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BACTERIAL DISEASES OF SILVER BIRCH (*BETULA PENDULA* ROTH.)

A.F. Goychuk[1], M.V. Shvets[2], I.M. Kulbanska[1], F.F. Markov[2], N.A. Muljukina[3] V.P. Patyka[3]

[1]National University of Life and Environmental Sciences of Ukraine, 19 General Rodimtsev Str., Kyiv, 03041, Ukraine
[2]Zhytomyr National Agroecological University, 7 Staryi Blvd, Zhytomyr, 10008, Ukraine
[3]Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine
e-mail: patykavolodymyr@gmail.com

A significant role in the pathogenesis of diseases of woody plants belongs to phytopathogenic bacteria and fungi. It has been scientifically confirmed that the organs and tissues of woody plants have a certain myco- and microbiota, the components of which are systematically interconnected both with each other and with the plant. The species composition and quantitative ratio are constantly changing both in the process of ontogenesis of the tree and with changes in its physiology. ***The aim*** *of the work was to study the species composition and the formation of diversity and systemic interactions of microbiota associated with bacterial dropsy in the pathology of Betula pendula Roth.* ***Methods****. Classical microbiological, phytopathological, biochemical, statistical methods were used in the work. Combined diagnostic methods were also used, in particular careful microscopic examination of the affected parts of plants, isolation and identification of the pathogen.* ***Results.*** *Bacterial origin of wet wood in the trunk of birches was detected. Samples of wood and exudate were taken for laboratory studies from plants that had pronounced signs of pathology (cracks, swellings). It has been experimentally proved that the causative agent of bacterial dropsy of silver birch is the phytopathogenic polybiotrophic bacterium Lelliottia nimipressuralis, which causes dropsy of coniferous and deciduous woody plants and experimentally found pathogenic properties to B. pendula. Xanthomonas campestris, Pantoea agglomerans and Bacillus subtilis are associated with bacterial dropsy pathology of B. pendula. The pathogenic properties of P. agglomerans and X. campestris on B. pendula are variable, which indicates the possibility of the expansion of the circle of plants sensitive for these species of bacteria. It was established that L. nimipressuralis both during spring and autumn inoculation showed high pathogenicity to B. pendula. In only one case, on isolated on the border of healthy and affected wood from young B. pendula (bast part) the results of artificial injury were less pronounced. Other bacteria isolated from bacterial dropsy, in particular X. campestris, were non-pathogenic for B. pendula. At the same time, we noted traces of artificial infection with X. campestris in the samples isolated on the border of healthy and affected wood from middle-age B. pendula (cambial part). This may indicate an expansion of the circle of sensitive plants or the increased sensitivity of certain forms of birch for the mentioned bacteria, which is quite likely, since the bacteria have a significant forms variety. In 10 places of inoculation no pathology caused by B. subtilis was found. Bacteria of the Bacillus genus were non-pathogenic for B. pendula in all experiment. Our studies have shown that they can be a regulatory factor in the development of bacterial dropsy.* ***Conclusions.*** *A certain variability of isolated strains in the assimilation of some carbohydrates and alcohols can be explained by the specific conditions of the existence of bacteria, including the influence of environmental factors on their biochemical properties. It is known that the ecological niche affects even the antigenic composition of bacteria; therefore, such an effect should also be expected on other properties. Our studies confirmed that causative agent of bacterial dropsy is L. nimipressuralis and clarified the information about this bacteria cells size.*

Keywords: Lelliottia nimipressuralis, phytopathogenic bacteria, Betula pendula, bacterial drops.

Each microorganism is a complex system of biochemical reactions, which changes its orientation depending on the living conditions [1]. The mechanisms by which the bacteria properties evolution is carried out are diverse and not fully understood, especially the process of transition of saprotrophic bacterial species to parasitism, their ability not only to initiate the development of the infectious process, but also to support it for a long time period [2, 3]. The presence of phytopathogenic bacteria in minimal quantities in healthy organs of plants as an integral component of microbiota was experimentally proved [4, 5]. Such bacteria are not only selected by the plant and accompany it at different stages of ontogenesis, but also perform a number of useful functions for it. However, under certain conditions, these components can cause a pathological process (disease) of a woody plant, which loses its healing properties. In particular, the buds and leaves of hanging birch are used in folk and scientific medicine, they have diuretic, choleretic, diaphoretic, purifying, bactericidal, anti-inflammatory and wound-healing effect. Birch buds are used in the manufacture of creams and other cosmetics. Essential oil from birch buds is used in alcoholic beverage production. In addition, birch sap – a pleasant refreshing drink, contains 0.5–2 % of sugars, organic acids, salts of potassium, calcium, iron, trace elements and has a beneficial effect on metabolism. The juice is also used to prepare "Birch" lotion and so on. Currently, these issues are relevant for both science and medical practice, forestry practice, in particular in terms of measures to protect plants from phytopathogenic bacteria. The above are led to the choice of our research.

