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LOW MOLECULAR WEIGHT *PSEUDOMONAS AERUGINOSA* BACTERIOCINS

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The aim of the work *was to study Pseudomonas aeruginosa ability to produce bacteriocins with low molecular weight, establishing their nature and belonging to a certain group of antimicrobial substances.* ***Methods.*** *The objects of the study were substances synthesized by six Pseudomonas aeruginosa strains – highly active bacteriocin producers. Isolation of substances with low molecular weight was carried out by dialysis through a semipermeable membrane with molecular weight cut-off (MWCO) 15 kDa. The belonging of the obtained substances to bacteriocins was checked by the influence on own producer strains, the treatment with trypsin (1 mg/ml), the detection of their absorption spectrum. The investigated substances were concentrated by ammonium sulphate, and the molecular weight was determined by SDS-PAGE.* ***Results.*** *The results of the dialysis of P. aeruginosa lysates for 3 and 10 days showed that the studied cultures are able to produce low-molecular substances that penetrate through the pores of dialysis membrane with MWCO 15 kDa. The duration of penetration and activity of these substances for each strain were different. But in all cases, dialysates of the 1st day were characterized by the maximum activity, and then the tendency to decrease these parameters in subsequent periods of sampling was observed. The detected substances did not penetrate through the dialysis membrane with MWCO 6-8 kDa, did not affect their own producer strains, and lost activity after treatment with trypsin (1 mg/ml). The peaks of their absorption were 205-210 nm, which is characteristic of peptide bonds of proteins. The received data allowed characterizing the detected substances as low molecular weight bacteriocins. The results of SDS-PAGE revealed a single peptide with molecular weight of 9 kDa.* ***Conclusions.*** *The investigated P. aeruginosa strains were characterized by production of low molecular weight microcin II-like bacteriocins with molecular weight about 9 kDa. These substances may be associated with S-type pycins, which are also synthesized by the same strains.*

Keywords: low molecular weight pyocins, microcin II-like bacteriocins, Pseudomonas aeruginosa, dialysis purification, absorption peak 205-210 nm.

For *Pseudomonas aeruginosa*, the production of bacteriocins (pyocins) of three types is described: R-, F- and S-types [1]. Pyocins of the R- and F-types are macromolecular structures, analogues of phage tails, which are constructed of several proteins and characterized by total molecular weight of about 1–10 MDa [2]. S-type pyocins belong to colicin-like or low-molecular-weight bacteriocins, which in most cases are a complex of two proteins – actually bacteriocin and immunity protein. Bacteriocin realizes killer activity, and cognate immunity protein protects the pyocin-producing cell [3].

In our previous studies it was shown that 30 and 40°C temperature treatment for 10 min increased the killer activity of S-type pyocins isolated from 10 of 11 investigated *P. aeruginosa* strains [4]. Temperature of 30°C effect on most bacteriocins stimulated 2-fold activity increase, and 40°C caused

4-fold rise in activity. In some cases the temperature influence on pyocins made possible to reach 8-16 times growth of activity indices. Meanwhile, the extension of duration of temperature impact doesn't stimulate the additional thermoactivation of bacteriocins. It was assumed in mentioned paper, that the studied S-type pyocins may be associated with other substances in an unstable complex, which, under the influence of temperature, destroys, improving the availability of active centers and increasing the activity of bacteriocins [4]. It is known that the molecular weight of S-type pyocins is in the range from 30 to 100 kDa [1, 2]. In the case of the existence of the association of S pyocins with other substances, the latter ones, obviously, should be characterized by low molecular weight. In general bacteriocins with molecular weight below 10 kDa are produced only 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) [2]. Therefore, the aim of this work was to study the ability of *Pseudomonas aeruginosa* to produce bacteriocins with low molecular weight, establishing their nature and belonging to a certain group of antimicrobial substances.

Materials and methods. The objects of investigation were bacteriocins with low molecular weight from six *P. aeruginosa* strains – UCM B-9, UCM B-330, UCM B-332, UCM B-333, UCM B-335, UCM B-353, maintained in the Ukrainian collection of microorganisms (UCM, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine). These microorganisms were previously described by us as highly active bacteriocin producers [5].

