https://doi.org/10.15407/microbiolj84.05.048

O.B. Balko[1*], L.B. Zelena[1], O.I. Balko[1],
L.O. Maksymenko[1], V.G. Voitsekhovsky[2], L.V. Avdeeva[1]

[1] Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Academika Zabolotnoho Str., Kyiv, 03143, Ukraine

[2] Bohomolets National Medical University,
13 Taras Shevchenko Blvd., Kyiv, 01601, Ukraine

[*] Author for correspondence; e-mail: oleksandrbalko@gmail.com

PROPERTIES OF PYOCIN S9 FROM *PSEUDOMONAS AERUGINOSA* UCM B-333

The deposited by us highly active bacteriocin producer Pseudomonas aeruginosa UCM B-333 synthesizes pyocins, which intensively inhibit phytopathogenic strains of Pseudomonas syringae — plant pathogens. This strain produces pyocins S1 and S5, as well as microcin-II-like bacteriocins. ***The aim of this work*** *was to check the presence of other pyocins in P. aeruginosa UCM B-333.* ***Methods.*** *The concentrated bacterial lysate of P. aeruginosa UCM B-333 was separated by ion-exchange chromatography on DEAE cellulose. The fraction with studied bacteriocin was further purified by gel filtration on Sephadex G-75. To determine the belonging of investigated pyocin to a certain subtype, its molecular weight, antimicrobial activity, kinetics of the effect on sensitive microorganisms, and serological homology with carotovoricins of Pectobacterium carotovorum were studied as well as the ability to interact with siderophore receptors and nuclease activity were tested.* ***Results.*** *The isolated pyocin is a protein with a molecular weight of the active part of pyocin of 43.4 kDa and an immune protein — of 9 kDa. This substance is characterized by nonspecific DNase activity and affects sensitive cells by the single-hit response kinetics of influence through binding to receptors that are not concerned with iron transport. The revealed pyocin is not related to carotovoricins, its activity spectrum is close to other pyocins' activities, and it affects clinical multidrug-resistant strains of Pseudomonas aeruginosa. The induction mechanism of this bacteriocin may be different from that described for other pyocins and not concerned with the RecA system. The determination of factors that stimulate the expression of pyocin S9 requires further study.* ***Conclusions.*** *According to the established properties, the studied substance is the closest to the foreseen pyocin S9.*

Keywords: *pyocin S9, Pseudomonas aeruginosa, nuclease activity, serological homology with carotovoricins, interaction with siderophore receptors.*

Citation: Balko O.B., Zelena L.B., Balko O.I., Maksymenko L.O., Voitsekhovsky V.G., Avdeeva L.V. Properties of Pyocin S9 from *Pseudomonas aeruginosa* UCM B-333. *Microbiological journal.* 2022 (1). P. 48—57. https://doi.org/10.15407/microbiolj84.05.048

Pseudomonas aeruginosa is one of the ESKAPE pathogens that pose a global threat to human health [1]. These microorganisms are considered able to cause nosocomial diseases that are difficult to treat in immunosuppressed patients with high incidence [2]. However, *P. aeruginosa* belongs to widespread bacterial species in the environment [3, 4]. These microorganisms can be isolated from different sources: water, soil, plants, and areas contaminated with carbohydrates [4—6]. Isolated from environment strains are considered non-pathogenic and are recommended for bioremediation and plant protection [7, 8]. *P. aeruginosa* is also intensively used in scientific research as one of the four model objects for the investigation of microorganisms and their metabolites vital activity [9]. The accumulation of a significant amount of data obtained at this model object provides the ability to extrapolate them to other species of bacteria [10].

Nowadays, in view of significant requirements for new antimicrobial agents, studies of pyocins — *P. aeruginosa* bacteriocins, have been intensively developed. Bacteriocins are antibiotic-like substances synthesized by bacteria for competitive antagonism against other closely related microorganisms [1, 11]. Among pyocins scientists classify high molecular weight structures, analogs of phage tails - R- and F-types pyocins and low-molecular proteins — S-type pyocins [12]. We also showed that *P. aeruginosa* can synthesize microcin-II-like bacteriocins — substances of the peptide nature [13, 14]. S-type pyocins are potentially used in medicine, veterinary medicine, or agriculture [11, 15]. Seven bacteriocins of this type — S1-S5, AP41, and M — have been previously described in the literature [16]. These substances were active against multidrug-resistant strains of *P. aeruginosa* — pathogens in humans [11, 17]. We have also established that pyocins can inhibit the growth of phytopathogenic *P. syringae* strains — causative agents of plant diseases [18]. Ghequire and De Mot have analyzed genomes of a significant number of *P. aeruginosa* strains and revealed sequences that may correspond to eight new, unexplored pyocins [12]. Most of them are foreseen *in silico* and only some were isolated in the pure form [19, 20].