Due to the specificity of biology and pathogenesis, phytopathogenic bacteria differ significantly in the nature of their effects from plant damage caused by fungi and harmful entomofauna. The key factors in the harmfulness of phytopathogenic bacteria are the aggressiveness of the pathogen, the similarity of the pathogen and the host plant, which are determined by many morphological, physiological-biochemical and molecular genetic properties of bacteria, their hosts (woody plants) and various environmental factors. Usually they are evolutionarily determined and are controlled by the time factor [6, 7].

The aim of the work was to study the species composition and the formation of diversity and systemic interactions of microbiota associated with bacterial dropsy in the pathology of *Betula pendula* Roth.

Materials and methods. Samples of birch affected by dropsy were collected in the investigated territories of "Emilchinske", "Korostenske", "Ovruchske", "Malinske", "Novograd-Volynske", "Olevske" State forestry enterprises.

Affected samples were taken from plants with different intensity of symptoms, different age groups (young, middle-age, pre-mature and mature plants) and from different parts of the trunk in cross and longitudinal sections – from bark, affected bast, on the border of healthy and affected wood and visually healthy tissue (Fig.1, Table 1).

The method of microscopic analysis was used to define the cause of the disease. All microscopic studies were performed on fresh plant material. If the bacterial nature of the disease was suspected,

F i g. 1. Bacterial dropsy of *B. pendula*: the formation of a pathological nucleus with subsequent healing of the lesion site (*in the left*) and a fragment of the dropsy distribution along the trunk (*in the middle and right*)

the diagnostic methods were used to establish the etiology of the disease: an accurate analysis of symptoms; a thorough microscopic examination of affected parts of the plants; isolation and identification of the pathogen [8–10].

To identify phytopathogenic bacteria, cultural properties were studied on a solid nutrient medium – potato agar (PA), on the Omelyansky medium with bromothymol blue aqueous indicator and the addition of 0.5 % carbohydrates [11].

Table 1

Symbol designation of strains and characteristics of samples (lesions) from selected localizations

Strain	Localization
Cb_{1-1}	Border of healthy and affected wood from middle-age *B. pendula* (cambial part)
Cb_{1-2}	Border of healthy and affected wood from pre-mature *B. pendula* (cambial part)
Bs_{2-1}	Affected wood from mature *B. pendula* (bast part)
Bs_{2-2}	Border of healthy and affected wood from middle-age *B. pendula* (bast part)
Bs_{2-3}	Healthy wood from young *B. pendula* (bast part)
Bs_{2-4}	Border of healthy and affected wood from young *B. pendula* (bast part)
Bs_{2-5}	Border of healthy and affected wood from pre-mature *B. pendula* (bast part)
Bs_{2-6}	Affected wood from mature *B. pendula* (bast part)
Br_{3-1}	Affected wood from middle-age *B. pendula* (bark)
Br_{3-2}	Border of healthy and affected wood from young *B. pendula* (bark)
Br_{3-3}	Healthy wood from mature *B. pendula* (bark)
Sp_{4-1}	Border of healthy and affected wood from pre-matured *B. pendula* (sapwood part)
Sp_{4-2}	Affected wood from middle-age *B. pendula* (sapwood part)

To check the pathogenic properties of bacteria, the method of artificial damage to plants (trunks and cut shoots) was used. For artificial damage, individual parts of the plant were used (including those isolated from the plant itself, namely leaves and young shoots) and whole separate plants [12, 13].

During the artificial infection, the trunks of 5 model birch trees aged 35–40 years were mechanically damaged. 2–3 wounds were made on each plant trunk. The place of injury was treated with alcohol using cotton swab, after that a suspension of a daily culture of microorganisms (8.6–9.9 x 10^{9} CFU/ml) was injected into the trunks in the amount of 5 ml per test plant.

Infection was also done by introducing a pure culture of bacterial mass (14.1–21.2 x 10^{9} CFU/ml) under the bark in places of artificial damage in the fall (October) at a temperature of + 8º C, humidity 57 %, light wind – 2 m/s, at 6 p.m., taking into account the circadian rhythms of plant resistance [14–17] to bacterial pathogens. The bacterial mass was introduced using bacterial loop preliminary sterilized over the flame of spirit lamp (dry alcohol) into the mechanical damage of the trunk section (incision).

Bacteria were identified by comparing their properties with the characteristics of collection strains, and according to the Bergey's Manual of Systematic Bacteriology, according to domestic and foreign researchers [8, 14, 15]. Data were statistically processed using Statistica 8.0.

Results. 187 samples were collected from the studied plants (from *B. pendula* trees affected by dropsy), from which 42 isolates were obtained and 19 pathogenic were selected for further studies (Table 2).

When isolating bacteria from the initial stages of bacterial pathologies, growth of the same type of colonies was observed on Petri dishes. This not only facilitated further work on the establishment of the pathogen, but also to a certain extent indicated the presence of *L. nimipressuralis* in the pathology of bacterial dropsy, that we established in further studies (Fig. 2).

