

THE SYNTHESIS OF PLANT GROWTH STIMULATORS BY PHYTOPATHOGENIC BACTERIA AS FACTOR OF PATHOGENICITY

DANKEVYCH, L.¹ – LEONOVA, N.¹ – DRAGOVOZ, I.¹ – PATYKA, V.¹ – KALINICHENKO, A.^{2,3} –
WŁODARCZYK, P.^{2*} – WŁODARCZYK, B.²

¹*Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine
154 Acad. Zabolotny str., Kyiv 03143, Ukraine*

²*Department of Process Engineering, Faculty of Natural Sciences and Technology,
University of Opole
Dmowskiego str. 7-9, 45-365 Opole, Poland*

³*Faculty of Economics and Management, Poltava State Agrarian Academy
Skovorody str. 1/3, Poltava 36003, Ukraine*

**Corresponding author*

e-mail: pawel.wlodarczyk@uni.opole.pl; phone: +48-77-401-6706

(Received 30th Sep 2017; accepted 12th Feb 2018)

Abstract. The environmental changes significantly influence the microorganisms and affect their properties, leading them to take uncharacteristic ecological niches. This study has focused on the ability of phytopathogenic bacteria that belongs to the genera *Pseudomonas*, *Curtobacterium*, *Ralstonia*, *Pantoea* and *Xanthomonas*, which are able to cause various diseases of legumes, to produce extracellular phytohormones with stimulatory action in vitro. The qualitative and quantitative composition of extracellular auxins and cytokinins has been determined by spectrodensitometric thin-layer chromatography. This research revealed that the synthesis of plant growth promoting phytohormones that are agents of different plant bacterial disease, play an important role in their pathogenicity and ecological plasticity. In particular, it has been established that the level of auxins synthesis by the studied bacteria, which cause diseases of legumes, correlates directly with their pathogenic properties. Also, a clear connection between the pathways of interaction with plant and the amount and spectrum of synthesized auxins and cytokinins has been revealed.

Keywords: *phytohormones, phytopathogenic bacteria, pathogens spreading, auxin, cytokinin, bacterial infection*

Introduction

It is known that the environmental changes associated with anthropogenic factor significantly affect both macro and microorganisms. Some researchers have shown that a change in the ecological conditions of microorganisms' existence leads to changes in their properties, allowing them to expand their area of presence. This tendency involves phytopathogenic bacteria. Pathogenic bacteria of polyphagous and monophagous nature expand the number of plant species that they affect. On the other hand, the bacteria, previously considered as opportunistic, increase their level of aggressiveness (Patyka and Pasichnyk, 2014; Zakharova et al., 2015; Patyka et al., 2015). Such changes of phytopathogen's properties occur due to accepting of new mechanisms of virulence, or to improvement of the ways of disease development.

Phytohormones play the regulatory role not only in the key physiological processes of plants, but they are involved also in plant–microbe interactions (Cui et al., 2009;

Boivin et al., 2016). Five main classes of compounds with phytohormonal activity can be distinguished, such as: auxins, cytokinins, gibberellins, ethylene and abscisic acid. Auxins, cytokinins and gibberellins have a stimulating effect on the key processes of plant ontogenesis, while ethylene and abscisic acid are hormones with inhibiting and anti-stress action. Auxins and cytokinins play an important role in the process of growth and development of plants as well as in their interactions with bacteria, including pathogens. Auxins are derivatives of indole compounds, the most known of which is indole-3-acetic acid (IAA). This compound is produced by plants and microorganisms *de novo* from tryptophan through many pathways (Woodward and Bartel, 2005; Spaepen et al., 2007). Synthesis of auxins and cytokinins as mechanisms to infect plant tissues is quite common in phytopathogenic bacteria. This process is thoroughly investigated in tumor-inducing species, in particular, *Agrobacterium tumefaciens*. In these bacteria the genes responsible for synthesis of cytokinins are transferred by bacterial Ti-plasmids, which are integrated into genome of plants (Jameson, 2000). The similar genes were found in cells of other phytopathogenic bacteria (Kakimoto, 2003; Frébort et al., 2011). But, besides the tumor- and gall-inducing species, other phytopathogenic bacteria, which cause necrosis, chlorosis, wilts or rots of plants, are also able to synthesize auxins and cytokinins (Jameson, 2000). Information about the role of auxins and cytokinins in plant–pathogen interaction is constantly supplemented with new data (Jin et al., 2003; Navarro et al., 2006; Chen et al., 2007; Ding et al., 2008).

Today, there are following concepts of the influence of auxins on disease development: first, exogenous auxins can weaken stiffness of the plant cell wall – a natural barrier against pathogens (Darley et al., 2001). They stimulate fast growth of plant organs by increasing the size of cells by stretching (“acid growth”). At the initial stage of infection process, pathogens synthesize IAA to stimulate the formation of enzymes of “acid growth” by a plant that induces hydrolysis of polysaccharides of the cell wall. Moreover, disaccharides and monosaccharides, which were released during this process, can serve as an ideal power source for pathogens for their survival and further spread (Lindow and Brandl, 2003). Second, exogenous auxins participate in the work of stomata, ensuring the pathways for invasion of some phytopathogens in plant tissues (Nico-Liu et al., 2006; Gottig et al., 2009). Finally, IAA-signaling can be antagonistic to the SAR-pathway (systemic acquired resistance) of formation of plant resistance to diseases (Chen et al., 2007; Pfeilmeier et al., 2016).

Synthesis of cytokinins as a mechanism for successful invasion into plant tissues is widespread among galls-forming bacteria, which usually have plasmid location of genes responsible for their synthesis. But physiological expedience of these hormones synthesis by non-tumors inducing species of phytopathogenic bacteria is not fully clear (Jameson, 2000).

The complete analysis of synthesis of plants growth promoting phytohormones by pathogenic bacteria, which cause various plant diseases, or differ by virulence properties, is rare. The present study is the first report about quantitative and qualitative analysis of extracellular auxins and cytokinins, synthesized by different species of bacteria pathogenic to legumes *in vitro*. This study can provide a new perspective on better understanding the compound role that stimulates plants’ growth and influences virulence of pathogens, plant infections and development of various diseases types. Also this research will hopefully improve the knowledge about different interactions between pathogen and plant.

Materials and methods

The subject of our research is the ability of various types of pathogenic bacteria for legume cultures to synthesize a wide range of extracellular auxins and cytokines. Objects of investigation were strains of phytopathogenic bacteria specified in *Table 1*. Strain *P. savastanoi* pv. *savastanoi* 9174^T, which is able to induce formation of tumors in plants was included in the study as a control.