We deposited *P. aeruginosa* UCM B-333 as a highly active producer of bacteriocins, which inhibited the growth of most laboratorial, clinical, and isolated from plants strains [21]. Previously, we isolated and studied pyocins S1 and S5, as well as microcin-II-like bacteriocins of this strain. The affiliation of these main active antimicrobial substances to the corresponding subtypes of bacteriocins was confirmed by the coincidence of their physicochemical properties with the RT-PCR data [13, 14, 18]. However, in the residual eluate, we revealed low activity of unknown nature. As shown in the literature, the most active producer-strains can secrete up to five bacteriocins simultaneously. Many new types of pyocins are foreseen only *in silico*, and primers to check their presence in the genome by PCR are not defined [12]. As *P. aeruginosa* UCM B-333 is a highly active producer of pyocins, we hypothesized that it is capable of synthesizing other bacteriocins that we have not previously identified. Therefore, the aim of this study was to test the presence of other pyocins in *P. aeruginosa* UCM B-333.

Materials and methods. The object of investigation was *P. aeruginosa* UCM B-333 from the Ukrainian collection of microorganisms (UCM, Zabolotnyi Institute of Microbiology and Virology (IMV), NAS of Ukraine). We deposited this strain in the Depositary of IMV under the number IMV B-7668 as a highly active producer of bacteriocins.

Isolation, separation, and purification of pyocins. Isolation of purified pyocins of *P. aeruginosa* UCM B-333 was performed as described previously [22]. The concentration of bacteriocins was conducted using 70% ammonium sulfate precipitation according to [14, 23]. Bacteriocins were separated by ion-exchange chro-

matography. For this, a column (25 by 130 mM) was filled with regenerated DEAE-cellulose and equilibrated with 600 mL of 20 mM Tris-HCl buffer (pH 7.5). 1 mL of bacteriocin sample was applied to the column and rinsed with 100 mL of the 20 mM Tris-HCl buffer. Then step-by-step elution was conducted with the same buffer containing 0—0.5 M NaCl. The eluates (5 mL) were collected into separate sterile tubes [14].

For repeated separation of bacteriocins, we used gel filtration. The column (5 by 430 mM) was filled with Sephadex G-75 and equilibrated with 100 mL of 20 mM Tris-HCl buffer (pH 7.5) with 0.35 M NaCl. 1 mL of bacteriocin sample was applied to the column; elution was conducted with 100 mL of the buffer. The eluates (3 mL) were collected into separate sterile tubes and stored at 4 °C. Chloroform was used as a preservative [24].

In both cases, protein concentration was determined by the Bradford method [25] via the measurement of absorbance at 280 nm against 20 mM Tris-HCl.

Pyocin antimicrobial activity. The antimicrobial activity of obtained fractions was tested by the two-layer agar method [17] at all stages of bacteriocin separation. Quantitative indices of activity were evaluated in units of activity — AU/mL or for convenience in $\times 10^3$ AU/mL [26]. As indicator cultures, we included in our study *P. aeruginosa* UCM B-3 and UCM B-10.

Kinetics of bacteriocin effect. Purified bacteriocin (100 μL) was added to 1.9 mL of 24 hr' suspension of *P. aeruginosa* UCM B-10, previously diluted with sterile nutrient broth 1:100. In other test samples, analogous volumes of bacteriocin (100 μL), previously diluted with 0.85% sodium chloride solution (PS) in 2, 3, and 4 times, were also added to the same volume of noted test culture. In the control sample, the same volume of 0.85% sodium chloride solution was added to the bacterial suspension. The control and test samples were incubated at 37 °C. To determine the titer of microorganisms, 100 μL of bacterial suspension was taken from the samples before adding bacteriocin (0 hr) and after 1 and 3 hr of cultivation. The survival index was determined by the percent ratio of the microorganism titer at the related time of cultivation to the titer at 0 h [23].

Interaction of pyocins with siderophore receptors. *P. aeruginosa* UCM B-10 was grown at 37 °C for 24 hr in nutrient broth or in nutrient broth with the addition of 40 μM Fe (III) citrate. 100 μL of fractions 25—31 after DEAE-cellulose, containing pyocin S5, so as 100 μL of the 7th fraction after gel filtration with Sephadex G-75, containing pyocin S9, was added to 1.9 mL of *P. aeruginosa* UCM B-10 daily suspensions, previously diluted to 1:100 with sterile nutrient broth. The suspensions were cultured at 37 °C for 2 hr. Before the addition of bacteriocins and after 1 and 2 hr of cultivation, 100 μL of bacterial suspension was taken from these samples to determine the culture titer [27].

SDS-PAGE. SDS-PAGE of *P. aeruginosa* bacteriocin proteins was conducted according to the Laemmli method [28] in 10% Tris-Glycine SDS-PAGE gel, 100 mA, 2 hr, using as markers PageRuler Plus Prestained Protein Ladder, 10-250 kDa (Thermo Scientific). The data were processed using the TotalLab software.