On the potato agar (PA), different morphotypes of colonies grew – shiny grayish-white, opaque white-cream and colonies with a yellow tint (Fig. 3). Pathogenic bacteria studied and isolated in the experiments of pure cultures were identified as the representatives of *Lelliottia, Bacillus* and

Xanthomonas genera. Spore-forming bacteria *B. subtilis* were identified as belonging to the genus *Bacillus*. On the PA they formed creamy-white oily colonies with uneven edges, which did not shine through.

Shiny grayish-white cream-colored colonies, which were assigned to the genus *Lelliottia*, were examined in details (Table 3).

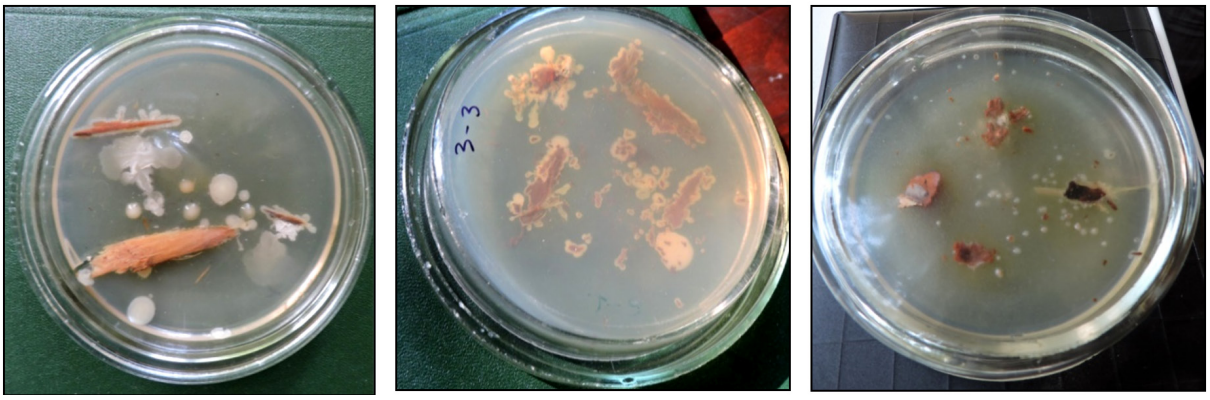
A significant variation in the number of morphotypes of bacteria isolated from various lesions and at different phases of the infection process has been established. Thus, when bacteria were isolated from the cortex of freshly affected peel, brilliant grayish-white colonies (classified as *L. nimipressuralis*) were predominant (63 %), the number of opaque creamy-white colonies (*B. subtilis*) was three times less. 16 % of colonies had other morphotypes (Table 4).

When isolating bacteria from a long-term lesion of the bast, opaque creamy-white colonies of *B. subtilis* (56 %) and slightly less grayish-white colonies of *L. nimipressuralis* (35 %) were obtained. Yellow pigmented bacteria were not detected, while the ammount of colonies with

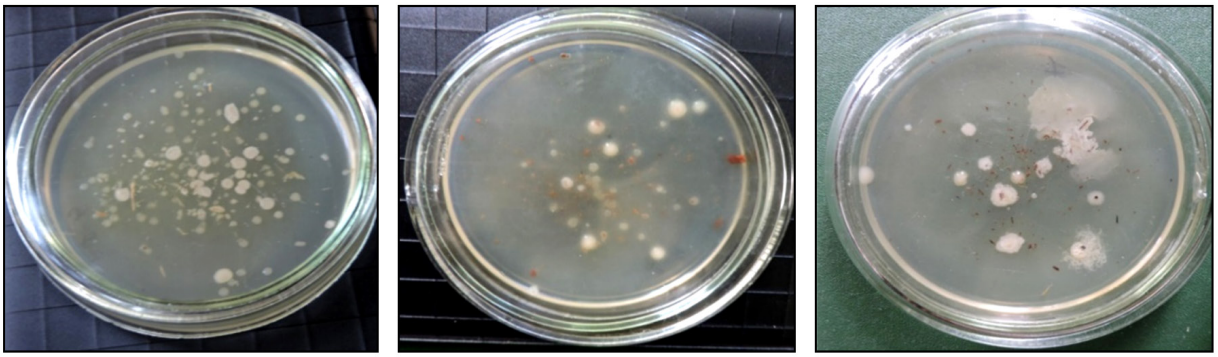
Table 2

The number of pathogenic bacteria studied and isolated in pure cultures in the experiments

Infection localization	The number of studied samples	The number of samples from which pathogenic bacteria were isolated	The number of selected pure cultures of pathogenic bacteria
Bast $_{2-1}$	11	1	1
Bark $_{3-1}$	7	1	1
Bark $_{3-2}$	4	3	2
Bast $_{2-4}$	13	6	3
Cambium $_{1-2}$	3	1	0
Bast $_{2-6}$	3	6	4
Sapwood $_{4-1}$	5	1	0



F i g. 2. Colonies of microorganisms isolated by fouling of affected tissues



F i g. 3. Colonies of microorganisms isolated from wood sawdust (a type of fouling method)

other morphotypes was 9%. Brilliant grayish-white colonies of *L. nimipressuralis* dominated in the freshly affected sample from the cambial part of birch (62 %), and there were half as many opaque creamy-white colonies of *B. subtilis*. Yellow pigmented bacteria were absent, while there were 6 % of other morphotypes.