Table 1. The strains of pathogenic for legumes bacteria used in research

Species, pathovar, strain	Place isolating, a year, the plant host
" <i>Pseudomonas lupini</i> " 8531, 8532, 8533, 8534, 8535	Ukraine, 1963-1971 years, lupine
" <i>Pseudomonas lupini</i> " 17, 6, 22	Ukraine, 2002-2003 years, lupine
" <i>Pseudomonas xanthochlora</i> " 8540	Ukraine, 1963-1971 years, lupine
" <i>Pseudomonas xanthochlora</i> " 3L, 9L	Ukraine, 2006-2007 years, lupine
<i>Pseudomonas syringae</i> pv. <i>syringae</i> * UCM B-1027 ^T (NCPPB 281, ICMP 3023 typical strain)	UK, 1950 year, lilac
<i>Pseudomonas savastanoi</i> pv. <i>phaseolicola</i> UCM B-1123 ^T (**NCPPB 52, ***ICMP 2740 pathotype strain)	Canada - ****, beans
<i>Pseudomonas syringae</i> pv. <i>pisi</i> 9177 ^T (ICMP 2452, NCPPB 2585 pathotype strain)	New Zealand, 1969 year, peas
<i>Pseudomonas savastanoi</i> pv. <i>savastanoi</i> 9174 ^T (NCPPB 639, ICMP 4352 pathotype strain)	Yugoslavia, 1959 year, oil
<i>Pseudomonas marginalis</i> pv. <i>marginalis</i> 9175 ^T (NCPPB 667, ICMP 3553 typical strain)	Belgium, chicory
<i>Pseudomonas savastanoi</i> pv. <i>glycinea</i> 8571 (NCPPB 1139)	Zimbabwe, soybeans
<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> 6564	Ukraine, 1952 year, beans
<i>Ralstonia solanacearum</i> UCM B-1109 ^T (NCPPB 325, ICMP 5712 typical strain)	USA, 1953 year, tomato
<i>Pantoea agglomerans</i> 8490	Romania, 1970 year, oats
<i>Pseudomonas syringae</i> pv. <i>tabaci</i> 225	Ukraine, 1946 year, tobacco
<i>Xanthomonas axonopodis</i> pv. <i>glycines</i> 8609	Ukraine, 1960 year, soybean

Notes: 1.*UCM – Ukrainian Collection of Microorganisms; 2.**NCPPB National Collection of Plant Pathogenic Bacteria, UK; 3.***ICMP – International Collection of Microorganisms from Plants, New Zealand; 4.**** – data available

Phytopathogenic bacteria of the genera: *Pseudomonas*, *Curtobacterium*, *Ralstonia*, *Pantoea* were cultured in the mineral Omelyansky medium (H₂O distilled – 1l.; K₂HPO₄ – 1.0 g; (NH₄)₂HPO₄ – 1.0 g; MgSO₄ – 0.5 g; CaCl₂ – 0.1 g; NaCl and FeSO₄ – trace amounts) for 24-48 h in 750 ml flasks on a shaker (220 rev min⁻¹) at 26-28 °C. Bacteria of the genus *Xanthomonas* were cultured in similar conditions in the synthetic Lich (Lili) medium (H₂O distilled – 1.0 l; casein – 2.0 g; fumaric acid – 1.3 g; K₂HPO₄ – 1.0 g; MgSO₄·7 H₂O – 0.5 g; Na₂CO₃ – 1.1 g; Fe₂(SO₄)₃ – 0.2 mg; ZnSO₄ – 0.2 mg; MgSO₄ – 0.1 mg; glucose – 15 g). To determine the spectrum of exogenous auxins the culture medium was added with 0.1% tryptophan.

In order to separate the biomass, the culture fluids of bacteria were centrifuged for 20 min at 9000 rev min⁻¹ and at 4 °C. The cells of bacteria were washed three times. The supernatants were used for further investigation to extract phytohormonal

compounds. The sediment of cells was suspended in distilled water and then dried at 103-105 °C in a desiccator, until constant weight. The amount of absolutely dry biomass (ADB) of microorganisms was determined gravimetrically. Determination of qualitative and quantitative composition of phytohormones in the culture fluids of phytopathogenic bacteria was performed by spectrodensitometric thin-layer chromatography (Pant and Agrawal, 2014; Leonova, 2015). Extracellular auxins and cytokinins were isolated from the supernatants of phytopathogenic bacteria by extraction of phytohormones with acetic acid ethyl ether at pH 3.0 and with n-butanol at pH 8.0. The obtained extracts were evaporated under vacuum at 40-45 °C. The dry sediment was dissolved in ethanol. Ethanol extracts of the supernatants of the studied bacteria were used for accumulative thin-layer chromatography. Quantitative determination of auxins and cytokinins was performed by using a Sorbfil scanning spectrodensitometer. In the research the synthetic standards of phytohormones were used, produced by companies *Sigma-Aldrich* and *Acros Organic*.

All experiments were performed in 6 iterations. The results obtained were processed statistically using computer program *Excel* from licensed *Microsoft Office 2010*. In the table and the figures the average values and standard errors ($M \pm m$) are presented. Values of $P < 0.05$ were considered to be significant.

Results and discussion

It has been shown that legumes pathogenic bacteria are able to produce a wide range of both auxins and cytokinins (*Fig. 1*).