Pyocin nuclease activity. To check the nuclease activity of bacteriocin from the 7th fraction after Sephadex G-75, 24 μL of this substance was mixed with the same volumes of phage λ DNA (43, 23, 9, and 2 ng/μL, «Fermentas»), exposed at 37 °C for 30 min, and then frozen at -18 °C. As a control, we used untreated DNA of phage λ (30 ng/μL) without pyocin and pyocin without DNA of phage λ. The availability of DNA and hydrolysis products was detected by electrophoretic separation in 1% agarose gel. The obtained digital images were processed by the TotalLab program [29].

Serological homology of pyocin with carotovoricins. Carotovoricins were isolated from *Pectobacterium carotovorum* strains [30]. Preparation of antiserum for bacteriocin and testing of

serological homology of the isolated pyocin with *P. carotovorum* carotovoricins were performed as described in [31].

Results. Separation of the lysate of the highly active bacteriocin producer *P. aeruginosa* UCM B-333 by ion-exchange chromatography with DEAE-cellulose revealed six groups of fractions with high protein concentration in the eluate: 7—14 fractions, the 23th fraction, 25—31 fractions, 33—36 fractions, 42—46 fractions, and 49—52 fractions (Fig. 1). Substances of the 23 th and 33—36 fractions did not affect sensitive cultures *P. aeruginosa* UCM B-3 and UCM B-10, which indicates the absence of active components in their composition. The total activity and protein concentration of 42—46 fractions were only twice higher than those in 49—52 fractions, and their adjacent location may indicate that this is the only stretched fraction group that contains similar active components. Thus, only three of the six detected groups of fractions may contain protein antimicrobial substances. We have previously shown that 7—14 and 25—31 fractions include pyocins S1 and S5, respectively [18]. However, the characteristics of substances of 42—52 fractions remain unknown.

Then the substances of 42—46 and 49—52 fractions were combined, and gel filtration with Sephadex G-75 was conducted. After elution, a single peak of protein concentration was revealed which coincided with the activity peak (Fig. 2). This fact confirms the supposition concerning single active substance in the 42—52 group of fractions.

A characteristic feature of the bacteriocin action on microorganisms is a single-hit response kinetics of influence [23]. The substance of peak fraction obtained on Sephadex G-75 provoked a linear decrease in the concentration of sensitive strain *P. aeruginosa* UCM B-10 during the observation period (Fig. 3).

A similar regularity was also observed in the samples with different concentrations of the studied substance. This fact indicates that the active component of the obtained fraction affects the used microorganisms like bacteriocins.

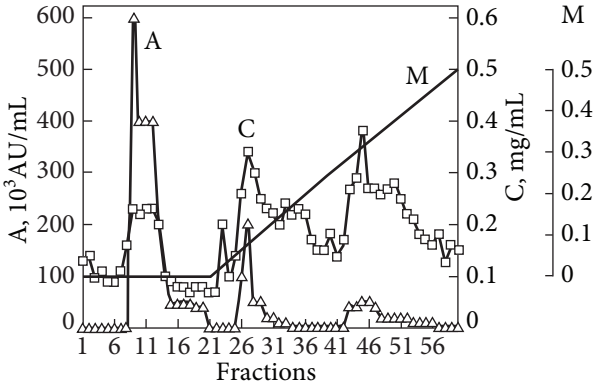

Fig. 1. Elution profile of pyocins of *Pseudomonas aeruginosa* UCM B-333 obtained by ion-exchange chromatography on DEAE cellulose using 20 mM Tris-HCl buffer with a gradient of sodium chloride concentration: A — the activity of pyocins, C — protein concentration, M — sodium chloride concentration

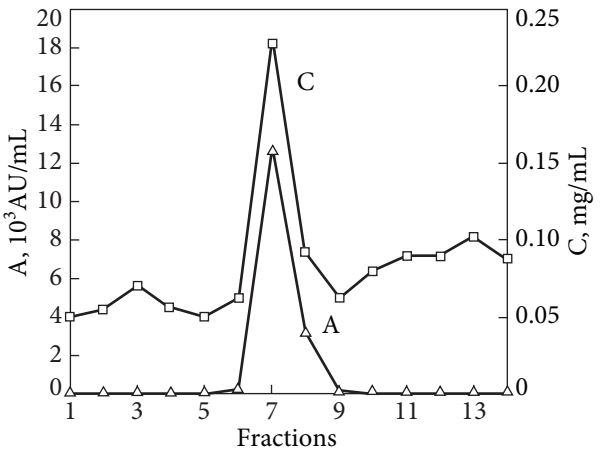

Fig. 2. Elution profile of pyocin of *Pseudomonas aeruginosa* UCM B-333 by gel filtration on Sephadex G-75: A — activity of pyocin, C — protein concentration

The fraction with the highest activity and protein concentration, obtained after gel filtration, was collected, concentrated with ammonium sulfate, dialyzed, and electrophoretically separated. Two proteins with molecular weights of 46.3 and 10 kDa were detected using SDS-PAGE (Fig. 4).