To obtain lysates of these *P. aeruginosa* strains, nalidixic acid was added to suspension of strain-producers in the logarithmic growth phase (final concentration 100 μg/ml). The used strains were sensitive to nalidixic acid at such concentration. After further incubation for 3 h, the induction was stopped by adding 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 [6].

In our study we tested the ability of *Pseudomonas aeruginosa* strains to produce bacteriocins with low molecular weight. The method of dialysis was used to isolate low molecular weight substances from obtained *P. aeruginosa* lysates. For dialysis we applied a semipermeable membrane of regenerated cellulose with molecular weight cut-off (MWCO) 15 kDa (Serva). Before conducting experiments semipermeable membrane was prepared and sterilized accordingly [7]. 1 ml of each lysate was added in the sacs of dialysis membrane. These sacs were dipped in 1 ml of 0.9% sodium chloride solution and kept at + 4°C for 24 hours. After mentioned period, the solution around the sac was aseptically sampled and marked as dialysate of the 1st day. After this, 1 ml of 0.9% sodium chloride solution was added to the vessel repeatedly and the dialysis was continued. Solutions sampled in the following days were marked as dialysate of the 2nd day, dialysate of the 3rd day, etc. The dialysis was conducted with two experiments for 3 and 10 days.

The obtained dialysates were tested for the presence of substances with antimicrobial activity against the indicator cultures for the detection of bacteriocins – *P. aeruginosa* UCM B-3 and UCM B-10. Qualitative and quantitative indices of killer activity were determined by the method of "two-layer agar", as described earlier [6, 8]. The obtained parameters were counted on 1 ml of dialysate and expressed in AU/ml or for convenience in 10^3 AU/ml.

A number of experiments were carried out to define belonging of the penetrated substances to bacteriocins. To determine penetration through a semipermeable membrane with MWCO 6–8 kDa, the dialysates of the 1st and 2nd days, received during 10-day dialysis, were carried into the sacs. The following procedure corresponded to the described above one used for dialysis. Determination of absorption spectra of dialysates was performed on Specord UV VIS. The samples were diluted with 20 mM Tris-HCl buffer (pH 7.5), brought into 0.2 cm thick cuvettes and registered against the mentioned buffer. The sensitivity of low molecular weight bacteriocins to proteolytic enzymes was tested by processing of dialysates with trypsin (1 mg/ml, Roth, 2500 USP-U/mg). After incubation of treated and untreated dialysates at 37°C for 1 h, their killer activity against the indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 was checked.

Concentration of bacteriocins was carried out by the ammonium sulphate precipitation to 20, 40, 60 and 80% saturation during 1 day at 4°C. The sediment was obtained at 30.000 g and 4°C for 30 min and resuspended in 6 ml of 20 mM Tris-HCl buffer (pH 7.5). The samples were dialyzed through dialysis membrane (3.5 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 means of low centrifugation at 4.000 g for 30 minutes [3].

SDS-PAGE of *P. aeruginosa* low molecular weight bacteriocins was carried out in 12,5% gel by the Laemmli method [9], using as markers the PageRuler Plus Prestained Protein Ladder, 10–250 kDa (Thermo Scientific).

The tests were conducted in three replications, statistical processing of experimental data was carried out using the Microsoft Excel program from the Microsoft Office suite of applications. Differences in average values were considered reliable at a level of significance $p < 0.05$. The error of the received data did not exceed 5%.

Results. It was established that during the dialysis of concentrated bacterial lysates of *P. aeruginosa* containing S-type pycins [7, 8], low molecular weight substances penetrate through the dialysis membrane. The dialysates containing these substances were selected and their antimicrobial activity against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 was determined. The killer activity test of dialysates taken for the first day (further dialysates of the 1st day) showed that the substances isolated from *P. aeruginosa* UCM B-9, UCM B-332 and UCM B-335 did not influence *P. aeruginosa* UCM B-3 (Fig. 1A, C, E). The killer activity of *P. aeruginosa* UCM B-330, UCM B-333 and UCM B-353 dialysates concerning this indicator culture was low and ranged from 0.8 to 12.8×10^3 AU/ml (Fig 1B, D, F). After the treatment of the other indicator culture *P. aeruginosa* UCM B-10 with these substances

the activity indices were some higher. So, the activity of UCM B-9 dialysate against UCM B-10 was 0.8×10^3 AU/ml, whereas these substances didn't influence UCM B-3. Dialysates from UCM B-330, UCM B-333 and UCM B-353 also were characterized by higher values of killer activity against UCM B-10, since activity indices of the substances in their composition were 51.2; 6.4 and 102.4×10^3 AU/ml, respectively. The substances in UCM B-332 and UCM B-335 dialysates also didn't influence indicator culture *P. aeruginosa* UCM B-10.