Brilliant grayish-white colonies of *L. nimipressuralis* predominated (59 %) among bacterial isolates from freshly affected sapwood, while opaque creamy-white colonies of *B. subtilis* and yellow pigmented (5 %) bacteria *X. campestris* were present in a smaller amount (9 %).

Table 3

Morphological and cultural properties of microorganisms isolated from affected woody organs of *B. pendula*

	Indicators of bacterial colonies (two-day)			
Sign	Shape	Round	Round	Round
	Profile	Flat	Convex	Convex
	Size (diameter)	4.3–5.8 mm (medium and large)	1.4–3.6 mm (small and medium)	0.8–2.1 mm (small)
	Color (pigment)	Yellow	Grayish-white	Creamy-white with a metallic tint
	Transparency	Translucent, shiny	Translucent, shiny	Opaque, matte
	Center	Raised, compacted	Raised	Raised
	Edges	Whole, flat	Whole, slightly wavy	Whole, slightly wavy
	Viscosity	–	Viscous	–
	Consistence	Dense	Mucous	Dense

Table 4

The ratio of bacterial colonies morphotypes isolated from lignified organs of *B. pendula* at different stages of the pathological process

Infection localization	The ratio of bacterial colonies (after 2 days) (%)			
	Grayish-white	Yellow pigmented	Opaque creamy-white	Others
Bark $_{3\text{-}1}$	63	0	21	16
Bark $_{3\text{-}2}$	52	0	34	14
Bark $_{3\text{-}3}$	59	11	27	3
Bast $_{2\text{-}1}$	28	0	11	61
Bast $_{2\text{-}2}$	0	68	8	24
Bast $_{2\text{-}3}$	40	12	39	9
Bast $_{2\text{-}4}$	67	0	29	4
Bast $_{2\text{-}5}$	35	0	56	9
Bast $_{2\text{-}6}$	59	5	9	1
Cambium $_{1\text{-}1}$	62	0	32	6
Cambium $_{1\text{-}2}$	8	0	7	85
Cambium $_{5\text{-}1}$	64	0	32	4
Sapwood $_{4\text{-}1}$	0	0	38	62
Sapwood $_{4\text{-}2}$	0	0	19	81

To determine the concentration (quantity) of microorganisms in a unit of volume, we plated the material on a solid nutrient medium (Koch's method) with the calculation of the grown colonies – 1 colony is usually formed by 1 cell (Table 5). As a result of the studies, the average values of CFU/ml that were isolated from the lignified affected organs of *B. pendula* ranged from 2 to 164 CFU/ml.

The largest number of colonies (164 and 127 CFU/ml, respectively) was formed by bacteria, which were further identified as *L. nimipressuralis*. Yellow pigment bacteria, classified as *X. campestris*, were isolated from only two studied samples – from the bark and bast part (23 and 16

CFU/ml, respectively). The permanent components in the pathology of bacterial dropsy were bacteria, which we classified as *B. subtilis.*

Various systematic and functional groups of microorganisms, from pathogenic to saprotrophic, participate in the pathological processes of forest woody plants dieback. The only reliable way that allows us to separate pathogens from saprotrophs is the determination of pathogenicity, that is, the ability of microorganism to infect living cells. Establishing the true causative agent of the disease is significantly complicated by the wide systemic interaction of microorganisms with all living components of the biogeocenosis against the background of constant changes in environmental conditions, ecological plasticity and variability of the phytopathogenic bacteria [18, 19]. Only true pathogens (with varying degrees of pathogenicity and virulence) are able to infect plants. Other components of the infectious process also participate in further pathology, which settle already on the prepared tissues and actively colonize the plant, completing its dieback [14].

Pathogens are present in minor amounts in every living organism, including woody plant. Usually in a healthy body they are depressed and certain changes in the plant are necessary to activate them. The experimental confirmation of this opinion [12, 14, 18] clearly indicates that not always pathogenic infection penetrates the plant from the outside.

Table 5

The number of CFU of bacteria isolated from the affected organs of *B. pendula*

Isolated strains of bacteria	Strains, the number of CFU/ml			
	L. nimipressuralis	*X. campestris*	*B. subtilis*	Others
Bast $_{2\text{-}1}$	164	0	54	–
Bark $_{3\text{-}1}$	68	0	27	15
Bark $_{3\text{-}2}$	19	23	18	39
Bast $_{2\text{-}4}$	41	16	21	24
Cambium $_{1\text{-}2}$	127	0	31	–
Bast $_{2\text{-}6}$	102	0	46	–
Sapwood $_{4\text{-}1}$	62	0	41	2
Bast $_{4\text{-}1}$	77	0	26	11

Inoculation experiments were aimed at the artificial damage of *B. pendula* tree trunks in the «field» conditions *(in vivo*) and infection of young shoots and birch leaves in the laboratory (*in vitro*). It is known that the isolation of bacteria from lesions does not yet indicate that they are the causative agents of bacteriosis.

