

## ANTIMICROBIAL, ENTOMOPATHOGENIC AND ANTIVIRAL ACTIVITY OF GAUPSIN BIOPREPARATION CREATED ON THE BASIS OF *Pseudomonas chlororaphis* STRAINS

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The aim of this review was to present the results of more than ten-year study of gaupsin biopreparation created on the basis of two strains *Pseudomonas chlororaphis* subsp. *aureofaciens* UCM B-111 and UCM B-306 with antifungal, entomopathogenic and antiviral activities. Data about antibiotic substances produced by these strains — phenazine and phenylpyrrole derivatives — are presented. Entomocidal properties against the wide spectrum of insect pests have been found out in the strains-producers. Antiviral activity of gaupsin due to the production of thermostable exopolymers containing neutral monosaccharides has been shown using the tobacco mosaic virus as a model. Lipopolysaccharides of the strains B-111 and B-306 also appeared to be highly active antiviral agents. Structure of their O-specific polysaccharides has been established. The last one are structurally heterogenic, presented by linear tri- and tetrasaccharide repeated links and have specific structure that has not been described previously.

**Key words:** gaupsin biopreparation, *Pseudomonas chlororaphis* subsp. *aureofaciens*, antibiotics — phenazines, pyrrolnitrine, entomopathogenic properties, antiviral activity.

Saprophytic bacteria *Pseudomonas chlororaphis* belong to one of the most active producers of antibiotic substances among various species of pseudomonads [1, 2]. In recent decades, significant progress has been made in their use for the biological protection of plants from pathogens [3, 4]. Preparations containing strains of this species are widely used throughout the world for plants protection of fungal diseases. It is enough to refer to the strain *Pseudomonas chlororaphis* MA 342, isolated in Sweden in 1991, offered to protect cereals of these diseases. Biopreparation based on this strain was patented by the Swedish company Bio Agri AB under the trade name Cedomon, registered and used in many European countries [5]. According to the US Environmental Protection Agency conclusion, *P. chlororaphis* strains are

non- pathogenic and non-toxic to humans, biota and environment [6].

At the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, the complex biopreparation gaupsin based on two strains of *P. chlororaphis* subsp. *aureofaciens*, which inhibits the growth of phytopathogenic bacteria and fungi, was created and patented in Ukraine in 2005 [7]. The strains of *P. chlororaphis* subsp. *aureofaciens* UCM B-111 and UCM B-306 (UCM — Ukrainian collection of microorganisms) in gaupsin composition were selected in a large-scale study of the antagonistic activity of a large collection of bacteria of the genus *Pseudomonas* against phytopathogenic fungi and bacteria, as well as study of their effect on some pests. The preparation is a cellular biomass of both

strains with a culture medium. In recent years, we have established some new properties of strains that are the gaupsin components, expanding the understanding of their biological activity and the prospects of their use in the pathogens and plant pests control.

Below we'll mention briefly some important features of the biological activity of *P. chlororaphis* subsp. *aureofaciens* strains — components of the biopreparation gaupsin and the substances produced by them and determining its activity. Some of these substances are found in many strains of the genus *Pseudomonas* and have been studied in detail; others have been discovered and studied by us for the first time.

#### *Antimicrobial substances of strains—components of the preparation gaupsin*

Up to the present, phytopathogenic fungi and, to a lesser extent, bacteria, are considered as the main target of the *P. chlororaphis* strains action. Siderophores — substances that carry out the iron transport, especially pseudobactin (pyoverdin) — the yellow-green fluorescent pigment of bacteria of the genus *Pseudomonas*, belong to the compounds that play an important role in limiting the number of pathogens [8]. Pyoverdin is the instrument of bacteria competition for iron with siderophores of phytopathogenic fungi, which have a lower iron binding constant. Thus, the fungistatic effect of pseudobactin is associated with the creation of iron deficiency for fungi — plant pathogens. Strains B-111 and B-306, which are the gaupsin components, actively synthesize pseudobactin, as evidenced by yellow-green fluorescence at their cultivation in both commonly used and special media.