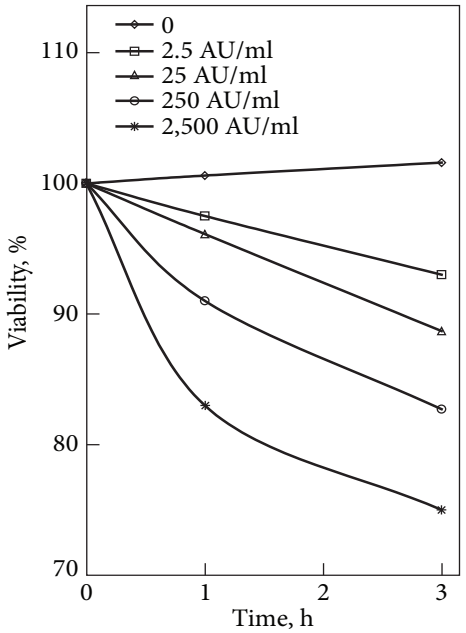

Fig. 3. The effect of *Pseudomonas aeruginosa* UCM B-333 pyocin on the viability of *Pseudomonas aeruginosa* UCM B-10. Pyocin of the indicated activity (AU/mL) was obtained after gel filtration on Sephadex G-75. The viability is the ratio of the number of survived cells after certain time to their initial number; 0 — culture not added with pyocin

As known, bacteriocins are made up of two proteins — the active protein and the immune protein. The active protein has a higher molecular weight and causes the destruction of sensitive cells. The immune protein is characterized by a lower molecular weight, does not provoke the destruction of sensitive cells, and is synthesized to protect producer cells from the killer effects of the active protein [11, 12, 15, 16]. So, the first protein of investigated pyocin with a molecular weight of 46.3 kDa is the active part of bacteriocin, and the second one with a molecular weight of 10 kDa is its immune protein. It should be noted that this pyocin with the noted molecular weight was revealed in a mixture of proteins at all stages of isolation, concentration, and separation, that is, in the initial lysate, after concentration by ammonium sulfate, and separation with DEAE-cellulose.

It is known that bacteriocins affect sensitive cells owing to nuclease or pore-forming activities [12]. To check the availability of these properties, the test substance was mixed with phage λ DNA

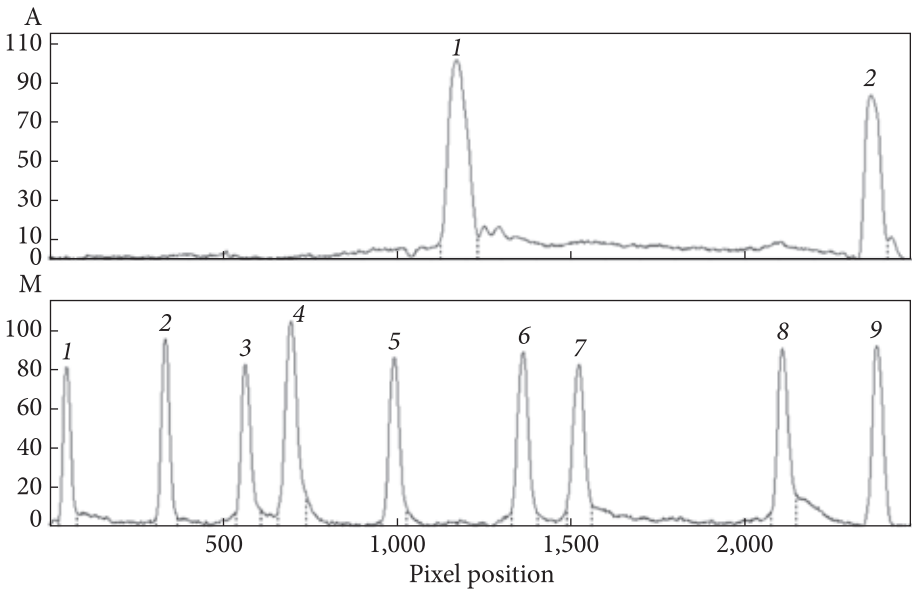

Fig. 4. Densitogram of *Pseudomonas aeruginosa* UCM B-333 (A) pyocin in the fraction obtained by gel filtration on Sephadex G-75: M — molecular weight markers (PageRuler Plus Prestained Protein Ladder, 10—250 kDa (Thermo Scientific): 1 — 250 kDa, 2 — 130 kDa, 3 — 100 kDa, 4 — 70 kDa, 5 — 55 kDa, 6 — 35 kDa, 7 — 25 kDa, 8 — 15 kDa, 9 — 10 kDa

of different concentrations. After the electrophoretic separation of used mixtures, tracks of cleaved DNA of different intensity were observed (Fig. 5).

