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INTERACTION BETWEEN S-TYPE PYOCINS AND MICROCIN-II-LIKE BACTERIOCINS IN *PSEUDOMONAS AERUGINOSA*

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According to our previous results, S-type bacteriocins of Pseudomonas aeruginosa are characterized by high activity against phytopathogenic Pseudomonas syringae strains. In addition to these pyocins producing strains are able to synthesize microcin-II-like bacteriocins. Presence of interaction between these two killer factors can determine methods of their use and activity increase of bacteriocins with antiphytopathogenic properties. ***The aim of the work*** *was to test possibility of interaction between S-type pyocins and microcin-II-like bacteriocins of P. aeruginosa.* ***Methods.*** *The objects of the study were pyocins produced by 6 P. aeruginosa strains. Killer factors in composition of induced lysates were concentrated by 70% ammonium sulphate precipitation, dialyzed through dialysis membrane with molecular weight cut-off (MWCO) 3.5 kDa. Then ion-exchange chromatography with DEAE-cellulose, gel filtration with Sephadex G-75 and ultracentrifugation at 215.000 g for 1 and 4 hours were used for their separation. Protein concentration and antimicrobial activity were determined in obtained fractions. Visualization of proteins in active fraction composition was conducted by electrophoresis according to the Laemmli method.* ***Results.*** *Under ion-exchange chromatography with DEAE-cellulose application elution of bacteriocins available in lysate composition occurs simultaneously. The highest indices of activity and protein concentration were in the 4th fraction, containing two protein bands with molecular weight near 58 and 9 kDa, which are typical for S5 pyocin and microcin-II-like bacteriocins of P. aeruginosa. Further gel filtration of sampled fractions through Sephadex G-75 allowed to separate noted killer factors and obtaine purified fraction containing microcin-II-like pyocins only. Application of ultracentrifugation during 1 hour didn't precipitate studied bacteriocins, whereas during 4 hours – lead to their separation. At the same time a twofold increase of activity indices for S-type pyocins in precipitates and for microcin-II-like killer factors – in supernatants were observed. However achieved concentration was characterized by short-term effect, since in 14 days activity of supernatants decreased by 4–16 times, and for precipitates – by 80–640 times. Then revealed tendency for activity decrease continued.* ***Conclusions.*** *S-type pyocins and microcin-II-like bacteriocins of P. aeruginosa interact with each other, that ensures their stabilization and protects again destruction. Application of methods that cause separation of these killer factors is inexpedient, since it results into considerable decrease of bacteriocin activity indices.*

Keywords: microcin-II-like bacteriocins, S-type pyocins, Pseudomonas aeruginosa, interaction.

It is well known that *Pseudomonas aeruginosa* strains are able to produce high-molecular bacteriocins and colicin-like bacteriocins – pyocins [1, 2]. R- and F-type high-molecular killer factors include structures with molecular weight 1–10 MDa, which resemble to phage tails and realize their killer activity through pore formation in cell walls of sensitive bacteria [3, 4]. Colicin-like S-type pyocins are characterized by molecular weight of 30–100 kDa and cause microorganism death by different mechanisms [5, 6]. Properties of these substances, peculiarities of their induction and killer action so as ability of some strains to produce them are described [7, 8]. Influence of induction conditions can lead to simultaneous synthesis of both types of bacteriocins by producer cultures [9, 10].

Previously we showed that *P. aeruginosa* strains can produce pyocins of another one type – low-molecular microcin-II-like killer factors with molecular weight near 9 kDa [11]. Bacteriocins with a molecular weight below 10 kDa are produced by *Escherichia coli* (microcins). They are divided into two classes: class I microcins are small peptides (<5 kDa) and class II microcins are larger (5 to 10 kDa) [12, 13]. However, the