The antibiotics of phenazine group are important representatives of antimicrobial compounds formed by *P. chlororaphis* [1, 2, 9, 10], studied in detail in three species of pseudomonads: soil isolates of *P. fluorescens* and *P. chlororaphis* and opportunistic human pathogen *P. aeruginosa*. Three phenazine pigments were isolated from the strains—components of the biopreparation gaupsin: yellow phenazine-1-carboxylic acid, orange 2-hydroxyphenazine-1-carboxylic acid and 2-hydroxyphenazine.

Chromato-mass spectrometric studies have shown the presence of phenazine derivatives both directly in gaupsin (on a production medium) and when growing of bacteria on King A medium, which is optimal for this group of pigments biosynthesis.

At the same time, the B-111 strain possessed a high phenazine-synthesizing activity, 520–600 mg/l of phenazine-1-carboxylic acid, 90–128 mg/l of 2-hydroxyphenazine-1-carboxylic acid and 20–35 mg of 2-oxyphenazine were present in the culture liquid. The same compounds were present in the culture liquid of strain B-306, but in smaller amounts (350–380, 60–75 and 15–20 mg/l, respectively).

Table 1 presents the results of studying the antifungal activity of phenazine pigments synthesized by strains B-111 and B-306. All of them inhibit the growth of phytopathogenic fungi (2-hydroxyphenazine-1-carboxylic acid is the most active among them). These substances inhibit also the growth of bacteria—plant pathogens: *Clavibacter michiganense*, *Agrobacterium tumefaciens*, and *Xanthomonas malvacearum*. Phenazine-1-carboxylic acid acts on them in concentrations of 100–200 µg/ml; 2-hydroxyphenazine-1-carboxylic acid — of 5–50 µg/ml, 2-hydroxyphenazine — of 50–20 µg/ml, respectively.

For a long time phenazines were considered only as antimicrobial compounds. However, in the last decades these ideas have been revised and supplemented by numerous experimental data demonstrating the important role of phenazines in the microbial cell: as electron carriers possessing a significant redox potential and affecting the reduction of  $Fe^{3+}$  to  $Fe^{2+}$  ions that are more accessible for both bacteria and plants, as signaling molecules that induce through the expression of genes the mechanisms of plant protection from infection, as well as agents that play an important role in the formation of microbial biofilm, and many other properties [1, 2, 10]. The formation of biofilms on the roots of plants with fluorescent bacteria of the genus *Pseudomonas* and the participation of phenazines in these processes has been the subject of numerous reports. It has been found that phenazine-1-carboxylic acid affects the growth of cells in the biofilm itself, and 2-hydroxyphenazine-1-carboxylic acid, synthesized by the strain *P. chlororaphis*, simultaneously with the above-mentioned compound affects on the adhesion of cells to surfaces [11]. All these properties along with antibiotic activity determine the use of pseudomonads for biological protection of plants and their productivity increase.

With the ability to synthesize antibiotics of the phenazine group (and possibly different chemical nature compounds as well), the action of *P. chlororaphis* strains on phytopathogenic nematodes, which inhabit the soil extensively

Table 1. Antifungal activity of phenazine pigments synthesized by *Pseudomonas chlororaphis* subsp. *aureofaciens* strains — gaupsin components [9]

Phytopathogenic fungi	Minimal fungi growth inhibitory concentration, µg/ml		
	Phenazine-1-carboxylic acid	2-Hydroxyphenazine-1-carboxylic acid	2-Hydroxyphenazine
<i>Fusarium avenaceum</i>	200	200	400
<i>Fusarium oxysporum</i>	200	200	–
<i>Mucor plumbeus</i>	200	100	200
<i>Drechslera graminea</i>	50	20	50
<i>Rhizopus arrhizus</i>	100	100	–
<i>Rhizoctonia solani</i>	200	200	–
<i>Monilia fructigena</i>	200	100	200
<i>Alternaria</i> sp.	100	50	–
<i>Botrytis cynerea</i>	50	20	100

Note: “–” — the activity against the test culture was not investigated.

and cause significant crop losses of up to 70%, is associated [12]. In our experiments, the strains of this species were the most active antagonists of the stem nematode of potato *Ditylenchus destructor* in comparison with other species of pseudomonads [13]. Pyrrolnitrine contributes to the antifungal effect of gaupsin; it is active antifungal agent of the phenylpyrrole group. We detected this antibiotic in a culture liquid of the strain B-306. It is effective against Gram-positive and Gram-negative bacteria and acts on many species of parasitic and saprophytic fungi in concentration of 0.19–10 µg/ml. This antibiotic was not detected in the culture liquid of the strain B-111.