The decrease in DNA content led to a decrease in the intensity of the formed tracks, while at a concentration of 2 ng/mL, the complete cleavage was observed. This fact indicates the presence of non-specific endonuclease activity in the substance studied.

As it is known from the literature, some pyocins, for example AP41 and S3, are characterized by serological homology with carotovoricins [32]. According to the standard Ouchterloni method, we studied the homology of isolated pyocin with carotovoricins of several *Pectobacterium carotovorum* strains. Obtained results showed the absence of serological homology between these substances.

It was determined that pyocins S2, S3, S4, and S5 affect sensitive cells through interaction with FpvAI, FpvAII, and FptA receptors, which are required for siderophores attaching [33, 34]. The ability to be bound with these receptors was tested for our bacteriocins. For this , we additionally used substances of 25—31 fractions separated on DEAE-cellulose, which contained pyocin S5 [18]. It was found that under cultivation in a medium with 40 μM iron (III) citrate, these receptors were blocked by siderophores and the activity of pyocin S5 decreased (Fig. 6). Instead, such an effect was not observed in the samples with the studied pyocin.

Discussion. Seven pyocins with partially investigated properties — S1, S2, S3, S4, S5, AP41, and M1 — have been described for *Pseudomonas aeruginosa* [16]. Moreover, the presence of eight other pyocins was foreseen in the genomes of these species' strains [12]. The existence of some of them has been confirmed by isolation from strain producers, while the others have not yet been obtained in the native state [19, 20]. However, the final evidence of the presence of the proper bacteriocin is its isolation in a purified form and the availability of data on its biologi-

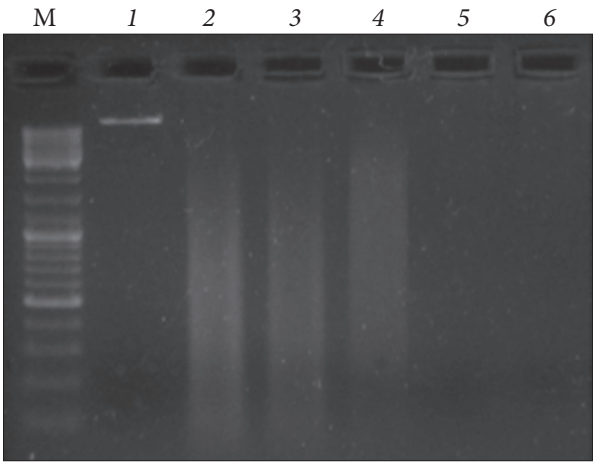

Fig. 5. The nuclease activity of *Pseudomonas aeruginosa* UCM B-333 pyocin during interaction with phage λ DNA added with the concentrations: 2 — 43 ng/μL, 3 — 23 ng/μL, 4 — 9 ng/μL, 5 — 2 ng/μL; M — DNA ladder, 1 — DNA of phage λ (30 ng/μL) not added with pyocin; 5 — pyocin not added with phage λ DNA

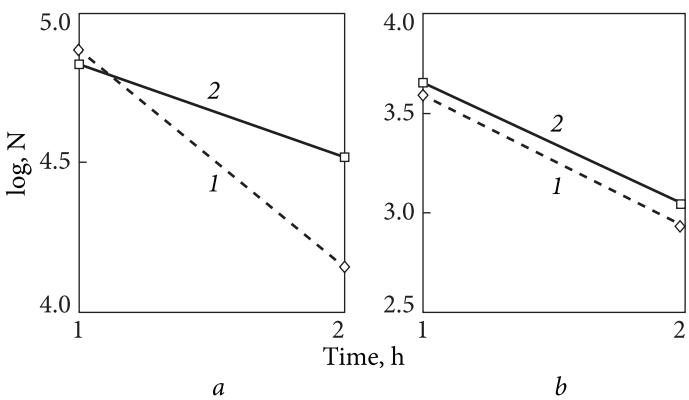

Fig. 6. Kinetics of the influence of pyocin S5 (A) and *Pseudomonas aeruginosa* UCM B-333 pyocin (B) on the titer (N) of *Pseudomonas aeruginosa* UCM B-10, grown in nutrient broth (1) and in nutrient broth with 40 μM Fe (III) citrate (2)

cal properties. It is difficult to perform this task since a number of pyocins can be produced by a narrow spectrum of producer-strains (eg pyocins S6 and S7), some of them require special conditions of induction (eg pyocin S5), and others may be characterized by a narrow activity spectrum and difficulty in selecting sensitive cultures (eg pyocin M1) [11, 12, 27]. The existence of defective bacteriocins also must not be ruled out. By analogy with defective bacteriophages or satellite viruses, the bacterial genome does not contain a complete set of specific genes needed for their production. The isolation of these defective agents requires the penetration of additional infectious agents. It should be noted that some of *P. aeruginosa* foreseen bacteriocins, eg pyocins S6 and S8, have been isolated in a purified form [19, 20], whereas information concerning the other ones, including pyocin S9, is lacking.