presence of such bacteriocins in *Pseudomonas syringae* has been shown only recently [14] and Ghequire and De Mot [1] theoretically predicted the possibility of the existence of similar substances in other pseudomonads. So they remain poorly studied. Studies of microcin-II-like bacteriocins *P. aeruginosa* are fundamentally new, because they have not been performed by other authors. We found that revealed substances are capable of penetrating through dialysis membrane with molecular weight cut-off (MWCO) 15 kDa but don't penetrate through the membrane with MWCO 6–8 kDa. They don't influence own producer strains, lose activity after trypsin treatment (1 mg/mL) and their absorption peaks amount to 205–210 nm, which is characteristic for peptide bond of proteins. We assumed that noted bacteriocins can be associated with S-type pyocins, produced by the same strains [15]. The relationship between different types of bacteriocins has been suggested by some authors [16], but not shown for pyocins. According to our previous results, S-type bacteriocins of *P. aeruginosa* are characterized by high activity against phytopathogenic *P. syringae* strains [17]. Presence of interaction between these two killer factors can determine methods of their use and activity increase of bacteriocins with antiphytopathogenic properties. That is why the aim of this work was to test possibility of interaction between S-type pyocins and microcin-II-like bacteriocins in *P. aeruginosa*.

Materials and methods. The objects of investigation were *P. aeruginosa* strains – UCM B-9, UCM B-330, UCM B-332, UCM B-333, UCM B-335, maintained in Ukrainian collection of microorganisms (UCM, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine). These strains of microorganisms are producers of highly active bacteriocins, corresponding data were published previously [18].

To obtain lysates of these *P. aeruginosa* strains, nalidixic acid was added to suspension of producer strains in the logarithmic growth phase (final concentration 100 μg/ml). After further incubation for 3 h, the induction was stopped by the addition of chloroform. The lysates were purified from bacterial detritus by low-speed centrifugation at 4.000 g for 30 min. The obtained supernatants were aseptically removed and stored in closed containers at 4–6 °C. Chloroform was used as a preserving agent [19].

Concentration of bacteriocins was conducted by 70% ammonium sulphate precipitation during 1 day at 4 °C. The sediment was obtained at 30.000 g and 4 °C for 30 min and resuspended in 2 mL of 20 mM Tris-HCl buffer (pH 7.5). The samples were dialyzed through dialysis membrane (MWCO 15 kDa) against 50 mL of 20 mM Tris-HCl buffer for 1 day at 4 °C with a single replacement of dialysis buffer. Purification from insoluble admixture was carried out by low centrifugation at 4.000 g for 30 minutes [11].

Bacteriocins were separated by ion-exchange chromatography. For this purpose column (24× 245 mm) was filled with regenerated DEAE-cellulose and equilibrated with 500 mL of 20 mM Tris-HCl buffer (pH 7.5). 10 ml of bacteriocin sample was applied to this column, flushed with 200 mL of 20 mM Tris-HCl buffer. Then step-by-step elution was conducted with 300 mL of 0–0.3 M NaCl in 20 mM Tris-HCl buffer. Eluates (10 mL) were collected into separate sterile tubes and kept at 4 °C with chloroform as a preserving agent.

For repeated bacteriocin separation we used gel filtration. In this case column (5×430 mm) was filled with Sephadex G-75, equilibrated with 100 mL of 20 mM Tris-HCl buffer (pH 7.5) with 0.15 M NaCl. 1 ml of bacteriocin sample was applied to this column; elution was conducted with 100 mL of previously mentioned buffer. Eluates (3 mL) were sampled in separate sterile tubes and stored at 4 °C. Chloroform was used as a preserving agent [20].

In both methods protein concentration in obtained fractions was determined by absorbance measurement at 280 nm in comparison with 20 mM Tris-HCl using SF-26. Protein content indices were determined by Bradford method [21].

For bacteriocin separation by ultracentrifugation according to Bradley [22] 12 mL of lysates were sampled and centrifuged at 215.000 g for 1 hour. At these parameters S-type pyocins pass into supernatants, marked as bacteriocin mixture. Obtained supernatants were precipitated repeatedly at 215.000 g for 4 hours. Then supernatants were selected separately, sediments were resuspended in 0.2 mL of TE buffer.