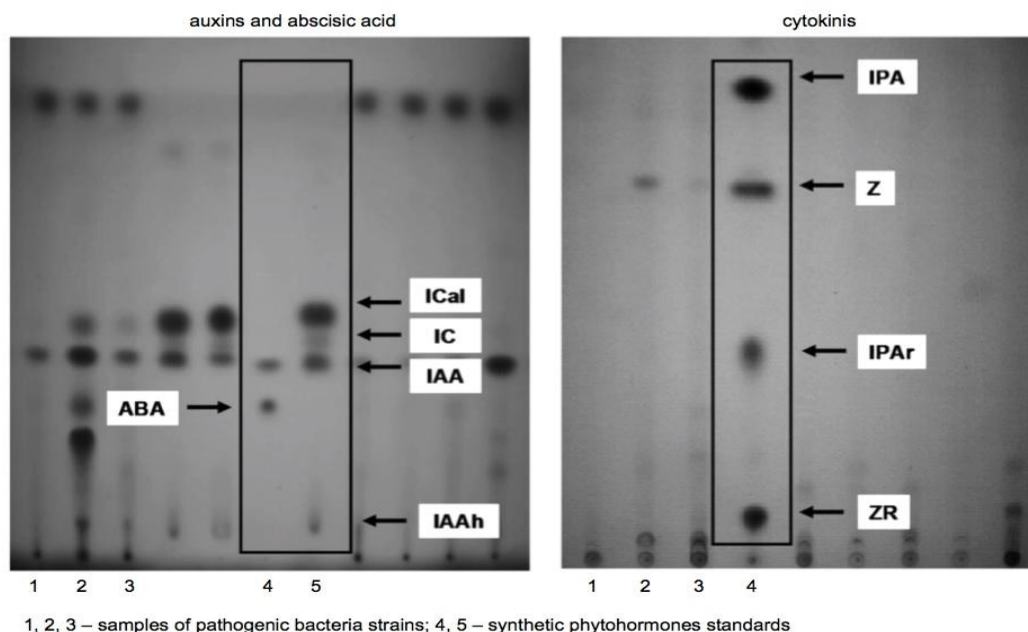


Figure 1. Detection of auxins, abscisic acid and cytokinins by TLC produced by phytopathogenic bacteria in thin layers of silicon oxide and aluminum oxide (Merck, UV₂₅₄): IAA – Indole-3-acetic acid; ICal – Indole-3-carboxaldehyde; IC – Indole-3-carbinol; ABA – abscisic acid; IAAh – Indole-3-acetic hydrazide; IPA – N⁶-(2-Isopentenyl)adenine; Z – zeatin; IPAr – N⁶-(2-Isopentenyl)adenosine; ZR – trans-Zeatin-riboside

The results show that the level of auxin synthesis by legumes pathogenic bacteria correlates directly with their pathogenic properties. Thus, the pathogens, which can affect a wide range of plants (polyphagous) – *P. syringae* pv. *syringae* B-1027^T, *P. marginalis* pv. *marginalis* 9175^T and *P. savastanoi* pv. *savastanoi* 9174^T synthesize higher levels of auxins (from 1987.91 to 1362.46 $\mu\text{g g}^{-1}$ ADB) compared with the phytopathogenic bacteria that cause disease in only one host plant (monophagous) – *P. savastanoi* pv. *phaseolicola* B-1123^T, *P. syringae* pv. *pisi* 9177^T (from 825.87 to 1267.5 $\mu\text{g g}^{-1}$ ADB) (Table 2).

Table 2. Analysis of extracellular auxins and cytokinins synthesis by pathogenic for legumes bacterial strains of the genus *Pseudomonas* with their pathogenic properties

Strains	Amount of phytohormones, $\mu\text{g/g}$ of absolutely dry biomass (ADB)		Pathogenic properties
	Auxins	Cytokinins	
<i>P. syringae</i> pv. <i>phaseolicola</i> UCM B-1123 ^T	1267.5	66.13	Monophagous, bean angular leaf spot
<i>P. syringae</i> pv. <i>pisi</i> 9177 ^T	825.87	92.03	Monophagous, pea bacterial blight
<i>P. syringae</i> pv. <i>syringae</i> UCM B-1027 ^T	1987.91	76.4	Polyphagous, cause different types of plant's disease belongs to more than 50 species
<i>P. marginalis</i> pv. <i>marginalis</i> 9175 ^T	1362.46	170.90	Polyphagous, cause disease of many plants species
<i>P. savastanoi</i> pv. <i>savastanoi</i> 9174 ^T (1 day cultivation)	344.67	331.97	Monophagous, causes tumours of plants which belongs to family <i>Oleaceae</i>

Furthermore, synthesis of auxins by tumor-inducing strain *P. savastanoi* pv. *savastanoi* 9174^T occurs gradually, respectively: 344.67 $\mu\text{g g}^{-1}$ ADB – the first day of culturing and 1893.24 $\mu\text{g g}^{-1}$ ADB – the second day of cultivation. This probably confirms the fact that the gradual synthesis of auxins causes stage-by-stage changes in hormonal regulation of plants, which ultimately leads to a gradual damage of plant tissue structure (Biovin et al., 2016; Jameson, 2000). Instead, already on the first day of cultivation, *P. syringae* pv. *syringae* B-1027^T, *P. marginalis* pv. *marginalis* 9175^T, significant amounts of auxins were synthesized, which indicates the possibility of using this indole compounds as the key virulence factors that quickly unbalance the hormonal regulation system of plants (Biovin et al., 2016; Jameson, 2000). Also, it should be noted that both polyphagous and monophagous differ in the spectrum of synthesized auxins (Fig. 2).

The representatives of pathovars of *phaseolicola*, *pisi*, *syringae*, included in the research, synthesize medium amounts of both IAA and indole-3-carboxylic acids, and indole-3-acetic acid hydrazide (from 41.5–58.3 to 39.71–53.1% of the total amount of auxins). Strain *P. savastanoi* pv. *savastanoi* 9174^T synthesizes almost equal numbers of both IAA and indole-3-carboxylic acids, and indole-3-carboxy-aldehyde (56.05 and 42.5% of the total amount of auxins). However almost 100% of auxins, synthesized by strain *P. marginalis* pv. *marginalis* 9175^T, accounted for IAA and indole-3-carboxylic acid (Fig. 2). No dependency was found between the number and range of synthesized

cytokinins and the pathogenic properties of polyphagous *P. syringae* pv. *syringae* B-1027^T, *P. marginalis* pv. *marginalis* 9175^T, as well as monophagous *P. syringae* pv. *phaseolicola* B-1123^T, *P. syringae* pv. *pisi* 9177^T (Fig. 3).