Thus, the compounds responsible for the antifungal and antibacterial activity of the preparation gaupsin are, according to our data, derivatives of phenazine and phenylpyrrole. The intensity of the biosynthesis of these antibiotics by the bacteria that are the part of the preparation and their activity against fungi and bacteria — causative agents of plant diseases is characterized.

#### *Effect of strains-components of the preparation gaupsin on insect-pests*

No less important than the antimicrobial, but much less studied is the entomopathogenic activity of *P. chlororaphis* strains. Ten

supported in the UCM (Ukrainian Collection of Microorganisms) strains of *P. chlororaphis* subsp. *aureofaciens* limited the number of apple moths caterpillars of the 5<sup>th</sup> age (mortality 26–85%) and the Colorado potato beetle larvae of the 4<sup>th</sup> age (mortality 25–60%). In this case, insects perished from septicemia and the action of an entomopathogenic toxin [7]. The most active strain was the UCM B-306; based on these experiments it was included into the composition of gaupsin. Many other insect-pests are sensitive to gaupsin (Table 2, 3).

For many decades, the point of view (which also was reflected in the diagnosis of the genus *Pseudomonas*) was generally accepted, that pseudomonads do not possess entomopathogenic activity. However, in 2006 a species of *P. entomophila*, highly pathogenic for many insects belonging to different orders, was described [14]. A complete sequencing of the genome of a type strain of the named species was carried out and the presence of an insecticidal toxin with high haemolytic activity, lipases and extracellular proteases involved in the death of insects was made in these bacteria. In 2012, by genetic analysis methods with the full sequencing of the genome of the *Pseudomonas chlororaphis* GP72 strain, used for biocontrol and colonizing the root system of plants, the Chinese authors identified an insecticidal toxin gene [15].

Table 2. Entomocide activity of *Pseudomonas chlororaphis* subsp. *aureofaciens* strains against caterpillars of apple moth and larvae of the Colorado potato beetle [7]

Strains <i>P. chlororaphis</i> subsp. <i>aureofaciens</i>	Apple moth caterpillars (L5)		Colorado potato beetle larvae (L4)	
	Total mortality, %	Imago yield, %	Total mortality, %	Imago yield, %
B-108	31	69	45	55
B-109	80**	20	30	70
B-110	30	70	30	70
B-111	70**	30	55**	45
B-112	35	65	25	75
B-113	40	60	30	70
B-114	62*	38	50*	50
B-306	85**	15	60**	40
B-360	26	74	30	70
B-361	62*	38	40	60
Control (without treatment)	25	75	25	74
HCP 0,95	12.2		22.0	
HCP 0,99	22.4		40.4	

Note. Hereinafter: \* —  $P < 0.05$  relative to control; \*\* —  $P < 0.01$  relative to control.

Table 3. Entomocidal activity ( $CK_{50}$ ) of *Pseudomonas chlororaphis* subsp. *aureofaciens* strains — gaupsin components [7]

Insect species	Development stage	Strains number	
		B-306	B-111
Lead-stripped leaf rollers ( <i>Ptycholoma lecheana</i> )	L 2	1.2	8.7
Meshleaf roller ( <i>Adoxophyes orana</i> )	L 2	0.6	11.7
Currant curled torturer ( <i>Pandemis cerasana</i> )	L 3	1.1	16.7
Bunch leaf roller ( <i>Lobesia botrana</i> )	L 2	0.9	2.0
Plum moth ( <i>Grapholita funebrana</i> )	L 5	0.4	1.4
Apple moth ( <i>Hyponomeuta malinella</i> )	L 3	0.4	1.3
Gooseberry fruit moth ( <i>Zophodia convolutella</i> )	L 4	0.6	10.7
Pudenitsa plum ( <i>Angerona prunaria</i> )	L 4	0.3	38.3
Colorado beetle ( <i>Leptinotarsa decemlineata</i> )	L 4	4.2	14.7

Note.  $CK_{50}$  is the number of cells (millions in ml) which causes the 50 % insects mortality

According to our data, some of the enzymes mentioned above are also present in strains of *P. chlororaphis* subsp. *aureofaciens* [13]. However, the nature of the toxin formed by representatives of this species has not yet been established and requires study.