Assuming that not all bacteria that were used in the experiments can cause bacterial diseases of silver birch, we conditionally divided them into 2 groups of isolated strains – pathogenic and nonpathogenic for living birch tissues.

In winter, inoculated plants remained unchanged in appearance. The first symptoms of the infectious process were observed 4 months after the artificial infection of birches and were characterized by very slow pathological processes. As a result, 6 months after inoculation, the dynamics of the infectious process and the final symptoms were similar to those from which pathogenic microorganisms were isolated. The control was test plants that had clear signs of pathology on the surface of the bark and under it. As a result of pathogenic effect of the causative agent of bacterial dropsy *L. nimipressuralis*, we noted in spring (April-May) the initial signs of the development of bacteriosis on perfectly healthy *B. pendula* in the forest stands: soft and slightly noticeable swelling on the trunks, the bark in such places was light brown in color, smaller leaf plates and a noticeable liquefaction of the crown.

We found certain differences in the intensity of the development of artificial bacterial pathology (lesion score, infectious class) [19] may be associated with individual resistance, and possibly with the varieties of the studied plants (Table 6). So, from 5 models the most intensive pathology development was found in 2 birches. We isolated bacteria in spring from experimental lesions during the period of intensive sap movement from inoculated test plants. Re-isolation of bacteria was a confirmation of the detected pathogen, which was identical in biological properties.

The yellow pigmented isolates that we isolated according to the pathology of bacterial dropsy and identified as the representatives of *Xanthomonas* genus did not practically had pathogenicity to *B. pendula* in the experiment.

Further studies of *Xanthomonas* genus bacteria (Br_{1-3} and Bs_{1-4} strains) isolated from the bark and bast of the affected trees showed unexpected sensitivity of birch *Pantoea agglomerans* Gav. to this pathogen, at the same time on other model trees there were only traces of lesions. In the literature [16, 21], this bacterium is noted in many bacterioses of forest woody plants as a concomitant microbiota. Gram-negative bacteria *P. agglomerans* (synonyms of *Enterobacter herbicola, Enterobacter agglomerans*) is a constant component of rhizospheric, epiphytic and endophytic microbiota of plants and affects crops under certain conditions [30]. Most of the *P. agglomerans* strains quickly lose their ability to infect any plants after preserving on artificial media. Bacteria penetrate the plant through various injuries, primarily in places of damage by insects. Tissues in these places are soften and capable of maceration as a result of hydrolysis of the intercellular lamella by the protopectinase enzyme produced by bacteria.

We found that *L. nimipressuralis* both after spring and autumn inoculation showed high pathogenicity to *B. pendula*. In only one case, on sample, isolated on the border of healthy and affected wood from young *B. pendula* (bast part), the results of artificial injury were less pronounced. Other bacteria isolated from bacterial dropsy, in particular *X. campestris*, were nonpathogenic for *B. pendula* [22, 23].

It is known that phytopathogenic bacteria in minimal quantities are components of the normal microbiota of plants, including woody ones, without showing noticeable pathology signs of the latter. Such an assumption is based both on our own studies and observations, as well as on the reports of other researchers [20, 22, 23]. It is emphasized in the studies of other authors [24], that pathogenic epiphytic and endophytic microorganisms with normal growth and development of trees do not cause visible signs of infection in their organs, because they are in such plants in a depressed state. In addition, in the tissues of healthy organs their amount is much (in several orders of magnitude) less than saprotrophs, and always less than the threshold concentration required to initiate the infectious process. So, according to the authors [12], this is not due to the lack of nutrients, but due to the other factors that regulate the reproduction of bacteria. However, for bacteria, especially phytopathogenic, their number is not so much important as their presence.

Under conditions favorable for phytopathogenic microorganisms in the system «woody plant-microbiota-environment» they can very quickly fill an ecological niche to a threshold concentration, thereby even causing epiphytoties [9], to a certain extent we can observe this in the modern phytosanitary state of *B.pendula*.

Our laboratory studies confirmed that the causative agent of bacterial dropsy is *L. nimipressuralis* and specified cell size data. They are small straight sticks that are located singly or in pairs, less often in chains or groups, rounded at the ends (somewhat elliptical in shape), polymorphic, ranging in size from 0.45 to 1.75 μm, and well stained. Bacteria are motile, do not form spores, gram-negative, have long peritrichous flagella. *L. nimipressuralis* – facultative anaerobes, grow well on PA, MPA, MPB.

They grow better on PA, where after 40-48 hours of growth they form round colonies up to 4 mm in diameter, the edge of which is elevated, hilly or slightly wavy, stands out more sharply from the middle. Colonies do not form water-soluble pigments, assimilate carbohydrates (with the formation of acid and gas), glucose (aerobic and anaerobic), maltose, rhamnose, sucrose, lactose; grow on sorbitol, but not on mannitol (Table 7).