Antimicrobial activity at all separation stages was tested by "two-layer agar" method [19]. Quantitative indices of lysate activities were determined by double serial dilutions method, substance activity was estimated according to the maximal dilution able to cause formation of lysis

zone. The obtained results were counted for 1 ml of studied lysate and expressed in activity units – AU/mL or for convenience in $\times 10^3$ AU/mL. *P. aeruginosa* UCM B-3 and UCM B-10 were used as indicator cultures [18].

SDS-PAGE of *P. aeruginosa* bacteriocin proteins was conducted according to the Laemmli method [23] in 10 % Tris-Glycine SDS-PAGE gel, 100 mA, 2 h, using as markers PageRuler Plus Prestained Protein Ladder, 10-250 kDa (Thermo Scientific). The data were processed using the TotalLab Quant software (v 2.0).

Results. The induction of *P. aeruginosa* UCM B-333 different type bacteriocins including microcin-II-like bacteriocins (M-II-LB) was conducted according to our optimized method, described previously [19]. Since this method was effective to obtain colicin-like S-type bacteriocins in high concentration, we decided to use it in these experiments. So, activity of *P. aeruginosa* UCM B-333 induced lysates was 2.9×10^6 AU/mL (Table 1).

Concentration of killer factors was carried out by ammonium sulphate precipitation, which was added to 70 % saturation. Bacteriocin activity in obtained precipitate increased by 9 times, protein content was equal to 3.7 mg/mL. Desalinization of concentrated lysates and insoluble components elimination was realized by dialysis with further low-speed centrifugation. The application of mentioned methods partially decreased samples specific activity to 5.5×10^6 AU/mg.

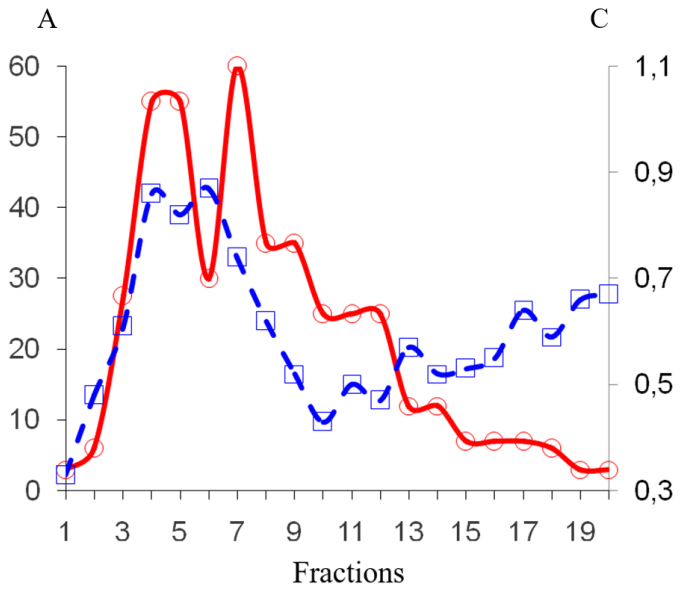
As it was shown previously, investigated *P. aeruginosa* strain is characterized by bacteriocin production multiplicity [18]. That is why for killer factor separation and to obtain purified M-II-LB fraction we applied ion-exchange chromatography with DEAE-cellulose. It was determined that available in lysates bacteriocins eluted simultaneously (Fig. 1).

Table 1

Stages of *P. aeruginosa* UCM B-333 pyocins purification

Sample	A,$\times10^3$ AU/mL	C, mg/mL	A_{sp},$\times10^3$ AU/mg
Induced lysate	2880	0.3	9600
Precipitate 70 % $(NH_4)_2SO_4$	25600	3.7	6919
Dialysate	11520	2.1	5486
DEAE-cellulose 4th fraction	55	0.86	64
Sephadex G-75 5th fraction	8	0.23	35

Legend: A – substance activity, C – protein concentration, A_{sp} – specific activity.