#### *Antiviral activity of the preparation gaupsin*

In 2010, studies were begun on the antiviral activity of gaupsin and the compounds responsible for it. It has been established that during three growth seasons gaupsin inhibited the development of the tobacco mosaic virus (TMV) of the strain U1 in experiments *in vivo* in *Datura stramonium* and *Nicotiana tabacum* cultures for 80–97% [9, 16, 17]. Active with respect to TMV were culture fluids obtained both with joint (gaupsin) and with separate growth of the strains B-111 and B-306 on the production medium, and on the medium King A as well.

Water-soluble thermostable preparations were obtained from the fermentation medium by evaporation, dialysis and lyophilization. Both dialyzed and non-dialyzed preparations exhibited similar antiviral activity, which indicated the high molecular weight of antiviral agents. The most significant effect (a decrease in the infectivity of TMV by 76.0–97.5%) on the leaves of tobacco and stramonium was obtained at a concentration of 10 mg/ml; the antiviral effect progressively decreased at concentrations of 1.0–0.1 mg/ml (40–57 and 24–27%, respectively).

The antiviral substances of gaupsin were not inactivated at 100 °C for 10 min and, apparently, do not contain protein components. The results of the analysis indicate that they are exopolymers containing neutral monosaccharides (fucose, mannose, galactose and glucose). Their chemical structure is not established.

Highly active antiviral agents were lipopolysaccharides (LPS), isolated from cells of the strains B-111 and B-306 [18–23]. They suppressed the infectivity of TMV on the model of three species of indicator plants (stramonium *Datura stramonium* L., tobacco *Nicotiana tabacum* L., variety Immune 580, and *Nicotiana sanderae* H.) at 0.001–10.0 mg/ml. The O-specific side chains of LPS of both strains did not inhibit TMV, and often stimulated the development of the virus. At the same time, core oligosaccharides inhibited the development of viral infection to varying degrees.

It is known that lipopolysaccharides of growth-stimulating rhizobacteria can act as signaling molecules, causing nonspecific activation of plant protective mechanisms (induced systemic resistance — ISR) [19]. It can be assumed that this mechanism may cause the antiviral effect of lipopolysaccharides of the strains — gaupsin components. At the same time, according to electron microscopy data, obtained during direct contact of LPS with the virus *in vitro*, the virions “stick together” forming “ligaments”, while in the control separate free virus particles were observed, which indicates a direct interaction between the lipopolysaccharides of the strains of *P. chlororaphis* subsp. *aureofaciens* and TMV.

Thus, for the first time, the antiphytoviral effect of the gaupsin preparation and the strains of *P. chlororaphis* subsp. *aureofaciens* in its composition has been found. It is shown that high antiviral activity is caused both by the synthesis of exopolysaccharides and by the lipopolysaccharides of the strains—gaupsin components, and in the latter one by core oligosaccharides. Further studies of *P. chlororaphis* subsp. *aureofaciens* LPS have demonstrated their high effect in respect of viruses of warm-blooded — influenza, herpes and hepatitis C [24].

The next stage of the work, carried out in 2015–2016, was to clarify the O-specific polysaccharides structure of the strains B-306 and B-111, as well as of the *P. chlororaphis* UCM B-106 type strain [21, 22].

It is shown that O-polysaccharides are structurally heterogeneous, represented by linear repeating tri- and tetrasaccharide units and possess a previously undescribed unique structure, and probably contribute to the high biological activity of *P. chlororaphis* lipopolysaccharides.

#### *Gaupsin practical application*

According to our data, the biopreparation gaupsin, as well as the strains of *P. chlororaphis* subsp. *aureofaciens*, has a wide range of properties that provide effective protection of agricultural plants. First of all, it is the synthesis of antifungal and antibacterial substances that inhibit the causative agents of plant diseases, which are thoroughly studied and widely used in the world practice of biological control, and also much less studied entomopathogenic properties (the nature of the insecticidal toxin has not been studied) and, finally, antiviral activity not known previously. The nature

and (partly) the structure of substances that inhibit phytoviruses have been established, but the role of these compounds as biocontrol instruments has to be evaluated.