Given the unequal absorption rate of certain alcohols and carbohydrates, the strains were first isolated and were divided into 2 groups. In addition to the general properties, strains from the first group isolated from *B. pendula* on the 5th day slowly absorbed lactose with acid and gas forming. The strains of this group do not metabolize inositol and glycerol; they do not grow on a medium with rhamnose.

Hydrogen sulfide is not produced, but litmus serum is acidified. The strains of the second group on glycerol form only acid, produce hydrogen sulfide, inositol is not absorbed, alkalize litmus serum with subsequent recovery. Characterizing the biochemical properties of bacteria of this species for Carter, Dye, R. I. Gvozdyak [14, 25], it should be noted that they grow well on the media of Ushinsky, Eykman, Liske, Fermi, with asparagine (they form a strongly pronounced or moderate turbidity, pellicle and sediment). In addition to the general properties, the strains of *B. subtilis, P. agglomerans*, and *X. campestris* isolated by us from *B. pendula* coagulated milk, assimilated

arabinose, glucose, maltose, lactose, and mannitol by forming acid, produced catalase. *B. subtilis* strains do not absorb inositol and glycerin; do not grow on medium with rhamnose. Hydrogen sulfide is not produced, but the litmus serum is acidified.

P. agglomerans associated with bacterial dropsy showed variability during artificial infection of birch; this pathogen was similar to those described in the literature according to the morphological and physiological-biochemical characteristics [18, 26].

Strains of *P. agglomerans* on glycerin showed variable properties, they produced hydrogen sulfide, did not assimilate inositol, and did not alkalize litmus serum. *P. agglomerans* cells were 0.5×3 μm in size, motile with peritrichous flagella. The optimum temperature for growth was +28° C. Whitish-gray

Table 6

The results of artificial bacteria inoculation of birch trunks

No. of test plants	Localization of isolated bacteria	The symbol of the strain	Identified species of bacterium	The results of inoculation
Artificial infection – October 12, 2018, record – April 15, 2019				
1.1	Bark	Br_{1-1}	*X. campestris*	–
	Bark	Br_{1-1}	*L. nimipressuralis*	+
	Cambium	Cb_{1-1}	*B. subtilis*	–
	Bast	Bs_{1-1}	*L. nimipressuralis*	+
	Sapwood	Sp_{1-1}	*X. campestris*	+;–
1.2	Bark	Br_{1-2}	*L. nimipressuralis*	+
	Bast	Bs_{1-2}	*B. subtilis*	–
	Cambium	Cb_{1-2}	*L. nimipressuralis*	+
	Sapwood	Sp_{1-2}	*X. campestris*	–
	Sapwood	Sp_{1-2}	*B. subtilis*	–
1.3	Bark	Br_{1-3}	*P. agglomerans*	+;–
	Cambium	Cb_{1-3}	*B. subtilis*	–
	Cambium	Cb_{1-3}	*L. nimipressuralis*	+
	Sapwood	Sp_{1-3}	*L. nimipressuralis*	+
1.4	Bark	Br_{1-4}	*B. subtilis*	–
	Cambium	Cb_{1-4}	*L. nimipressuralis*	+; –
	Sapwood	Sp_{1-4}	*X. campestris*	–
1.5	Bark	Br_{1-5}	*L. nimipressuralis*	+;–
	Cambium	Cb_{1-5}	*L. nimipressuralis*	+;–
	Sapwood	Sp_{1-5}	*L. nimipressuralis*	+
Artificial infection – May 05, 2019, record – September 19, 2019				
1.1	Bark	Br_{1-1}	*X. campestris*	–
	Bark	Br_{1-1}	*L. nimipressuralis*	+;–
	Cambium	Cb_{1-1}	*B. subtilis*	–
	Bast	Bs_{1-1}	*L. nimipressuralis*	–
	Sapwood	Sp_{1-1}	*X. campestris*	–
1.2	Bark	Br_{1-2}	*L. nimipressuralis*	+;–
	Bast	Bs_{1-2}	*B. subtilis*	–
	Cambium	Cb_{1-2}	*L. nimipressuralis*	+;–
	Sapwood	Sp_{1-2}	*X. campestris*	–
	Sapwood	Sp_{1-2}	*B. subtilis*	–
1.3	Bark	Br_{1-3}	*P. agglomerans*	+;–
	Cambium	Cb_{1-3}	*B. subtilis*	–
	Bast	Bs_{1-3}	*L. nimipressuralis*	+
	Sapwood	Sp_{1-3}	*L. nimipressuralis*	+
1.4	Bark	Br_{1-4}	*B. subtilis*	–
	Cambium	Cb_{1-4}	*L. nimipressuralis*	–
	Sapwood	Sp_{1-4}	*X. campestris*	–
1.5	Bark	Brv_{1-5}	*L. nimipressuralis*	+;–
	Bast	Bs_{1-5}	*X. campestris*	–

colonies were formed on the 4th day. Strains of *X. campestris* absorbed inositol, did not produce hydrogen sulfide. They showed variable ability to produce pectinase, formed catalase (Table 8).