F i g. 1. Elution profile of *P. aeruginosa* UCM B-333 pyocins obtained by ion-exchange chromatography with DEAE-cellulose: A – activity, $\times10^3$ AU/mL; C – protein concentration, mg/mL

Peaks of activity indices were revealed in adjacent fractions 4.5 and 7 and were equal to 5.5×10^4 and 6×10^4 AU/mL, respectively. Activity of substances in the 6th fraction was twice lower – 3.0×10^4 AU/mL. But 4th and 6th fractions had the highest protein concentrations. Increased activity indices were also observed in following fractions, but they were characterized by low protein content. Thus, discordance in peaks of activity and protein concentration, expanded fraction profile indicated that these fractions contained unseparated substances of the same type.

To determine the belonging of obtained bacteriocins to certain subtype, SDS-PAGE was conducted (Fig. 2A). For this purpose we used substances from the 4th fraction, which had the highest activity indices and the highest protein concentration simultaneously. After electrophoretic separation two protein bands with molecular weight near 58 and 9 кDa were revealed.

As we showed previously, genome of investigated strain contained genes of bacteriocin subtype S5, and their expression increased considerably under influence of nalidixic acid. According to Ling et al molecular weight of S5 pyocin is equal to 57.6 kDa [5, 24]. In other our studies it was revealed that molecular weight of M-II-LB was equal to 9 kDa. So obtained fraction contained S5 pyocins and M-II-LB of *P. aeruginosa*.

To separate noted killer factors, substances from the 4th fraction were subjected to gel filtration through Sephadex G-75 (Fig. 3). The results of this method revealed maximal protein concentration – 0.23 μg/mL in the 5th fraction. Activity indices of this fraction were the highest – 8×10^3 AU/mL. Other activity peaks or protein concentration maximum were not determined in neighbouring fractions. So it was suggested that gel filtration succeeded in separation of P. aeruginosa killer factors. Taking into account the difference between molecular weights of S5 pyocins and M-II-LB, obtained fraction must contain substances with lower molecular weight or M-II-LB. To confirm this assumption the secondary SDS-PAGE was conducted (Fig. 2B). After electrophoretic separation a single protein band with molecular weight near 9 kDa, which is typical for *P. aeruginosa* M-II-LB, was revealed in the 5th fraction.

Thus isolation of purified M-II-LB from concentrated pyocin mixture of *P. aeruginosa* UCM B-333 is possible due to application of ion-exchange chromatography with DEAE-cellulose and further gel filtration with Sephadex G-75.

Other method which allows to separate substances with different molecular weight is ultracentrifugation. In this case M-II-LB isolation was conducted from some *P. aeruginosa* strains. Bradley showed that ultracentrifugation at 215.000 g for 1 hour didn't precipitate colicin-like bacteriocins. We used centrifugation under noted parameters for purification of S-type pyocins and M-II-LB from high-molecular killer factors and admixture, possible present in composition of initial lysates. Obtained supernatants were signed as mixture (Table 2). Activity of supernatants from different strains was approximately the same and was equal to $12.8–51.2\times10^3$ AU/mL against *P. aeruginosa* UCM B-3 and $51.2–204.8\times10^3$ AU/mL – against *P. aeruginosa* UCM B-10.

According to Bradley, application of repeated ultracentrifugation at 215.000 g for 4 hours creates conditions for precipitation of S-type pyocins. Increase multiplicity of bacteriocin activity in precipitates, relative to mixture activity (index n), was equal to 80-320 against *P. aeruginosa* UCM B-3 and 40-80 – against *P. aeruginosa* UCM B-10. M-II-LB are characterized by 7 times lower molecular weight then in S-type pyocins. Therefore