Primers for only seven more investigated pyocins, namely S1-S5, AP41, and M1, are presented in the literature and GenBank. PCR with those primers allowed detecting pyocins S1 and S5 in the genome of *P. aeruginosa* UCM B-333. These bacteriocins have been previously identified in the composition of 7—14 and 25—31 fractions, respectively [18]. Consequently, substances of fractions 42—52 contain a pyocin of another type. Additional evidence of this fact is lack of serological homology between the investigated pyocin and carotovoricins, which is characteristic for pyocins AP41 and S3 [32]. The isolated bacteriocin had the nuclease activity, which is not inherent for S4, S5, and M1 pyocins [16, 17]. The molecular weight of the active part of studied bacteriocin is 46.3 kDa, while that of pyocin M1 is 31.8 kDa, and of the other pyocins are from 56.1 kDa (pyocin S5) to 83.9 kDa (pyocin AP41) [12, 17, 23]. The isolated substance did not change its activity after the addition of iron (III) salt to the medium, which may indicate binding to non-iron transport receptors. This property is not characteristic for pyocins S2-S5 [16, 27, 34]. All these facts indicate that obtained bacteriocin does not correspond to any of the studied pyocins: S1-S5, AP41, and M1.

The eight pyocins, namely S6-S12 and M4, predicted *in silico*, have different properties, the comparison with which would allow us to classify the isolated bacteriocin to a certain type. It is worth noting that only pyocins S8, S9, and S10 are characterized by DNase activity [12]. The predicted molecular weight of the immune protein of pyocin S10 is 17.3 kDa, and that of its active part — 56.9 kDa, which do not correspond to such parameters of the studied bacteriocin. The hypothetical molecular weight of the active part of pyocin S8 is 84.9 kDa, which also differs from the isolated pyocin. Thus, our isolated bacteriocin is the closest to the predicted pyocin S9.

The results of antimicrobial property studies revealed that the activity spectrum of isolated bacteriocin is similar to the analogous parameters of pyocins S1 and S5, which are also produced by *P. aeruginosa* UCM B-333. These substances inhibited laboratory strains and affected clinical isolates of *P. aeruginosa*. However, the initial activity of pyocin S9 in the lysate composition was significantly lower than the activities of pyocins S1 and S5, which may be due to the peculiarities of this bacteriocin induction. It is known that the recA system is responsible for the induction regulation of most pyocins, including pyocin S1 [16, 27]. For bacteriocin induction, we used nalidixic acid, the inducing effect of which on microorganisms is due to the damage to nucleic acids that activate the RecA-protein, which cleaves the repressor of the *prtN* gene. This leads to the synthesis of PrtN protein, which is bound to the P-box structure and triggers the transcription of pyocins [12]. However, pyocin S5 does not have the regulatory sequence P-box required for binding of the transcriptional activator PrtN. An increase in pyocin S5 transcription is stimulated by oxidative stress [27]. Therefore, the use of inductors that partially damage the nucleic acid of bacteria does not lead to this bacteriocin

production. In our work, pyocin was induced through the activation of the recA system. Based on the above, it can be assumed that the synthesis of pyocin S9, as well as pyocin S5, may not be related to the RecA system.

It is well known that pyocin S5 interacts with siderophore receptors [33, 34]. Therefore, during the cultivation of strains, sensitive to these bacteriocins, in a medium with 40 μM iron (III) salt, the receptors are blocked and pyocin activity can be significantly reduced. The absence of this effect in the case of pyocin S9 suggests that the receptors for this substance may be other structures, not concerned with iron transport. A similar regularity was also observed for some other bacteriocins. Thus, pyocin S6 receptors remain unidentified, but it has been shown that their interaction is not related to the structure of FpvA [12, 19].

Thus, the isolated pyocin is a protein with a molecular weight of their active part of 43.4 kDa and immune protein — of 9 kDa. This substance is characterized by nonspecific DNase activity and affects sensitive cells by the single-hit response kinetics of influence through binding to receptors that are not concerned with iron transport. The revealed pyocin is not related to carotovoricins; its activity spectrum is close to other pyocins' activities. Studied pyocin affects clinical multidrug-resistant strains of *Pseudomonas aeruginosa*. According to the established properties, the studied substance is the closest to the foreseen pyocin S9. The induction mechanism of this bacteriocin may be different from that described for other pyocins and not concerned with the RecA system. The determination of factors that stimulate the expression of pyocin S9 requires further study.