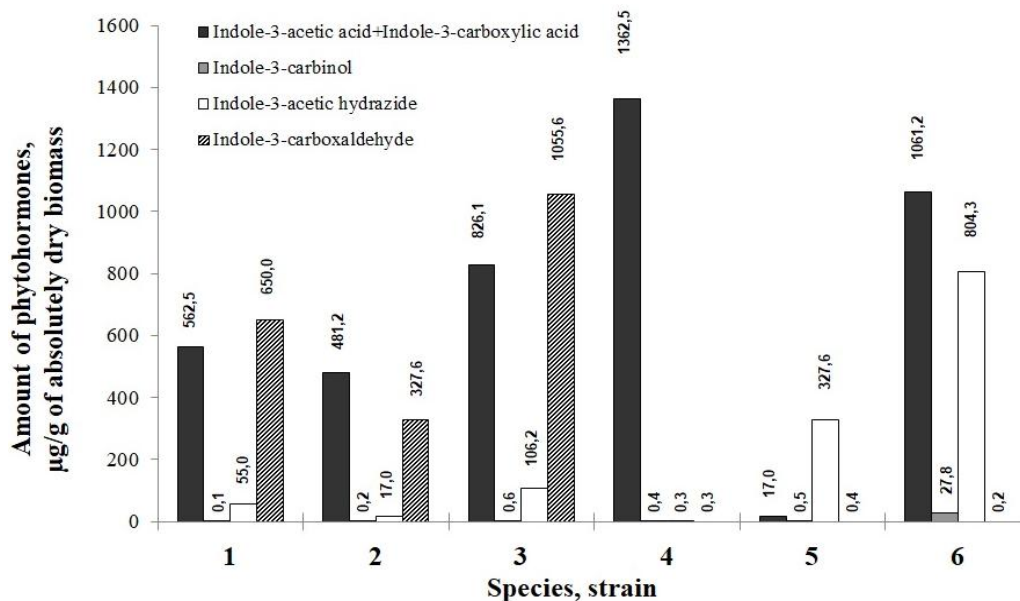


Figure 2. Synthesis of extracellular auxins by pathogenic for legume representatives of genus *Pseudomonas*: 1 – *P. syringae* pv. *phaseolicola* UCM B-1123^T, 2 – *P. syringae* pv. *pisi* 9177^T, 3 – *P. syringae* pv. *syringae* UCM B-1027^T, 4 – *P. marginalis* pv. *marginalis* 9175^T, 5 – *P. savastanoi* pv. *savastanoi* 9174^T (1 day cultivation), 6 – *P. savastanoi* pv. *savastanoi* 9174^T (2 days cultivation). Here and after: $M \pm m$, $n = 6$; $P < 0.05$ vs. control

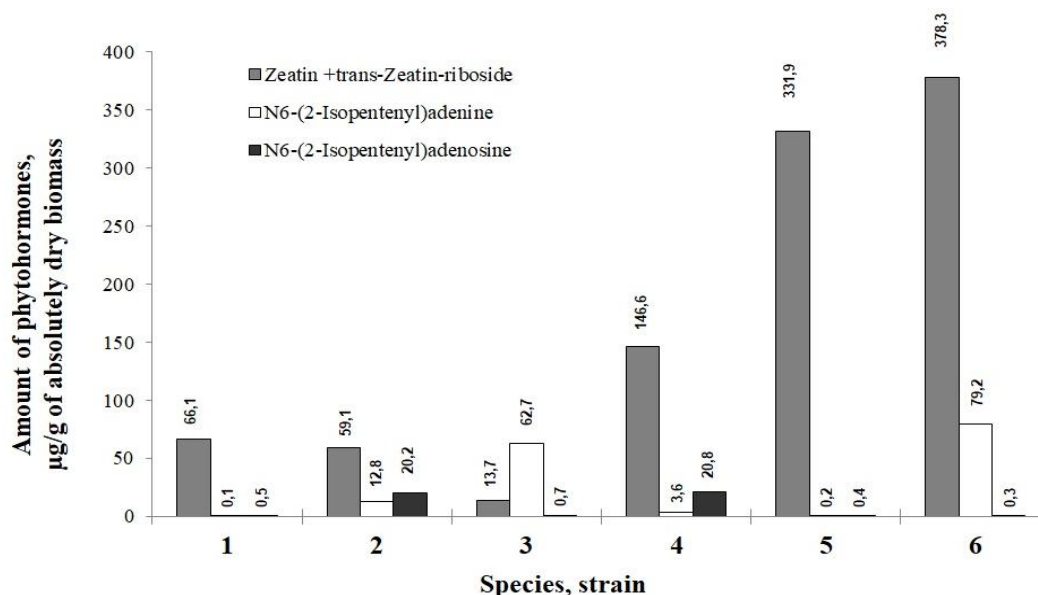


Figure 3. Synthesis of extracellular cytokinins by pathogenic for legume representatives of genus *Pseudomonas*: 1 – *P. syringae* pv. *phaseolicola* UCM B-1123^T, 2 – *P. syringae* pv. *pisi* 9177^T, 3 – *P. syringae* pv. *syringae* UCM B-1027^T, 4 – *P. marginalis* pv. *marginalis* 9175^T, 5 – *P. savastanoi* pv. *savastanoi* 9174^T (1 day cultivation), 6 – *P. savastanoi* pv. *savastanoi* 9174^T (2 days cultivation)

The highest levels of cytokinin synthesis were observed in tumor-inducing strain *P. savastanoi* pv. *savastanoi* 9174^T, both on the first and on the second day of culturing (331.97-457.51 µg g⁻¹ ADB). Also, it should be noted that this strain is able to synthesize the largest amount of such forms of cytokinins as zeatin and zeatin-riboside (Fig. 3).

During the research, on the example of two pathogens of bacterial diseases of lupine: “*Pseudomonas lupini*” (brown bacterial spot) and “*Pseudomonas xanthochlora*” (wet watery rot), a direct correlation between the level of aggressiveness of a strain on plants and the amount of synthesized auxins has been revealed (Table 3).

Table 3. Comparative characteristics of the amounts of extracellular auxins and cytokinins what synthesized by not valid taxa of pathogenic bacteria of the genus *Pseudomonas* with their level of aggressiveness on the zoned lupine’s varieties

Strains	Amount of phytohormones, µg/g of absolutely dry biomass (ADB)		Intermediate aggressive strains on host plants, scores
	Auxins	Cytokinins	
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 8531	1554.82	Not determined	6.6±0.23
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 8532	586.36	94.52	5.6±0.10
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 8533	562.83	Not determined	4.4±0.19
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 8534	914.05		4.4±0.12
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 8535	1168.08		4.5±0.25
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 6	2124.89	102.23	8.5±0.40
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 17	2074.77	Not determined	8.2±0.50
<i>P. syringae</i> (“ <i>P. lupine</i> ”) 22	98.46	4.56	2.0±0.10
“ <i>P. xanthochlora</i> ” 8540	1207.68	27.66	7.7±0.25
“ <i>P. xanthochlora</i> ” 3L	1201.22	23.26	7.0±0.1
“ <i>P. xanthochlora</i> ” 9L	391.99	tr.	2.0±0.5