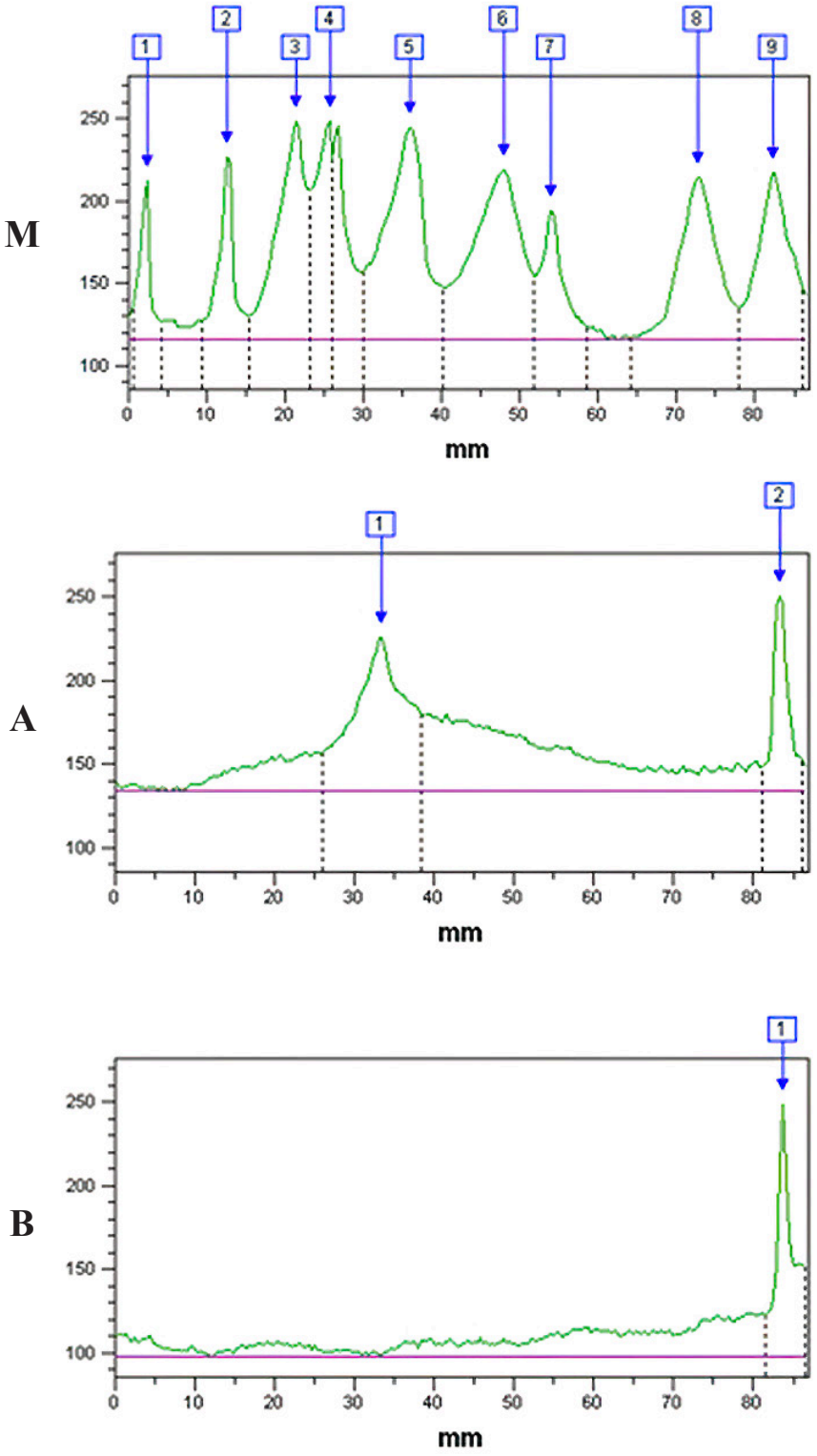
Table 2

Activity of *P. aeruginosa* bacteriocins against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 before and after ultracentrifugation at 215000 g for 4 hours