REFERENCES

1. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, et al. Antimicrobial Resistance in ESKAPE Pathogens. Clin Microbiol Rev. 2020; 33:e00181-19.
2. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, et al. Epidemiology and treatment of multidrug-resistant and extensively drugresistant *Pseudomonas aeruginosa* infections. Clin Microbiol Rev. 2019; 32:e00031-19.
3. Kaszab E, Radó J, Kriszt B, Pászti J, Lesinszki V, et al. Groundwater, soil and compost, as possible sources of virulent and antibiotic-resistant *Pseudomonas aeruginosa*. Int J Environ Health Res. 2021; 31(7):848—860.
4. Garrity GM, Brenner DJ, Krieg NR, Staley JR. Bergey's manual of systematic bacteriology. 2nd ed. Vol. 2B. The Gammaproteobacteria. New York: Springer; 2005.
5. Cabot G, Zamorano L, Moyà B, Juan C, Navas A, et al. Evolution of *Pseudomonas aeruginosa* antimicrobial resistance and fitness under low and high mutation rates. Antimicrob Agents Chemother. 2016; 60(3):1767—1778.
6. Kaszab E, Kriszt B, Atzél B, Szabó G, Szabó I, et al. The occurrence of multidrug-resistant *Pseudomonas aeruginosa* on hydrocarbon-contaminated sites. Microb Ecol. 2010; 59(1):37—45.
7. Ebadi A, Khoshkholgh SNA, Olamaee M, Hashemi M, Ghorbani NR. Effective bioremediation of a petroleum-polluted saline soil by a surfactant-producing *Pseudomonas aeruginosa* consortium. J Adv Res. 2017; 8:627—633.
8. Yasmin S, Hafeez FY, Mirza MS, Rasul M, Arshad HMI, et al. Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by rhizospheric *Pseudomonas aeruginosa* BRp3. Front Microbiol. 2017; 8:1895.
9. Kato J, Kim HE, Takiguchi N, Kuroda A, Ohtake H. *Pseudomonas aeruginosa* as a model microorganism for investigation of chemotactic behaviors in ecosystem. J Biosci Bioeng. 2008; 106(1):1—7.
10. Frank DW. Research topic on *Pseudomonas aeruginosa*, biology, genetics, and host—pathogen interactions. Front Microbiol. 2012; 3:4—5.
11. Soltani S, Hammami R, Cotter PD, Rebuffat S, Ben Said L, et al. Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. FEMS Microbiol Lett. 2021; 45(1):fuaa039.
12. Ghequire mgK, De Mot R. Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. FEMS Microbiol Rev. 2014; 38:38523—38568.
13. Balko OB. Low molecular weight *Pseudomonas aeruginosa* bacteriocins. Mikrobiol Z. 2019; 81(6):97—109.
14. Balko OB. Interaction between S-Type Pyocins and Microcin-II-Like Bacteriocins in *Pseudomonas aeruginosa*. Mikrobiol Z. 2021; 83(3):72—80.

15. Rooney WM, Chai R, Milner JJ, Walker D Bacteriocins Targeting Gram-Negative Phytopathogenic Bacteria: Plantibiotics of the Future. Front Microbiol. 2020; 11:575981.
16. Michel-Briand Y, Baysse C. The pyocins of *Pseudomonas aeruginosa*. Biochimie. 2002; 84:499—510.
17. Ling H, Saeidi N, Rasouliha BH, Chang MW. A predicted S-type pyocin shows a bactericidal activity against clinical *Pseudomonas aeruginosa* isolates through membrane damage. FEBS Lett. 2010; 584(15):3354—3358.
18. Balko OB, Balko OI, Zelena LB, Pasichnyk LA, Avdeeva LV. Method of plant protecting from bacterial pathogen *Pseudomonas syringae.* Pat. 120295 UA, MPK A01N 25/00, C12R 1/38, C12R 1/385, C12N 1/20 — Publ. 11.11.2019, Bul. № 21.
19. Dingemans J, Ghequire mgK, Craggs M, De Mot R, Cornelis P. Identification and functional analysis of a bacteriocin, pyocin S6, with ribonuclease activity from a *Pseudomonas aeruginosa* cystic fibrosis clinical isolate. MicrobiologyOpen. 2016; 5(3):413—23.
20. Turano H, Gomes F, Barros-Carvalho GA, Lopes R, Cerdeira L, et al. Tn *6350*, a Novel Transposon Carrying Pyocin S8 Genes Encoding a Bacteriocin with Activity against Carbapenemase-Producing *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2017; 61(5):e00100-17.
21. Balko AB, Avdeeva LV. [Screening of producers of bacteriocin-like substances active against *Pseudomonas aeruginosa*]. Mikrobiol Z. 2012; 74(2):8—13. Russian.
22. Balko AB, Vidasov VV, Avdeeva LV. [Optimization of conditions of *Pseudomonas aeruginosa* bacteriocin induction]. Mikrobiol Z. 2013; 75(1):79—85. Russian.
23. Sano Y, Kageyama M. Purification and Properties of an S-Type Pyocin, Pyocin AP41. J Bacteriol. 1981; 146(2):733—739.
24. Ito S, Kageyama M, Egami F. Isolation and characterization of pyocins from several trains of *Pseudomonas aeruginosa*. J Gen Appl Microbiol. 1970; 16(3):205—214.
25. Kruger NJ. The Bradford Method for Protein Quantitation. In: Walker JM, editor. Basic Protein and Peptide Protocols. Methods in Molecular Biology, vol. 32. Humana Press; 1994. p. 9—15.
26. Saeed S, Rasool AJ, Ahmed S, Khanum T, Khan MB, et al. New insight in Staphylococcin reseach: Bacteriocin and/or BLIS produced by *S. aureus* AB188. W J Microbial Biotech. 2006; 22(7):713—722.
27. Elfarash A, Dingemans J, Ye L, Hassan AA, Craggs M, et al. Pore-forming pyocin S5 utilizes the FptA ferripyochelin receptor to kill *Pseudomonas aeruginosa*. Microbiology. 2014; 2:261—269.
28. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature. 1970; 227(5259):680—685.
29. Sano Y, Matsui H, Kobayashi M, Kageyama M. Molecular structures and functions of pyocins S1 and S2 in *Pseudomonas aeruginosa*. J Bacteriol. 1993; 175(10):2907—16.
30. Tovkach FI. De Defective lysogeny in *Erwinia carotovora*. Mikrobiologiia. 2002; 71(3):359—367.
31. Maksymenko LO. Morpho-structural, killer and serological properties of macromolecular bacteriocins of *Pectobacterium carotovorum* subsp. *carotovorum* and their relatedness to bacteriophage ZF40. Microbiology&Biotechnology. 2020; 2:44—56.
32. Chuang D-Y, Chien Y-C, Wu H-P. Cloning and Expression of the *Erwinia carotovora* subsp. *carotovora* Gene Encoding the Low-Molecular-Weight Bacteriocin Carocin S1. J Bacteriol. 2007; 189(2):620—626.
33. Behrens HM, Lowe ED, Gault J, Housden ng, Kaminska R, et al. Pyocin S5 Import into *Pseudomonas aeruginosa* Reveals a Generic Mode of Bacteriocin Transport. MBio. 2020; 11: e03230-19.
34. White P, Joshi A, Rassam P, Housden ng, Kaminska R, et al. Exploitation of an iron transporter for bacterial protein antibiotic import. PNAS. 2017; 114(45):12051—12056.