Table 7

Physiological and biochemical properties of bacterial strains isolated from bacterial dropsy of *B. pendula*

Biochemical tests	Bacterial strains								
	Br_{3-1}	Br_{3-2}	Cb_{1-2}	Cb_{5-1}	Bs_{2-1}	Bs_{2-4}	Bs_{2-6}	Sp_{4-1}	Sp_{4-2}
Acetoin formation	+	+	–	+	–	+	+	+	+
Protopectinase	–	–	–	–	–	–	–	–	–
Catalase	+	+	–	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+
Oxidase	–	–	–	–	–	–	–	–	–
Cellulase	+	+	+	+	+	+	+	+	+
Amylase	–	–	–	–	–	–	–	–	–
Indole	–	–	–	–	–	–	–	–	–
Ammonia	–	–	+	–	+	–	–	–	–
Fermentation of sugar:									
glucose	ag	ag	a	ag	g	a	ag	ag	ag
saccharose	a	a	a	a	a	a	a	a	a
lactose	ag	ag	g	ag	g	ag	ag	ag	ag
rhamnose	a	a	a	a	a	a	a	a	a
sorbitol	ag	ag	ag	ag	ag	ag	ag	ag	ag
mannitol	–	–	–	–	–	–	–	–	–

Legend: (+) – the presence of properties; (–) – lack of properties; (+,–) – variable properties; *(a) – acid formation; (ak) –alkali formation; (g) – gas formation.

Table 8

Physiological and biochemical properties of bacteria associated with bacterial dropsy of birch

Test	*B. subtilis*	*P. agglomerans*	*X. campestris*
Gram staining	+	–	–
Spores formation	+	–	–
Milk coagulation	+	+	+
Peptonization	+	–	+
Litmus serum	+	–	
Formation:			
indole, ammonia	–	–	
hydrogen sulfide	–	+	–
Assimilation of carbohydrates and alcohols:			
arabinose, glucose, maltose, lactose, mannitol	a	a	a
mannose, raffinose, fructose	–	a	a
salicin	–	+,–	–
glycerin	–	+,–	
rhamnose	–	a	
dulcite, inositol	–	–	+
xylose, sorbitol	–	–	
Formation:			
amylase	+	–	
pectinase	–	–	+;–
oxidase	–	+	–
catalase	+	+	+
proteinase	–	+	+

Legend: (+) – the presence of properties; (–) – lack of properties; (+,–) – variable properties; *(a) – acid formation; (ak) – alkali formation; (g) – gas formation; (r) – reduction; (*) – individual strains have other properties.

They synthesize proteinases. Cells 0.8×2.6 μm in size, straight rods, monotrich. The optimum temperature for growth is $+27^0$ C. Large yellow mucous colonies formed on the PA.

Discussion. Research was directed on the study of induction of microorganisms of different dominant functional orientation and the formation of conditions for their activity in the rhizosphere of plants. The ability of bacteria to level phytopathogens can also be due to the high rate of occupation of their ecological niche in the rhizosphere, and the biosynthesis of antibiotics and other antifungal metabolites [2, 4, 20, 26].

The causative agent of bacterial dropsy is an integrated part of healthy plant organs as epiphytic and endophytic myco- and microbiota [12, 15, 24]. Therefore, in the presence of pathogens in the organs of healthy plants, it is inappropriate to talk about the incubation period, because we are talking about the so-called vital obligates, as integral components of healthy plants' microbiota. According to morphotypes, brilliant grayish-white, opaque white-cream and yellow pigment colonies were identified from the pathological tissues of *B. pendula* affected with bacterial dropsy, which we assigned to the genera *Lelliottia, Bacillus, Xanthomonas* and *Pantoea.* Phytopathogenic bacterium *L. nimipressuralis* was pathogenic for *B. pendula* in the experiments. The pathogenic properties of *P. agglomerans* and *X. campestris* were variable (*X. campestris* showed weak pathogenic properties in only one variant). Isolated strains of bacteria according to the main characteristics were similar to those described in the literature [27, 28]. Definitely, the variability of some *L. nimipressuralis* isolates in the assimilation of carbohydrates and alcohols may be related to the specific conditions of bacteria existence. Associated with bacterial dropsy *P. agglomerans*, which found variability in the case of artificial infection of birch, according to the morphological, physiological and biochemical characteristics also similar to that described in the literature [14, 23].

The development of epiphytoties in the context of environmental science and the theoretical basis of integrated protection against diseases requires a detailed study of the complex interactions between pathogens and plants, ecology studies of the formation and functioning of biological systems, starting from the cell level and above. At the same time, systems of the highest level of organization have their own specific properties. This position in general ecology is known as the principle of functional integration. In the context of the problem, the main intellectual efforts in the current study were aimed at investigation mainly the features of the life cycle and the development of the pathogen – its localization in organs. The patterns of infectious pathology – the reaction of cells, organs and plants to the colonization of the pathogen were also partially investigated.