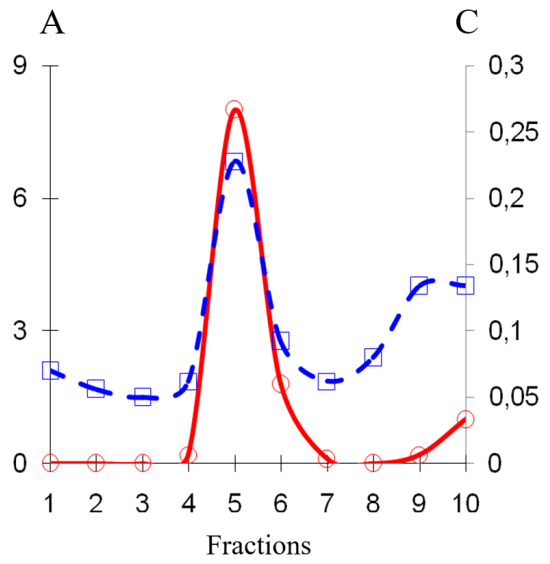
Strain	Mixture		Precipitate				Supernatant			
	B-3	B-10	B-3		B-10		B-3		B-10	
	A		A	n	A	n	A	n	A	n
B-9	12.8	102.4	1024	+80	8192	+80	25.6	+2	102.4	0
B-330	51.2	204.8	8192	+160	8192	+40	102.4	+2	409.6	+2
B-332	25.6	102.4	8192	+160	8192	+80	102.4	+2	102.4	0
B-333	25.6	102.4	8192	+320	8192	+80	51.2	+2	204.8	+2
B-335	12.8	51.2	1024	+80	4096	+80	25.6	+2	102.4	+2

Legend: A – killer activity, $\times10^3$ AU/mL; n – increase multiplicity of bacteriocin activity after ultracentrifugation relative to their activity in mixture composition.

under used ultracentrifugation conditions these substances can't be precipitated and remain in supernatants. Also it was noted twice increase of supernatant activity indices against both indicator cultures (n=2). Thus use of ultracentrifugation made possible not only to separate S-type pyocins and M-II-LB, but also to increase their activity indices.



F i g. 2. Densitograms of *P. aeruginosa* bacteriocins contained in the 4th fraction (A, peaks 1 and 2) after ion-exchange chromatography with DEAE-cellulose and in the 5th fraction (B, peak 1) after gel filtration through 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



F i g. 3. Elution profile of *P. aeruginosa* UCM B-333 pyocins obtained by gel filtration through Sephadex G-75: A – activity, ×10³ AU/mL; C – protein concentration, mg/mL

To estimate efficiency of this method application for increase of bacteriocin activity, indices of obtained precipitates and supernatants were determined during a period of their storage. In 14 days after ultracentrifugation supernatant activities decreased approximately by 4–16 times, and for precipitates – by 80–640 times as compared with indices at the moment of their isolation (Table 3).

Under further observation detected tendency continued. So, in 90 days it was observed decrease in supernatants activities by 8–32 times, and for precipitates – by 320–1280 times according to their initial parameters (Table 4). But on the other hand, killer activity indices of bacteriocin mixture, which contained the same but unseparated killer factors, didn't decrease or decreased minimally – by 2 times against indicator culture *P. aeruginosa* UCM B-3 and by 8 times against UCM B-10.

This fact indicated that application of noted method for increase of bacteriocin activity has short-term result. During further storage of killer factors their activity decreased substantially.

Table 3

Activity of *P. aeruginosa* bacteriocins contained in unseparated and separated by ultracentrifugation samples against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 after storage during 14 days

Strain	14 days mixture				14 days precipitate				14 days supernatant			
	B-3		B-10		B-3		B-10		B-3		B-10	
	A	n	A	n	A	n	A	n	A	n	A	n
B-9	12.8	0	51.2	-2	12.8	-80	51.2	-160	6.4	-4	6.4	-16
B-330	25.6	-2	102.4	-2	12.8	-640	51.2	-160	25.6	-4	25.6	-16
B-332	25.6	0	102.4	0	51.2	-160	51.2	-160	51.2	-2	25.6	-4
B-333	25.6	0	102.4	0	51.2	-160	51.2	-160	12.8	-4	12.8	-16
B-335	12.8	0	25.6	-2	12.8	-80	12.8	-320	12.8	-2	51.2	-2

Legend: A – killer activity, ×10³ AU/mL; n – decrease multiplicity of bacteriocin activity after storage during 14 days relative to their activity in composition of corresponding initial samples.