To estimate the duration of penetration of low molecular weight substances, the subsequent sampling of dialysates was carried out. The substances taken for the second day of dialysis (further dialysates of the 2nd day) from UCM B-9 and UCM B-335, as well as their dialysates of the 1st day, didn't influence indicator culture *P.aeruginosa* UCM B-3 (Fig 1A, E). On the second day the activity of other dialysates UCM B-330 and UCM B-353 against this culture fell and amounted to 3.2 and 6.4×10^3 AU/ml, respectively (Fig 1B, F), but it was absent in substances from B-333 (Fig 1D).

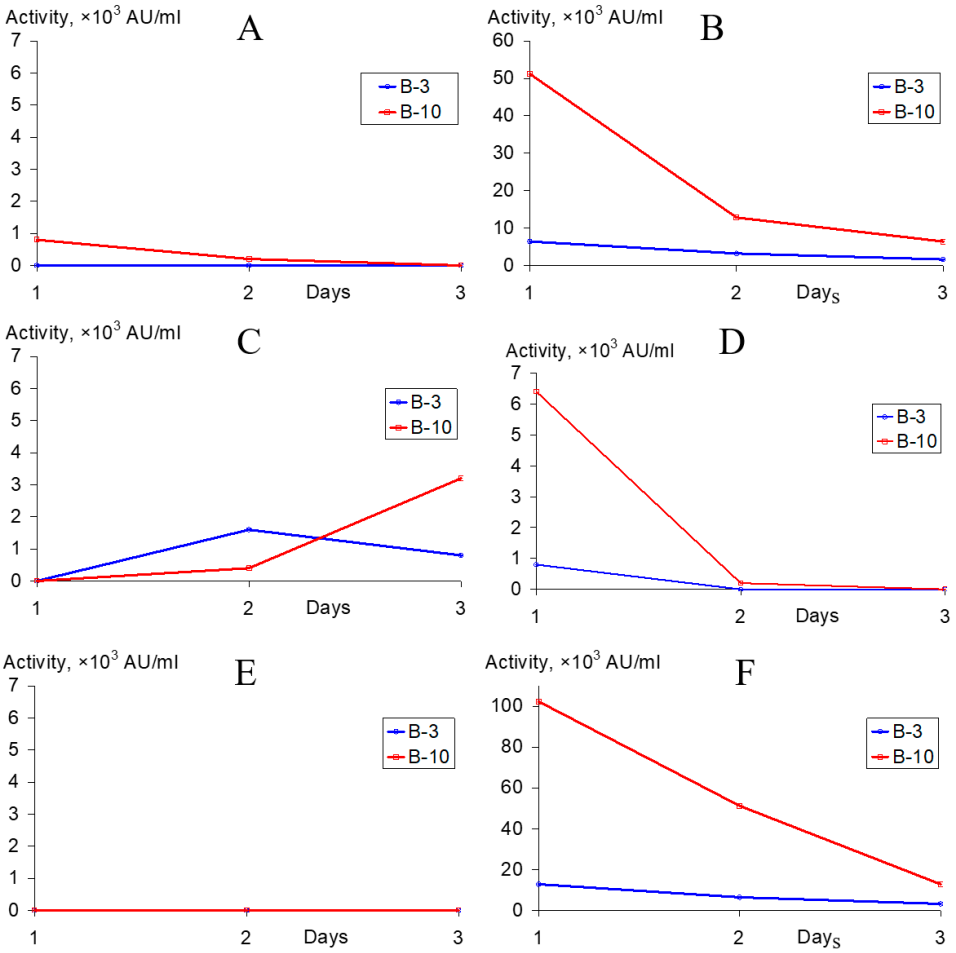

Fig. 1. The activity of low molecular weight bacteriocins in the composition of selected during 3 days dialysates of *P. aeruginosa* UCM B-9 (A), UCM B-330 (B), UCM B-332 (C), UCM B-333 (D), UCM B-335 (E) and UCM B-353 (F) against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10

However, the activity of UCM B-332 substances increased from zero values on the first day of selection to 1.6×10^3 AU/ml – on the second day (Fig 1C).