Gaupsin activity is directed against a wide range of pathogens. Most of them are also a target of the action of chemical plant protection products used in agriculture. All studied strains of *P. chlororaphis* were resistant to modern chemical fungicides and insect growth regulators [25]. The resistance of bacteria to these herbicides suggests the possibility of their joint use, which could lead to a net beneficial effect, as well as the reduction in chemical preparations consumption, and significant reduction of the pesticide press to the biocenosis, and the production of output yield free from harmful chemicals.

At present, the biopreparation gaupsin is produced by a number of Ukrainian enterprises in accordance with the licensing agreements for release and sale, concluded with the developer of the gaupsin — Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. Our partners — SE Enzyme, Ltd. “Research and Production Center Cherkasy Bio Protection”, Ltd. “Company BioNik”, Ltd. “Agricultural biotechnology” — established the production of the preparation, the main consumers of which are small and medium-sized farms, as well as private individuals.

Since the release of the preparation on the agricultural market up to the present day, numerous data have been obtained, confirming the high effectiveness of gaupsin as a polyfunctional biological agent with pronounced fungicidal, insecticidal and growth-stimulating activity. Below we will confirm this conclusion with several examples.



Thus, Table 4 illustrates the fungicidal effect of the preparation for the protection of soybean from a number of the most common fungal diseases in western Polissya (2014). Soybean treatment with gaupsin (5 l/ha) inhibited the development of diseases at a level similar to, or slightly below, the Abacus fungicide (BASF). This chemical preparation is a two-component fungicide of the new generation, containing epoxiconazole and pyraclostrobin. It is used to control dangerous diseases of cereals, corn, soybeans and sugar beet and presented in the Ukrainian market as a premium fungicide for soybean use.

Gaupsin also shows a high activity for the protection of plants from insect pests: codling moth, aphids, pyralid moths (snout moths, grass moths, bud moth or flyweed), bugs, cicadas, moths, Colorado beetle, Owllet moths, wheat thrips, ground beetles, geometer moths, pyralid moths (snout moths or grass moths), mites. The effectiveness of gaupsin applying to protect apple trees from red spider mites (8 l/ha + EPPA-10 0.25 l/ha) was shown by production researches conducted in 2013 at the Ltd. “Agro firm Bilozerske” of the Kherson region (Table 5).

As a control, a tank mixture of chemical preparations Omite (2 l/ha) and Apollo (0.4 l/ha) was chosen. The active ingredients of the aforementioned acaricides of contact action are propargite and clofentezine, respectively, which are evaluated as highly dangerous and moderately dangerous substances for humans. The efficiency of a tank mixture of chemical preparations was 84.9 % compared to 82.7 % after treatment with gaupsin. Subsequent treatments of apple trees with chemical preparations increased the rate of insecticide application and led to an increase in pest resistance.



*Pseudomonas* spp. and biopreparat Gaupsin

Table 4. Gaupsin antifungal efficacy on soybean

Preparation	Tier	Cercosporosis			Ascochitosis			Peronosporosis			Septoriosis		
		growth, %	spreading, %	efficiency, %	growth, %	spreading, %	efficiency, %	growth, %	spreading, %	efficiency, %	growth, %	spreading, %	efficiency, %
Control preparations (without fungicidal treatment)	Bottom	7.5±0.4	32.0±1.5		8.0±0.6	25.0±1.5		12.5±1.1	100		3.5±0.4	16.2±1.3	
	Top	12.5±0.5	45.0±2.3		15.0±1.2	48.2±2.4		16.0±1.3	100		5.2±0.4	10.1±0.9	
Gaupsin 5.0 l/ha	Bottom	3.2±0.2*	22.0±1.6	57.3±3.4	3.5±0.3*	30.0±1.9	56.3±3.3	4.2±0.2**	100	66.4±5.9	2.0±0.1	10.0±0.9*	42.9±3.7
	Top	11.5±0.5	32.1±1.7	8.0±0.4	13.8±1.2	35.5±2.3	8.0±0.4	8.5±0.7*	100	46.9±3.7	3.2±0.3	8.0±0.6	38.5±3.3
Abacus 1.5 l/ha	Bottom	3.0±0.08*	25.0±1.6	60.0±3.7	3.0±0.3*	30.0±2.2	62.5±3.6	3.5±0.3**	100	72.0±6.8	2.2±0.3	10.5±1.0*	62.9±4.8
	Top	10.0±0.4	25.7±1.6*	20.0±0.8	13.3±1.4*	29.8±1.9	11.3±1.2	8.0±0.4**	100	50±4.2	3.0±0.3	7.5±0.6*	42.3±3.1