Table 4

Activity of *P. aeruginosa* bacteriocins contained in unseparated and separated by ultracentrifugation samples against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 after storage during 90 days

Strain	90 days mixture				90 days precipitate				90 days supernatant			
	B-3		B-10		B-3		B-10		B-3		B-10	
	A	n	A	n	A	n	A	n	A	n	A	n
B-9	6.4	-2	12.8	-8	1.6	-640	6.4	-1280	1.6	-16	3.2	-32
B-330	25.6	-2	51.2	-4	6.4	-1280	12.8	-640	12.8	-8	25.6	-16
B-332	25.6	0	25.6	-4	6.4	-1280	25.6	-320	12.8	-8	12.8	-8
B-333	25.6	0	25.6	-4	12.8	-640	25.6	-320	3.2	-16	6.4	-32
B-335	6.4	-2	12.8	-4	1.6	-640	3.2	-1280	3.2	-8	25.6	-4

Legend: A – killer activity, ×10³ AU/mL; n – decrease multiplicity of bacteriocin activity after storage during 90 days relative to their activity in composition of corresponding initial samples.

Thus ultracentrifugation allows to separate S-type pyocins and M-II-LB, but application of this method for their activities increase is inexpedient.

Discussion. In our previous studies it was shown that M-II-LB are able to penetrate through a semipermeable membrane with MWCO 15 kDa [11]. We took advantage of revealed ability for their isolation separately from other killer factors. That is why, in this work, to prevent the loss of low-molecular bacteriocins, lysate purification was conducted using membrane with MWCO 3 kDa. As a result, bacteriocin mixture applied to column with DEAE-cellulose contained both S-type pyocins and M-II-LB.

It is known that molecules of S1 pyocins are characterized by weak positive charge; therefore they don't fix with DEAE-cellulose and flush by pure buffer during elution. We didn't select these fractions; therefore availability of this type pyocins weren't confirmed. Properties of our isolated M-II-LB hadn't been studied. But their absorption peak was in near ultraviolet range at wave length 205-210 nm. It indicates the absence of tyrosine, tryptophan and in less measure – histidine and phenylalanine [15]. Similar properties are characteristic for microcins B17, C7 and H47. Their total surface charge is equal to zero or weak positive. DEAE-cellulose is one of anion-exchange sorbates, which binds substances with negative surface charge. But during elution of S5 pyocins and M-II-LB mixture through noted carrier, these substances remain bound with each other. Adduced facts can indicate availability of weak electrostatic interaction between these killer factors. It is known that substance separation by gel filtration, ultracentrifugation and dialysis is not connected with their molecule charge, and based exclusively on differences in size and molecular weight. The results described in this work and obtained in previous studies indicated that application of noted methods allows to separate S-type pyocins with higher molecular weight from low molecular weight M-II-LB. If mentioned killer factors interact with each other in the way, different from electrostatic one, their separation will be limited or impossible.

It was shown in the study that the use of ultracentrifugation in most cases led to increase of bacteriocin activity in precipitates by 80–160 times. But their volume comparing with volume of bacteriocin mixture decreased only by 60 times. So we observed additional, not connected with decrease of sample volume, activity increase of S-type pyocins by 1.5–2.5 times. However supernatants volume in comparison with volume of bacteriocin mixture practically didn't change. As the result of S-type bacteriocin precipitation, it could be expected that supernatant activity will decrease. But we observed inverse relationship – double increase of their activity indices. Thus application of ultracentrifugation resulted in the same increase of S-type bacteriocins and M-II-LB. The revealed dependence additionally confirmed previous assumption about interaction of these killer factors with each other. Their separation probably release lysis domain and improve interaction of each type bacteriocins with receptors of sensitive cells.

Testing of separated by ultracentrifugation precipitates and supernatants showed sharp decrease of their activity indices after 14 days of storage. Detected dependence continued farther during 90 days of observation but wasn't so evident. It should be noted that comparing with intensive activity fall of S-type pyocins in precipitates, indices of M-II-LB in supernatants decreased considerably less. At the same time activity of mixture contained unseparated killer factors of both types didn't practically change. So, S-type pyocins and M-II-LB of *P. aeruginosa* interact with each other, that ensures their stabilization and protects again destruction.

