aspect). Lviv : Naukoviy visnik NLTU, 26/7, 179-185 (in Ukrainian).
17. Weather archive (WMO ID) 33415 [Electronic resource] – Resource access mode: htth/rp5.ru.

А. Ф. Гойчук, В. Ф. Дрозда, І. М. Кульбанська, М. В. Швець. Фітопатогенні бактерії в патології лісових деревних рослин Полісся та Лісостепу України

Наведено результати багаторічних експериментальних досліджень бактеріальної патології лісових деревних рослин в Україні. Підкреслюється, що на лісових деревах у світі описано кілька десятків бактеріозів із різним ступенем пошкодження, що викликаються бактеріями родів Pseudomonas, Xanthomonas, Enterobacter, Erwinia, Agrobacterium, Brenneria, Xylella, Rhizobium, Corynebacterium, Bacillus, Clostridium і ін. Наголошується на необхідності поглиблення досліджень аутомікробіоти, зокрема патогенних її складників, у контексті розуміння як загальнобіологічних проблем патології, так і з метою напрацювання лісозахисних заходів.

Ключові слова: *бактеріальні захворювання, фітопатогенні бактерії, етіологія, симптоматика, патогенез, антагонізм, системну взаємодію, Pinus sylvestris, Quercus robur, Fraxinus excelsior, Betula pendula.*

А. Ф. Гойчук, В. Ф. Дрозда, И. М. Кульбанская, М. В. Швец. Фитопатогенные бактерии в патологии лесных древесных растений Полесья и Лесостепи Украины

Приведены результаты многолетних экспериментальных исследований бактериальной патологии лесных древесных растений в Украине. Подчеркивается, что на лесных деревьях в мире описано несколько десятков бактериозов с различной степенью повреждения, вызываемых бактериями родов Pseudomonas, Xanthomonas, Enterobacter, Erwinia, Agrobacterium, Brenneria, Xylella, Rhizobium, Corynebacterium, Bacillus, Clostridium и др. Отмечается необходимость в углубленных исследованиях аутомикробиоты, в частности патогенных ее составляющих, в контексте понимания как общебиологических проблем патологии, так и с целью выработки лесозащитных мероприятий.

Ключевые слова: *бактериальные заболевания, фитопатогенные бактерии, этиология, симптоматика, патогенез, антагонизм, системное взаимодействие, Pinus sylvestris, Quercus robur, Fraxinus excelsior, Betula pendula.*