Table 5. Gaupsin effectiveness at apple trees protecting from red spider mites

Processing date	Amount/leaf									
	Imago			Larvae			Eggs			death, %
	living	dead	death, %	living	dead	death, %	living	dead	death, %	
	Gaupsin									
09.08	1.1 ± 0.1	2.4 ± 0.3	68.6 ± 5.9	–	–	–	8.75 ± 0.4	15.45 ± 1.2	63.8 ± 6.3	
13.08	4.3 ± 0.4**	20 ± 2.2**	82.7 ± 7.2**	1.0 ± 0.09	2.1 ± 0.2	67.7 ± 6.3	12.25 ± 1.3	18.5 ± 1.7	59.7 ± 5.6	
	Omite + Apollo									
09.08	4.1 ± 0.3**	23.2 ± 2.3**	84.9 ± 7.8	2.1 ± 0.1	2.8 ± 0.3	57.1 ± 5.4	9.3 ± 0.8	17.6 ± 1.4	65.4 ± 6.3	

At the same time, the cost of chemical treatment was 3 times higher than of biological treatment.

Thus, a wide spectrum of biological activity favorably distinguishes gaupsin from numerous monovalent preparations directed against one (or one group) of plant pests. Current issues remain related to the substances responsible for the beneficial effect of gaupsin: the elucidation of the chemical structure of exopolymers responsible for the antiviral effect of the preparation; the study of the mechanisms of its action and effectiveness in field experiments against economically important pathogens of viral diseases of agricultural plants in Ukraine; the elucidation of the nature of the entomopathogenic toxin formed by bacteria; the further improvement of biotechnology and various forms of gaupsin usage.

#### REFERENCES

1. Chincholkar S., Tomashov L. Microbial Phenazines: Biosynthesis, Agriculture and Health. *New York: Springer Berlin Heidelberg*. 2013, 248 p.
2. Bernd H., Rehm A. Pseudomonas. Model Organism, Pathogen, Cell Factory. *Wiley-VCH*. 2008, 395 p.
3. Weller D. Pseudomonas Biocontrol Agents of Soil-born Pathogens: Looking Back over 30 years. *Phytopathology*. 2007, V. 97, P. 250–256. doi: 10.1094/PHYTO-97-2-0250.
4. Mercado-Blanco J., Bakker P. Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection. *Antonie van Leeuwenhoek*. 2007, 92 (4), 367–89. doi: 10.1007/s10482-007-9167-1.
5. Tombolini R., van der Gaag D., Gerhardson B., Jansson J. Colonization Pattern of the Biocontrol Strain *Pseudomonas chlororaphis* MA 342 on Barley Seeds Visualized by Using Green Fluorescent Protein. *Applied and Environmental Microbiology*. 1999, 65 (8), 3674–3680.
6. US Environmental Protection Agency. *Pseudomonas chlororaphis* strain 63-28 (006478) Fact Sheet. Cited by [http://www.epa.gov/opppbd1/biopesticides/ingredients/factsheets/factsheet\\_006478](http://www.epa.gov/opppbd1/biopesticides/ingredients/factsheets/factsheet_006478).
7. Kiprianova E., Goral S. Insectofungicidal preparation gaupsin for the control of pests and agricultural plants diseases. *Patent of Ukraine* № 73682 AO 1N 63/00 C 12 N 1/20, publ. 15. 08. 2005. (In Ukrainian).