Note: “tr.” - found trace amounts

In particular, low aggressive strains of *Pseudomonas xanthochlora* produced amounts of auxins 3 times or more smaller than the highly aggressive strains. A similar regularity can be observed for *Pseudomonas lupini*. Thus, low aggressive strain “*Pseudomonas lupini*” 22 synthesizes the amount of auxins 21 or more times less than highly aggressive strains *Pseudomonas lupini* 6 and 17. However, in the medium aggressive and low aggressive collection strains of *Pseudomonas lupini*, this correlation between the level of aggressiveness and the total amount of synthesized auxins is not observed so clearly (Table 3). No direct connection between the range of indole compounds and aggressiveness of the strains has been found. Also, it should be noted that more than 90% of all auxins synthesized by low aggressive strain *Pseudomonas xanthochlora* 9L accounted for IAA, which re-affirms the primary role of the compound in initiation and development of microbe–plant interaction (Fig. 4). In addition, it was established that there is a correlation between the level of aggressiveness of a strain and the amount of synthesized cytokinins. Thus, the

smallest amounts of these compounds are synthesized by low aggressive strains *Pseudomonas xanthochlora* 9L and *Pseudomonas lupini* 22 (from trace amounts to 4.56 $\mu\text{g g}^{-1}$ ADB). Also, it should be noted that in all of the investigated strains the level of zeatin and its transport form zeatin-riboside makes the highest percentage of the pool of synthesized cytokinins (Fig. 5).

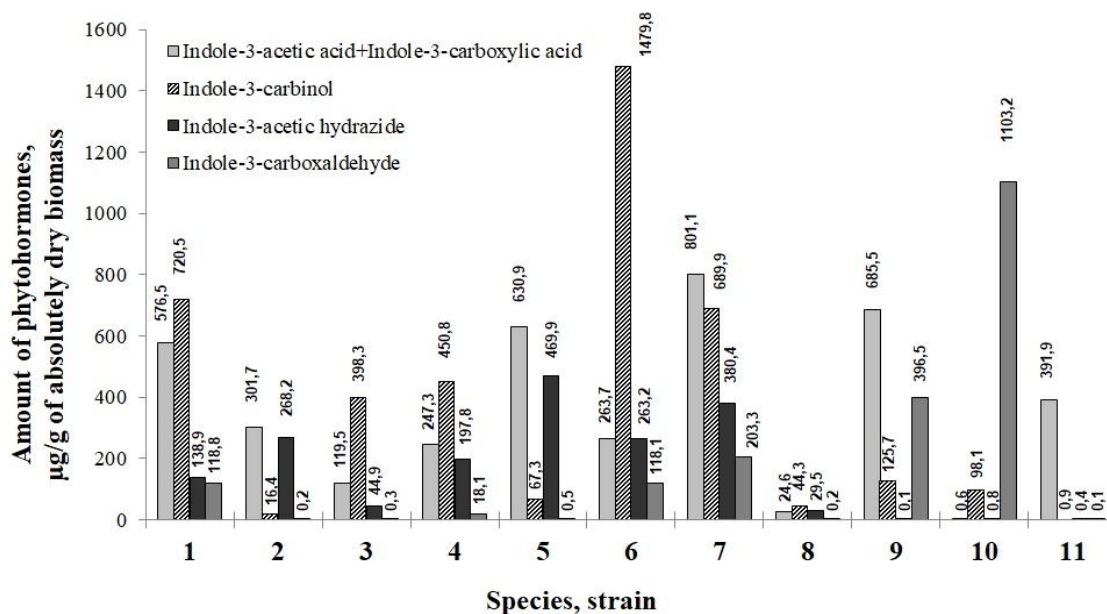


Figure 4. Synthesis of extracellular auxins by pathogenic for lupine representatives of genus *Pseudomonas*: (A) – 1, 2, 3, 4, 5, 6, 7, 8 – “*P. lupini*” 8531, 8532, 8533, 8534, 8535, 6, 17, 22 and 9, 10, 11 – “*P. xanthochora*” 8540, 3L, 9L

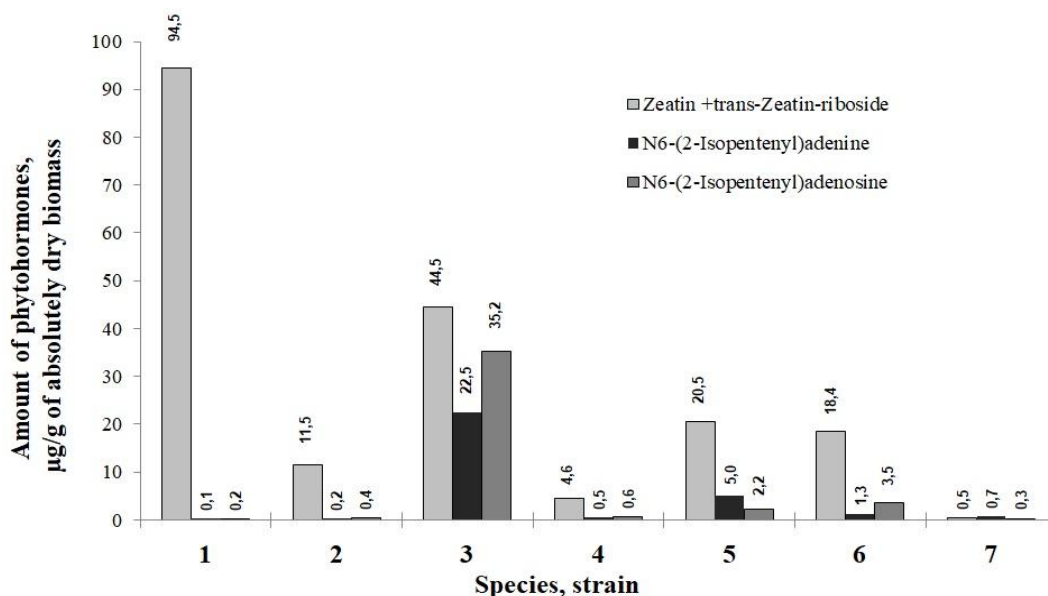


Figure 5. Synthesis of extracellular cytokinin by pathogenic for lupine representatives of genus *Pseudomonas*: 1, 2, 3, 4 – “*P. lupini*” 8532, 8535, 6, 22 and 5, 6, 7 – “*P. xanthochora*” 8540, 3L, 9L

We have studied an ability to synthesize extracellular auxins and cytokinins by pathogens of various types of bacterial diseases of soybean. It has been established that an amount of synthesized auxins and their range depends on the pathogen's properties and the features of its interaction with a plant. Polyphagous *R. solanacearum* UCM B-1109^T, *P. syringae* pv. *tabaci* 225, *C. flaccumfaciens* pv. *flaccumfaciens* 6564 produce large amounts of auxins (from 1512.29 up to 948.8 $\mu\text{g}\cdot\text{g}^{-1}$ ADB) (Table 4) Monophagous *X. axonopodis* pv. *glycines* 8609 and *P. savastanoi* pv. *glycinea* 8571 synthesize more smaller amounts of auxins (from 445.09 to 147.38 $\mu\text{g}\cdot\text{g}^{-1}$ ADB). The highest levels of synthesis of these indole compounds were observed in strains *R. solanacearum* UCM B-1109^T (1256.81 $\mu\text{g}\cdot\text{g}^{-1}$ ADB), *P. agglomerans* 8490 (1327.0 $\mu\text{g}\cdot\text{g}^{-1}$ ADB) and *C. flaccumfaciens* pv. *flaccumfaciens* 6564 (1512.29 $\mu\text{g}\cdot\text{g}^{-1}$ ADB).