Conclusions. A certain variability of isolated strains in the assimilation of certain carbohydrates and alcohols can be explained by the specific conditions of the existence of bacteria, including the influence of environmental factors on their biochemical properties. It is known that the ecological niche even affects the antigenic composition of bacteria; therefore, such an effect should also be expected on other properties.

To determine the concentration (quantity) of microorganisms in a unit of volume, we investigated the material in a solid nutrient medium (Koch's method) with the calculation of the grown colonies – 1 colony is usually formed by 1 cell. As a result of the studies, the average values of CFU/ml that were isolated from the lignified affected organs of *B. pendula* ranged from 2 to 164 CFU/ml.

Our studies in the laboratory confirmed that *L. nimipressuralis* is the causative agent of bacterial edema and updated data on cell size. *Xanthomonas campestris, Pantoea agglomerans* and *Bacillus subtilis* also participate in the pathology of bacterial dropsy of *B. pendula.*

БАКТЕРІАЛЬНІ ХВОРОБИ ПОВИСЛОЇ БЕРЕЗИ (*BETULA PENDULA* ROTH.)

А.Ф. Гойчук[1], М.В. Швець[2], І. М. Кульбанська[1], Ф.Ф. Марков[2], Н.А. Мульюкіна[3], В.П. Патика[3]

[1]Національний університет біоресурсів і природокористування України, вул. Генерала Родімцева, 19, Київ, 03041, Україна
[2]Житомирський національний агроекологічний університет, Старий бульвар, 7, Житомир, 10008, Україна
[3]Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

Резюме

Значна роль у патогенезі хвороб деревних рослин належить бактеріальним структурам. На-

уково підтверджено, що органам і тканинам деревних рослин притаманна певна міко- та мікробіота, складники якої системно взаємопов'язані як між собою, так і з рослиною. При цьому видовий склад і кількісне співвідношення постійно змінюються як у процесі онтогенезу дерева, так і зі змінами його фізіології. **Метою** роботи є дослідження видового складу і формування різноманітності та системної взаємодії асоційованих з бактеріальною водянкою мікробіоти у патології *Betula pendula*. **Методи**. В роботі використано класичні мікробіологічні, фітопатологічні, біохімічні, статистичні методи. За підозри на бактеріальну природу хвороби використовували поєднані методи діагностики, за допомогою яких встановили етіологію захворювання: точний аналіз симптомів; ретельне мікроскопічне дослідження уражених частин рослин; виділення і ідентифікацію збудника. **Результати.** Встановлено, що мокра деревина в стовбурі берез – бактеріального походження. Відібрано зразки деревини і ексудату для лабораторних досліджень із рослин, які мали яскраво виражені ознаки патології (тріщини, здуття). Експериментально доведено, що збудником бактеріозу берези повислої є фітопатогенна бактерія-полібіотроф *Lelliottia nimipressuralis*, яка спричинює водянку хвойних і листяних лісових деревних рослин і в експерименті виявила патогенні властивості до *B. pendula.* У патології бактеріальної водянки *B. pendula* виявлені асоційовані з нею бактерії *Xanthomonas campestris, Pantoea agglomerans* та *Bacillus subtilis*. Виявлені варіабельні патогенні властивості *P. agglomerans* і *X. campestris* на *B. pendula*, що вказує на можливість розширення живильних рослин для даних видів бактерій. Встановлено, що *L. nimipressuralis* як при весняній, так і при осінній інокуляції виявила високу патогенність до *B. pendula*. Тільки в одному випадку виділені на межі здорової і ураженої деревини з молодої *B. pendula* (луб'яна частина) результати штучного ураження були менш вираженими. Інші ж ізольовані з бактеріальної водянки бактерії, зокрема *X. campestris*, у переважній більшості були непатогенними для *B. pendula*. Разом з тим, виділені на межі здорової і ураженої деревини із середньовікової *B. pendula* (камбіальна частина), на*ми* відмічені сліди від штучного ураження *X. campestris.* Це може свідчити про розширення кола живильних рослин або про дещо підвищену чутливість певних форм берези до згаданих видів бактерій, що цілком ймовірно, оскільки бактерії мають значну видову різноманітність. Так, у місцях інокуляції з 10 уражень жодних ознак патології від *B. subtilis* не встановлено. Бактерії роду *Bacillus* виявились непатогенними для *B. pendula* у всіх варіантах досліду. Як показали наші дослідження, вони певною мірою можуть бути регуляторним чинником у розвитку бактеріальної водянки. **Висновки.** Певну варіабельність ізольованих штамів у засвоєнні деяких вуглеводів та спиртів можна пояснити конкретними умовами існування бактерій, у тому числі і впливом екологічних чинників на їх біохімічні властивості. Відомо, що екологічна ніша впливає навіть на антигенний склад бактерій, тому слід очікувати такого впливу й на інші властивості. Нашими дослідженнями в лабораторних умовах підтверджений збудник бактеріальної водянки – *L. nimipressuralis* та уточнені дані щодо розмірів його клітин.

Ключові слова: *Lelliottia nimipressuralis*, фітопатогенні бактерії, *Betula pendula*, бактеріальна водянка.

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