8. Cézard C., Farvacques N., Sonnet P. (2015-01-01). Chemistry and biology of pyoverdines, *Pseudomonas* primary siderophores. *Curr. Med. Chem.* 2015, 22 (2), 165–186. doi: 10.2174/0929867321666141011194624.
9. Kiprianova E., Shepelevich V., Klochko V., Ostapchuk A., Varbanets L., Scoklyuk L., Berezkinas A., Avdeeva L. Antifungal and antiviral substances of *Pseudomonas chlororaphis* subsp. *aureofaciens* strains — components of gaupsin. *Microbiol. Zh.* 2013, 75 (6), 28–35. (In Russian).
10. Pierson L., Pierson E. Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. *Appl. Microbiol. Biotechnol.* 2010, 86 (6), 1659–1670. doi: 10.1007/s00253-010-2509-3.
11. Wang D., Yu J. M., Dorosky R. J., Pierson L. S., Pierson E. A. The Phenazine 2-Hydroxyphenazine-1-carbonic acid promotes extracellular DNA release and has broad transcriptomic consequences in *Pseudomonas chlororaphis* 30-84. *PLoS One.* 2016, 26, 11 (1), e0148003. doi: 10.1371/journal.pone.0148003.
12. Lee J., Ma K., Ko S., Kang B., Kim I., Kim Y. Nematicidal activity of a nonpathogenic biocontrol bacterium, *Pseudomonas chlororaphis* O6. *Curr. Microbiol.* 2011, 62 (3), 746–751. doi: 10.1007/s00284-010-9779-y.
13. Smirnov V., Kiprianova E. Bacteria of the *Pseudomonas* genus. *Kyiv: Naukova dumka.* 1990, 263 p. (In Russian).
14. Vodovar N., Vallenet D., Cruveiller S., Rouy Z., Barbe V., Acosta C., Cattolico L., Jubin C., Lajus A., Segurens B., Vacherie B., Wincker P., Weissenbach J., Lemaître B., Medigue C., Boccard F. Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. *Nat. Biotechnol.* 2006, 24 (6), 673–679.
15. Xuemei S., Mingmin C., Hongbo H., Wei W., Huasong P., Ping X., Xuehong Z. Genome Sequence of *Pseudomonas chlororaphis* GP72, a Root-Colonizing Biocontrol Strain. *J. Bacteriol.* 2012, P. 1269–1270. doi: 10.1128/JB.06713-11.
16. Balko O., Kiprianova O., Kovalenko O., Shepelevitch V., Avdeeva L. Antiphytoviral activity of biopreparation gaupsin. *Microbiol. i Biotechnol.* 2010, N 2, P. 51–57. (In Ukrainian).
17. Shepelevitch V., Shubchynskyy V., Varbanets L., Kiprianova E. Antiviral Activity of Carbohydrate-containing Biopolymers of *Pseudomonas chlororaphis* subsp. *aureofaciens*. Twenty-Fours International Conference on Antiviral Research (ICAR). Sofia, Bulgaria (May 8–11, 2011). 2011, P. 64.
18. Kiprianova E., Varbanets L., Shepelevitch V., Voychuk S. Antiviral activity of *Pseudomonas chlororaphis* subsp. *aureofaciens* lipopolysaccharides. *Biotechnologia Acta.* 2013, 6 (2), 68–73. (In Russian). doi: 10.15407/biotech6.02.068.
19. Van Wees S., van der Ent S., Pieterse C. Plant immune responses triggered by beneficial microbes. *Curr. Opin. Plant. Biol.* 2008, 11 (4), 443–448. doi: 10.1016/j.pbi.2008.05.005.
20. Varbanets L., Zdorovenko E., Kiprianova E., Avdeeva L., Brovarskaya O., Rybalko S. Characteristics of *Pseudomonas chlororaphis* lipopolysaccharides. *Microbiology.* 2015, 84 (6), 680–690. (In Russian).
21. Zdorovenko E., Varbanets L., Shaskov A., Kiprianova E., Knirel Y. Structure of the O-polysaccharide of the lipopolysaccharide of *Pseudomonas chlororaphis* subsp. *aureofaciens* UCM B-306. *Carbohydr. Res.* 2015, V. 410, P. 47–50. doi: 10.1016/j.carres.2015.03.019.
22. Zdorovenko E., Kadykova A., Varbanets D., Shashkov A., Kiprianova E., Brovarskaya O., Knirel Y. Structure of the O-specific polysaccharides of *Pseudomonas chlororaphis* subsp. *chlororaphis* UCM B-106. *Carbohydr. Res.* 2016, V. 433, P. 1–4. doi: org/10.1016/j.carres.2016.06.013.
23. Kiprianova E., Varbanets L., Rybalko S., Shepelevich V. Strain *Pseudomonas chlororaphis* subsp. *aureofaciens* producing lipopolysaccharide with antiviral activity. *Patent of Ukraine № 76689, MPK 2013.01, C12N 1/00*, publ. 10. 01. 2013. (In Ukrainian).
24. Varbanets L., Kiprianova E., Rybalko S. Strain *Pseudomonas chlororaphis* subsp. *aureofaciens* producing lipopolysaccharide with activity against viruses of influenza, herpes and hepatitis C. *Patent of Ukraine № 103797, MPK 2015.01, C12N 7/00*, publ. 25. 12. 2015. (In Ukrainian).
25. Shepelevich V., Kiprianova E., Yaroshenko L., Avdeeva L. Sensitivity of *Pseudomonas chlororaphis* strains to antibiotics and tools of plant protection. *Microbiol. Zhurnal.* 2012, 74 (6), 24–28 (In Russian).