Table 4. Comparative analysis of extracellular auxins and cytokinins synthesis by agents of soybean's bacterial diseases with their pathogenic properties

Strains	Amount of phytohormones, $\mu\text{g/g}$ of absolutely dry biomass (ADB)		Pathogenic properties
	Auxins	Cytokinins	
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i> 6564	573.29	50.81	Polyphagous, bacterial tan spot of soybean and bacterial wilt of bean
<i>R. solanacearum</i> UCM B-1109 ^T	1256.81	222.1	Polyphagous, bacterial wilting of many plants
<i>P. agglomerans</i> 8490	1327.0	323.51	Polyphagous, disease like bacterial stem stripe and wet spottiness a of various crops
<i>P. syringae</i> pv. <i>tabaci</i> 225	510.18	194.00	Polyphagous, wildfire of soybean and tobacco
<i>P. savastanoi</i> pv. <i>glycinea</i> 8571	445.09	75.30	Monophagous, angular leaf spot of soybean
<i>X. axonopodis</i> pv. <i>glycines</i> 8609	586.0	102.27	Monophagous, soybean bacterial pustule

Pathogens of bacterial diseases of soybean differ not only by quantitative, but also by the spectrum of synthesized auxins. In particular, the highest levels of IAA and indole-3-carboxylic acid synthesis were found in both monophagous *X. axonopodis* pv. *glycines* 8609 (near 100% of the total amount of auxins) and polyphagous *C. flaccumfaciens* pv. *flaccumfaciens* (over 90%). Also, it should be noted that high level of production of both IAA and indole-3-carboxylic acid was found in *P. agglomerans* 8490 (over 50%). However, low levels of synthesis of IAA and indole-3-carboxylic acid were observed in both monophagous *P. savastanoi* pv. *glycinea* 8571 (about 48%) and polyphagous *P. syringae* pv. *tabaci* 225 (about 28%) and *R. solanacearum* UCM B-1109^T (over 29%) (Fig. 6).

In the investigation of cytokinin activity of strains – pathogens of soybean bacterial diseases, we have shown that the high level of synthesis of cytokinins is observed in polyphagous *R. solanacearum* UCM B-1109^T (222.1 $\mu\text{g}\cdot\text{g}^{-1}$ ADB) and *P. agglomerans* 8490 (323.51 $\mu\text{g}\cdot\text{g}^{-1}$ ADB) (Table 4). Also, it should be noted that both *R. solanacearum*

UCM B-1109^T and *P. agglomerans* 8490 synthesize rather large amounts of a transport form of zeatin – zeatin-riboside (65.17–32.46% of the total amount of cytokinins respectively) (Fig. 6). Instead, the other studied strains have lower levels of synthesis of exogenous cytokinins (from 194.00 to 50.81 µg·g⁻¹ ADB) compared with the above-mentioned strains. In addition, *P. syringae* pv. *tabaci* 225, *C. flaccumfaciens* pv. *flaccumfaciens* 6564 and *X. axonopodis* pv. *glycines* 8609 are also able to synthesize significant amounts of zeatin–riboside (from 22.04 to 51.14%) (Fig. 7).

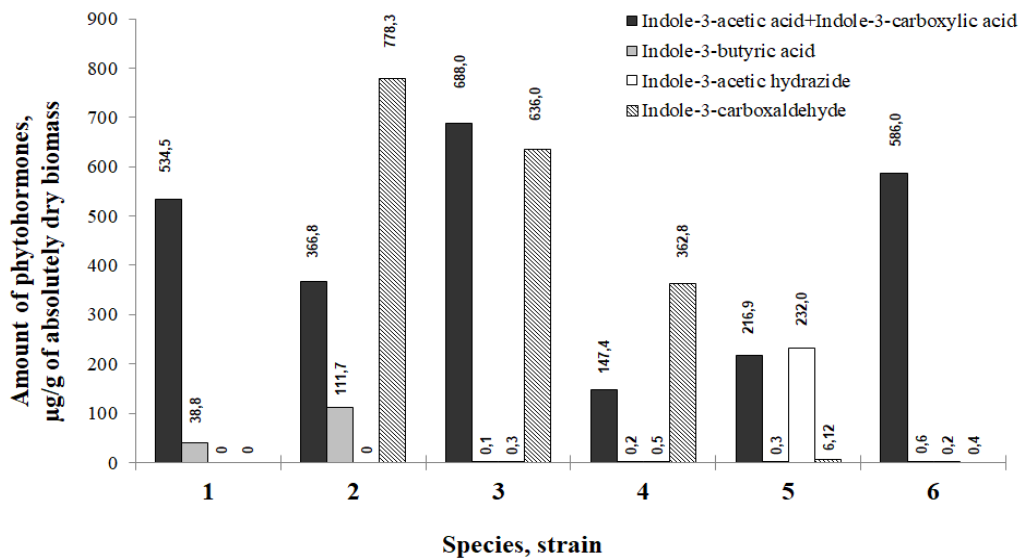


Figure 6. Synthesis of extracellular auxins by pathogenic for soybean bacteria: 1 – *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* 6564, 2 – *Ralstonia solanacearum* UCM B-1109^T, 3 – *Pantoea agglomerans* 8490, 4 – *Pseudomonas syringae* pv. *tabaci* 225, 5 – *Pseudomonas savastanoi* pv. *glycinea* 8571, 6 – *Xanthomonas axonopodis* pv. *glycines* 8609

Discussion

It has been shown that the polyphagous and strains with a high level of aggressiveness synthesize much higher levels of auxins, compared with the monophagous and strains with a low level of aggressiveness. This fact does not contradict the literature data. In particular, certain researchers had shown that spontaneous mutant *Pseudomonas fluorescens* HP72 synthesizes only a small amount of IAA and has a low ability to colonize roots of bent grass (Biovin et al., 2016). The strain of *Erwinia chrysanthemi*, which is mutant by the synthesis of IAA, has very low levels of synthesis of pectate lyase that worsens local maceration of plant's tissues (Yang et al., 2007).