The test of dialysate activity revealed that on the second day the killer properties of substances against *P. aeruginosa* UCM B-10 was also substantially lower. For example, the activity of UCM B-9, UCM B-333 dialysates was minimal – 0.2×10^3 AU/ml, and the dialysate of UCM B-335 didn't influence UCM B-10. For UCM B-330 and UCM B-353 dialysates the decrease of killer activity level to 12.8×10^3 AU/ml was noted in both cases. But, activity indices of dialysate substances from UCM B-332 against UCM B-10, as well as UCM B-3, also partially increased and amounted to 0.4×10^3 AU/ml. Thus, in most cases, the activity of substances from dialysates of 2nd day, comparing with their analogues in dialysates of 1st day, significantly decreased.

The test of killer activity of dialysates selected on the 3rd day revealed regularities similar to described above (Fig 1). The substances from UCM B-9, UCM B-333 and UCM B-335 didn't influence indicator culture *P. aeruginosa* UCM B-3, but the activity indices of UCM B-330, UCM B-332 and UCM B-353 dialysates decreased twice. The substances from UCM B-9, UCM B-333 and UCM B-335 did not exhibit activity against *P. aeruginosa* UCM B-10, and indices of UCM B-330 and UCM B-353 fell in 2 and 4 times, respectively. Thus, during all selection period, a tendency to activity decrease was observed for most dialysates. However, the activity of UCM B-332 substances increased and amounted to 3.2×10^3 AU/ml for dialysate of 3rd day against UCM B-10. In contrast to the most dialysates, substances of UCM B-9 didn't influence UCM B-3, and UCM B-335 dialysates didn't cause the growth impediment of both indicator cultures.

To find out the reasons of detected deviations and to establish regularities of *P. aeruginosa* penetration of low molecular weight substances, a repeated dialysis of concentrated lysates was performed for 10 days. It was shown that the substances from UCM B-9 were released during 7 days (Fig. 2A). Their activity against indicator culture UCM B-3 was minimal – $0.4–0.8\times10^3$ AU/ml. Concerning *P. aeruginosa* UCM B-10 the lowering of indices was noted from 6.4×10^3 AU/ml – in dialysate of the 1st day to 1.6×10^3 AU/ml – in the substances of the 7th day. From the 8th day and in the subsequent, the activity of selected UCM B-9 dialysates wasn't revealed.

The substances of UCM B-330, as compared to the substances of UCM B-9, were characterized by significantly higher activity indices against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 – 12.8 and 51.2×10^3 AU/ml, respectively (Fig. 2B). During the observation period, their activity decreased. The penetration of UCM B-330 substances active against *P. aeruginosa* UCM B-3 as well as UCM B-9 dialysates was also observed only up to 7 days. Instead, concerning UCM B-10, the substances of UCM B-330 maintained activity 1.6×10^3 AU/ml even on the 10th day of sampling. The activity of UCM B-332 for the 1st day against UCM B-3 was similar to that in substances of UCM B-330, and concerning UCM B-10 – twice lower (Fig. 2C). The penetration of active substances in UCM B-332 was observed up to 7 days, and their activity as compared with indices for the 1st day decreased in 16 times. For UCM B-333, duration of activity detection in dialysates was the least prolonged (Fig. 2D). Thus, on the first day of sampling, the activity of UCM B-333 dialysates was rather high and approached to UCM B-330 indices – 6.4 та 51.2×10^3 AU/ml