**АНТИМІКРОБНА, ЕНТОМОПАТОГЕННА  
ТА ПРОТИВІРУСНА АКТИВНІСТЬ  
БІОПРЕПАРАТУ ГАУПСИН  
НА ОСНОВІ ШТАМІВ  
*Pseudomonas chlororaphis***

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Метою огляду було подати результати більш ніж десятирічного дослідження біопрепарату гаупсин, створеного на основі двох штамів *Pseudomonas chlororaphis* subsp. *aureofaciens* УКМ В-111 і УКМ В-306, що має антифунгальну, ентомопатогенну та противірусну активність. Наведено дані щодо синтезу цими штамми антибіотичних речовин — похідних феназину і фенілпіролу. У штамів-компонентів гаупсину виявлено ентомоцидні властивості щодо широкого спектра комах-шкідників. На моделі вірусу тютюнової мозаїки встановлено антивірусну активність гаупсину, що пов'язана з утворенням термостабільних екзополімерів, які містять нейтральні моносахариди. Високоактивними противірусними агентами виявились також ліпополісахариди штамів В-111 і В-306. Встановлено будову їхніх О-специфічних полісахаридів. Останні структурно гетерогенні, представлені лінійними повторюваними три- та тетрасахаридними ланками і мають раніше не описану специфічну будову.

**Ключові слова:** біопрепарат гаупсин, *Pseudomonas chlororaphis* subsp. *aureofaciens*, антибіотики-феназини, піролнітрин, ентомопатогенні властивості, противірусна активність.

**АНТИМІКРОБНАЯ, ЭНТОМОПАТОГЕННАЯ  
И ПРОТИВОВИРУСНАЯ АКТИВНОСТЬ  
БИОПРЕПАРАТА ГАУПСИН  
НА ОСНОВЕ ШТАММОВ  
*Pseudomonas chlororaphis***

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Целью обзора было представить результаты более чем десятилетнего исследования биопрепарата гаупсин, созданного на основе двух штаммов *Pseudomonas chlororaphis* subsp. *aureofaciens* УКМ В-111 и УКМ В-306 и обладающего антифунгальной, энтомопатогенной и противовирусной активностью. Представлены данные о синтезируемых этими штаммами антибиотических веществах — производных феназина и фенилпиррола. У штаммов-продуцентов гаупсина обнаружены энтомоцидные свойства в отношении широкого спектра насекомых-вредителей. На модели вируса табачной мозаики установлена антивирусная активность гаупсина, связанная с образованием термостабильных экзополимеров, содержащих нейтральные моносахариды. Высокоактивными противовирусными агентами оказались также липополисахариды штаммов В-111 и В-306. Установлено строение их О-специфических полисахаридов. Последние структурно гетерогенны, представлены линейными повторяющимися три- и тетрасахаридными звеньями и имеют ранее не описанное специфическое строение.

**Ключевые слова:** биопрепарат гаупсин, *Pseudomonas chlororaphis* subsp. *aureofaciens*, антибиотики-феназины, пирролнитрин, энтомопатогенные свойства, противовирусная активность.