It has been established that an amount of synthesized auxins and their range depends on the biology of a pathogen and the features of its interaction with a plant. In particular, strains *P. syringae* pv. *syringae* B-1027^T, *P. savastanoi* pv. *phaseolicola* B-1123^T, *P. syringae* pv. *pisi* 9177^T, *P. savastanoi* pv. *glycinea* 8571, *P. syringae* pv. *tabaci* 225 and *R. solanacearum* B1109^T synthesize low and medium amounts of both IAA and indole-3-carboxylic acids. It is known that representatives of pathovars of *glycinea*, *phaseolicola*, *pisi*, *syringae*, *tabaci* are able to synthesize specific pathogenicity factors, in particular, toxins, synthesis of which may correlate with production of IAA. Thus, it was shown that strains of *P. syringae* pv. *syringae*, mutant

relating to IAA synthesis, significantly reduced production of syringomycin and, consequently, the virulence (Hwang et al., 2005). In addition, researchers have shown that synthesis of IAA by *R. solanacearum* and *P. syringae* is directly related to HRP-gene-encoded type III secretion system (Type III secretion systems (T3SSs)), which is directly transcribed into effector proteins (hairpins) in the host plant cells and it is the key to development of infection process and disease symptoms (Jin et al., 2003; Fouts et al., 2002). Probably, rather high level of synthesis of auxins, in particular, IAA, by *P. agglomerans* 8490 is related to the ability of this species to colonize all components of phyllosphere, because the interaction with a plant is a characteristic feature of the biology of this species. Also, it is known that *P. agglomerans* are able to synthesize IAA in three ways (Spaepen et al., 2007). It appears that the presented above facts fully explain the research results. The auxins for these strains play a role of signaling molecules that start a cascade of other pathogenicity reactions rather than the role of key factors of virulence, probably. However, in strains *P. marginalis* pv. *marginalis* 9175^T, *X. axonopodis* pv. *glycines* 8609 and *C. flaccumfaciens* pv. *flaccumfaciens* 6564 synthesis of the mentioned above indole compounds plays the crucial role in mechanisms of their interaction with a plant (the increased permeability of the plant cell wall, disturbance in stomata work, inhibition of responses of plant protection, etc.), because, according to the literature, information about them having specific pathogenicity factors and their relationship to synthesis of auxins is almost absent.

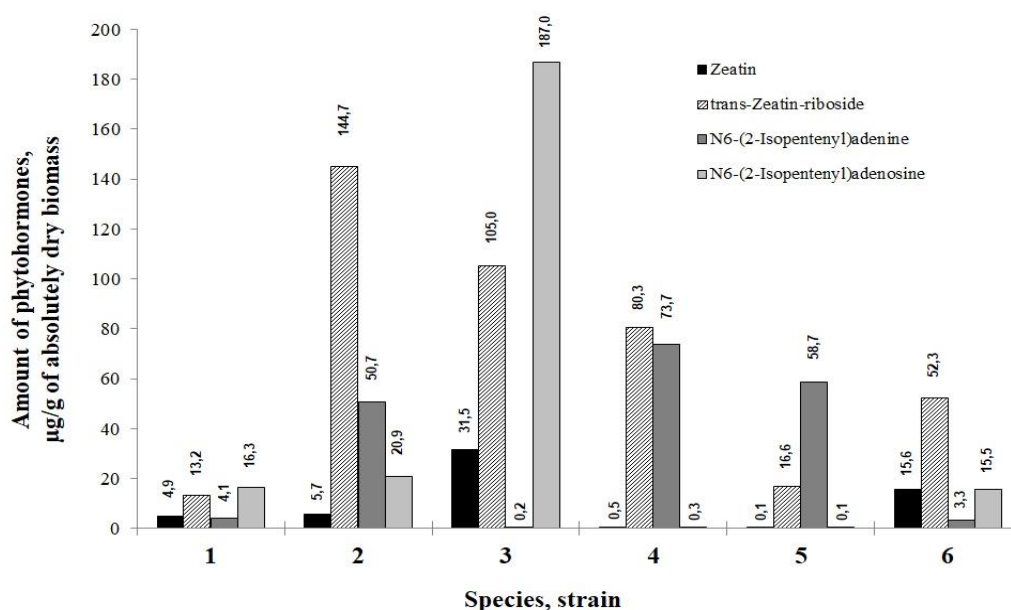


Figure 7. Synthesis of extracellular cytokinins by pathogenic for soybean bacteria: 1 – *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* 6564, 2 – *Ralstonia solanacearum* UCM B-1109^T, 3 – *Pantoea agglomerans* 8490, 4 – *Pseudomonas syringae* pv. *tabaci* 225, 5 – *Pseudomonas savastanoi* pv. *glycinea* 8571, 6 – *Xanthomonas axonopodis* pv. *glycines* 8609

According to the literature, bacteria synthesize cytokinins, using them primarily as a chemical signal to interact with plant or as a mechanism for successful invasion into plant tissues (Giron et al., 2013). Using the example of *R. solanacearum*, a connection between auxin and cytokinin signaling and plant roots susceptibility to this pathogen has been revealed (Kakimoto, 2003; Giron et al., 2013). The high level of synthesis of

cytokinins by *P. agglomerans* 8490 is likely to be connected with peculiarities of the biology of this species. Moreover, as in the case of tumor-inducing species, in representatives of species *P. agglomerans* and *R. solanacearum* the genes *trm/tzs*, responsible for synthesis of auxins and cytokinins, are localized in plasmids. Therefore, the number of their copies is much larger, compared to the species with nucleoid location of the similar genes (Kakimoto, 2003).

Conclusion

It has been determined that the level of synthesized auxins produced by the studied bacteria correlates directly with their pathogenic properties. A connection between the ways of plants' infection and the amount and spectrum of synthesized auxins and cytokinins has been established. In particular, certain types of phytopathogenic bacteria, in which formation of specific pathogenicity factors (toxins, enzymes) is connected to production of auxins, synthesize low and medium amounts of indole-3-acetic acid and indole-3-carboxylic acid. For these pathogens, these compounds probably act as signaling molecules, triggering reactions of other mechanisms of pathogenesis. However, the other species, for which there is not any known connection of synthesis of specific pathogenicity factors with auxin signaling, are able to synthesize significant amounts of mentioned above indole compounds, which probably play a crucial role in their interaction with the plant.