Received 10.04.2022

О.Б. Балко[1], Л.Б. Зелена[1], О.І. Балко[1],
Л.О. Максименко[1], В.Г. Войцеховський[2], Л.В. Авдєєва[1]

[1] Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, 03143, Україна
[2] Національний медичний університет імені О.О. Богомольця
бульвар Тараса Шевченка, 13, Київ, 01601, Україна

ВЛАСТИВОСТІ ПІОЦИНА S9, ОТРИМАНОГО З *PSEUDOMONAS AERUGINOSA* УКМ В-333

Задепонований нами високоактивний продуцент бактеріоцинів *Pseudomonas aeruginosa* УКМ В-333 синтезує піоцини, які інтенсивно пригнічують розмноження фітопатогенних культур *Pseudomonas syringae* — збудників захворювання рослин. Цей штам виділяє піоцини S1 та S5 типу, а також мікроцин-II-подібні бактеріоцини. **Метою** даної роботи була перевірка наявності у складі *P. aeruginosa* УКМ В-333 інших піоцинів. **Методи.** Концентрований бактеріальний лізат *P. aeruginosa* УКМ В-333 було розділено за допомогою іонообмінної хроматографії на ДЕАЕ-целюлозі. Фракцію з досліджуваним бактеріоцином додатково очищали гель-фільтрацією на сефадексі G-75. Для встановлення приналежності виявленого піоцину до відповідного підтипу визначали його молекулярну масу, антимікробну активність і кінетику впливу на чутливі мікроорганізми, а також спорідненість із каротоворіцинами *Pectobacterium carotovorum* і перевіряли здатність взаємодіяти з рецепторами для сидерофорів і наявність нуклеазної активності. **Результати.** Виділений піоцин є білком з молекулярною масою власне піоцина 43.4 kDa та білка імунності — 9 kDa. Дана речовина характеризуються неспецифічною ДНКазною активністю і впливає на чутливі клітини за одноударною кінетикою через зв'язування з рецепторами, які не пов'язані з транспортом заліза. Він не споріднений із каротоворіцинами, характеризується близьким до інших піоцинів спектром дії і проявляє активність щодо клінічних мультирезистентних штамів *Pseudomonas aeruginosa*. Механізм його індукції може бути відмінним від описаного для інших піоцинів і не пов'язаним із RecA системою. Встановлення факторів, які стимулюють посилення його експресії, потребує подальшого вивчення. **Висновки.** За встановленими властивостями досліджувана речовина є найближчою до передбаченого піоцина S9.

Ключові слова: *піоцин S9, Pseudomonas aeruginosa, нуклеазна активність, серологічна спорідненість із каротоворіцинами, взаємодія з рецепторами сидерофорів.*