Comparing described methods of purified M-II-LB isolation it should be noted that application of ion-exchange chromatography with DEAE-cellulose with following gel filtration through Sephadex G-75 so as ultracentrifugation enables to attain assigned task. But they are technologically complicated; require long-term stages of sample preparation. Application of ultracentrifugation is inexpedient, since it leads to decrease of bacteriocin activity under further storage. In contrast to noted approaches, our previously described method of dialysis for *P. aeruginosa* concentrated lysates through a semipermeable membrane with MWCO 15 kDa [15] can be considered not only effective in the same measure but also more accessible to wide application.

Conclusions. S-type pyocins and microcin-II-like bacteriocins of *P. aeruginosa* interact with each other, that ensures their stabilization and protects again destruction. Application of methods that cause separation of these killer factors is inexpedient, since it results into considerable decrease of bacteriocin activity indices.

ВЗАЄМОДІЯ ПІОЦИНІВ S-ТИПУ І МІКРОЦИН-ІІ-ПОДІБНИХ БАКТЕРІОЦИНІВ *PSEUDOMONAS AERUGINOSA*

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Резюме

За результатами наших попередніх досліджень було показано, що бактеріоцини S-типу *Pseudomonas aeruginosa* характеризуються високою активністю щодо фітопатогенних штамів *Pseudomonas syringae*. Окрім даних піоцинів, штами-продуценти здатні виділяти мікроцин-ІІ-подібні бактеріоцини. Наявність взаємодії між цими кілерними факторами може визначати методи їх використання і підвищення активності бактеріоцинів з антифітопатогенними властивостями. **Метою роботи** було перевірити можливість взаємодії піоцинів S-типу і коліцин-ІІ-подібних бактеріоцинів *P. aeruginosa*. **Методи.** Об'єктом дослідження були піоцини, синтезовані 6 штамами *P. aeruginosa* . Кілерні фактори у складі індукованих лізатів концентрували висолюванням 70%-ним сульфатом амонію, діалізували через діалізну мембрану з порогом відсічення 3,5 кДа, після чого для їх розділення використовували іонообмінну хроматографію на ДЕАЕ-целюлозі, гель-фільтрацію на сефадексі G-75 та ультрацентрифугування при 215.000 g протягом 1 і 4 год. В отриманих фракціях визначали вміст білка та антимікробну активність. Візуалізацію білків у складі активних фракцій проводили методом електрофорезу за Laemmli. **Результати.** При використанні іонообмінної хроматографії на ДЕАЕ-целюлозі елюція наявних у складі лізатів бактеріоцинів відбувалась одночасно. Найвищі показники активності і концентрації білка виявлялись у фракції 4, яка містила дві білкові полоси з молекулярними масами близько 58 і 9 кДа, характерні S5 піоцинам і мікроцин-ІІ-подібним бактеріоцинам *P. aeruginosa*. Подальше гель-фільтрування відібраних фракцій через сефадекс G-75 дозволило розділити вказані кілерні фактори і отримати очищену фракцію, яка містила виключно мікроцин-ІІ-подібні піоцини. Використання ультрацентрифугування протягом 1 год не осаджувало досліджувані бактеріоцини, тоді як протягом 4 год – призводило до їх розділення. При цьому спостерігалось двократне підвищення показників активності піоцинів S-типу у складі осаду, а мікроцин-ІІ-подібних кілерних факторів – в супернатантах. Однак досягнуте концентрування характеризувалось лише короткостроковим ефектом, оскільки вже через 14 діб активність супернатантів знижувалась у 4–16 разів, а осадів – у 80–640 разів. В подальшому виявлена тенденція до зниження активності зберігалась. **Висновки.** Піоцини S-типу і мікроцин-ІІ-подібні бактеріоцини *P. aeruginosa* взаємодіють між собою, що забезпечує їх стабілізацію і захищає від руйнування. Використання методів, які спричиняють розділення даних кілерних факторів, є недоцільним, оскільки призводять до значного зниження показників активності бактеріоцинів.

Ключові слова: мікроцин-ІІ-подібні бактеріоцини, піоцини S-типу, *Pseudomonas aeruginosa*, взаємодія.

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