Our investigation of quality and quantity phytohormones compositions synthesized by different pathogenic for plants bacteria will improve the understanding of pathogen-plant interactions and, thus, will create preconditions for developing strategies to prevent the pathogens spreading.

REFERENCES

- [1] Boivin, S., Fonouni-Farde, C., Frugie, F. (2016): How auxin and cytokinin phytohormones modulate root microbe interactions. – *Front. Plant Sci.* 7: 1–12.
- [2] Chen, Z., Agnew, J. L., Cohen, J. D., He, P., Shan, L., Sheen, J., Kunkel, B. N. (2007): *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. – *Proceedings of the National Academy of Sciences of the United States of America* 104(50): 20131–20136.
- [3] Cui, H., Xiang, T., Zhou, J-M. (2009): Plant immunity: a lesson from pathogenic bacterial effector proteins. – *Cellular Microbiology* 11(10): 1453–1461.
- [4] Darley, C. P., Forrester, A. M., McQueen-Mason, S. J. (2001): The molecular basis of plant cell wall extension. – *Plant Mol. Biol.* 47: 179–195.
- [5] Ding, X., Cao, Y., Huang, L., Zhao, J., Xu, C., Li, X., Wang, Sh. (2008): Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independent basal immunity in rice. – *Plant Cell* 20(1): 228–240.
- [6] Fouts, D. E., Abramovitch, R. B., Alfano, J. R., Baldo, A. M., Buell, C. R., Cartinhour, S., Chatterjee, A. K., D'Ascenzo, M., Gwinn, M. L., Lazarowitz, S. G., Lin, N. C., Martin, G. B., Rehm, A. H., Schneider, D. J., Dijk, K., Tang, X., Collmer, A. (2002): Genomewide identification of *Pseudomonas syringae* pv. tomato DC3000 promoters controlled by the HrpL alternative sigma factor. – *Proceedings of the National Academy of Sciences of the United States of America* 99(4): 2275–2280.

- [7] Frébort, I., Kowalska, M., Hluska, T., Frébortová, J., Galuszka, P. (2011): Evolution of cytokinin biosynthesis and degradation. – *J. Exp. Bot.* 62(8): 2431–2452.
- [8] Giron, D., Frago, E., Glevarec, G., Pieterse, C. M. J., Dicke, M. (2013): Plant-Microbe-Insect Interactions. Cytokinins as key regulators in plant–microbe–insect interactions: connecting plant growth and defence. – *Functional Ecology* 27: 599–609.
- [9] Gottig, N., Garavaglia, B. S., Garofalo, C. G., Orellano, E. G., Ottado, J. A. (2009): A filamentous hemagglutinin-like protein of *Xanthomonas axonopodis* pv. *citri*, the phytopathogen responsible for citrus canker, is involved in bacterial virulence. – *PLoS ONE Journal* 4(2): e4358.
- [10] Hwang, M. S. H., Morgan, R. L., Sarkar, S. F., Wang, P. W., Guttman, D. S. (2005): Phylogenetic characterization of virulence and resistance phenotypes of *Pseudomonas syringae*. – *Appl. Environ. Microbiol.* 71(9): 5182–5191.
- [11] Jameson, P. E. (2000): Cytokinins and auxins in plant–pathogen interactions – an overview. – *Plant Growth Regul.* 32: 369–380.
- [12] Jin, Q. L., Thilmony, R., Zwiesler-Vollick, J., He, S. Y. (2003): Type III protein secretion in *Pseudomonas syringae*. – *Microbes and Infection Journal* 5(4): 301–310.
- [13] Kakimoto, T. (2003): Biosynthesis of cytokinins. – *J. Plant Res.* 116(3): 233–239.
- [14] Leonova, N. O. (2015): Abscisic acid and ethylene production by biotechnological strains of *Bradyrhizobium japonicum*. – *Biotechnologia Acta* 8(5): 64–70.
- [15] Lindow, S. E., Brandl, M. T. (2003): Microbiology of the phyllosphere. – *Appl. Environ. Microbiol.* 69(4): 1875–1883.
- [16] Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O., Jones, J. D. (2006): A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. – *Science* 312(5772): 436–439.
- [17] Nico-Liu, D. O., Ronald, P. C., Bogdanove, A. J. (2006): *Xanthomonas oryzae* pathovars: model pathogens of a model crop. – *Molecular Plant Pathology* 7(5): 303–324.
- [18] Pant, G., Agrawal, P. K. (2014): Isolation and characterization of indole acetic acid production plant growth promoting rhizobacteria from rhizospheric soil of *Withania somnifera*. – *Journal of Biological and Scientific Opinion* 2(6): 373–383.
- [19] Patyka, V. P., Pasichnyk, L. A. (2014): Phytopathogenic bacteria in the system of modern agriculture. – *Mikrobiology Zhurnal* 76(1): 21–26.
- [20] Patyka, W., Gnatiuk, T., Zhytkevych, N., Kalinichenko, A., Frączek, K. (2015): Occurrence of the pathogenic bacteria *Pantoea agglomerans* in soybean cultivation. – *Progress in Plant Protection* 55(3): 281–285.
- [21] Pfeilmeier, S., Cally, D., Malone, J. G. (2016): Bacterial pathogenesis of plants: future challenges from a microbial perspective. – *Molecular Plant Pathology* 17(8): 1298–1313.
- [22] Spaepen, S., Vanderleyden, J., Remans, R. (2007): Indole-3-acetic acid in microbial and microorganism – plant signaling. – *FEMS Microbiology Reviews* 31(4): 425–448.
- [23] Woodward, A. W., Bartel, B. (2005): Auxin: regulation, action and interaction. – *Ann. Bot.* 95(5): 707–735.
- [24] Yang, S., Zhang, Q., Guo, J., Charkowski, A. O., Glick, B. R., Ibekwe, A. M., Cooksey, D. A., Yang, Ch.-H. (2007): Global effect of indole-3-acetic acid biosynthesis on multiple virulence factors of *Erwinia chrysanthemi* 3937. – *Appl. Environ. Microbiol.* 73(4): 1079–1088.
- [25] Zakharova, O., Kalinichenko, A., Dankevich, L., Patyka, V. (2015): Rapeseeds bacterial diseases and their REP-PCR analysis. – *Journal of Pure and Applied Microbiology* 9(1): 205–210.