against UCM B-3 and UCM B-10, respectively. However, on the second day it was observed the 8-fold decrease of UCM B-333 activity concerning the UCM B-3, after which on the 3rd day these substances didn't influence the mentioned indicator culture. A similar sharp decrease in activity was noted against UCM B-10, although even dialysates of 4th day caused slight growth inhibition. The most prolonged penetration of substances was characteristic for UCM B-335 (Fig. 2E). In this case, the activity, even at insignificant level, was detected against both strains to 10 days of sampling. For UCM B-353 the penetration of substances was similar to that of UCM B-333 (Fig. 2F). The activity of UCM B-353 dialysates of the 1st day was the highest of all investigated substances – 204.8 and 819.2×10^3 AU/ml against UCM B-3 and UCM B-10, respectively. Next two days a 4-fold decrease in activity was observed, and on the 4th day the activity of substances fell in 8 times. The dialysates of the 7th day didn't influence UCM B-3, and on the 9th day of sampling these substances weren't active against both used indicator cultures.

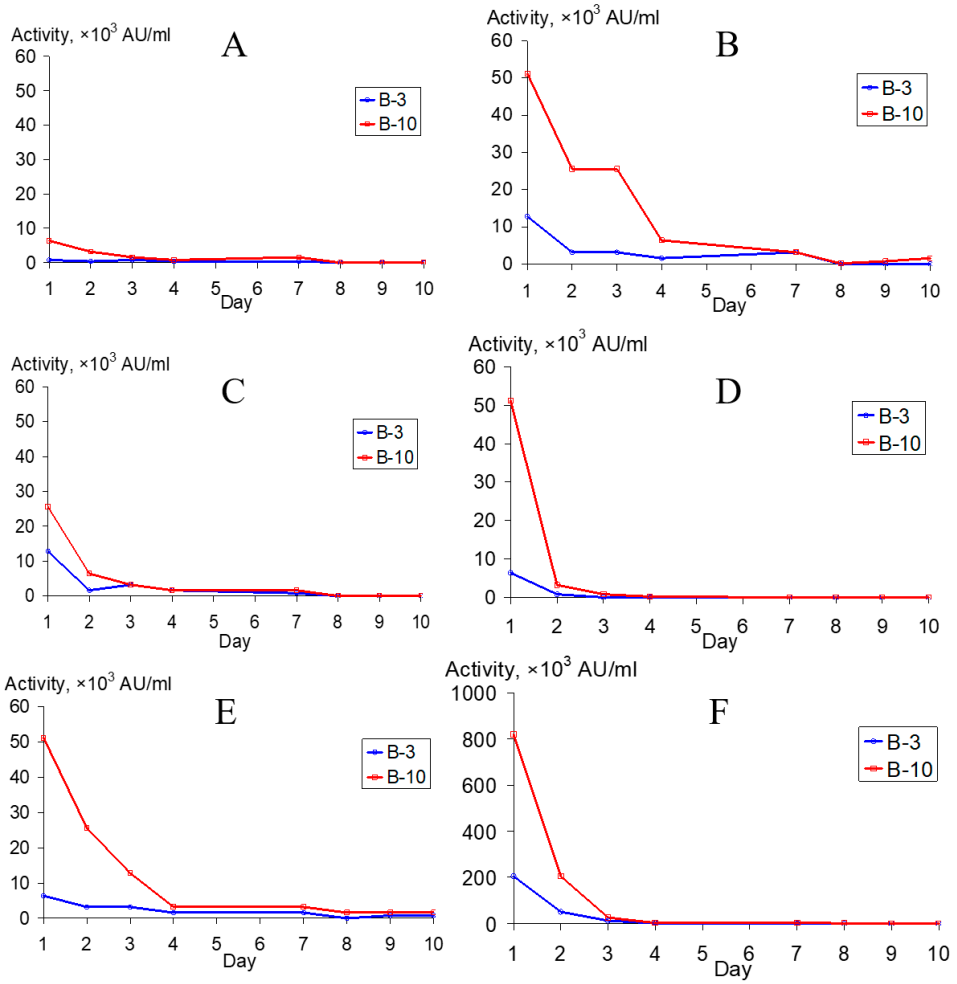

Fig. 2. The activity of low molecular weight bacteriocins in the composition of selected during 10 days dialysates of *P. aeruginosa* UCM B-9 (A), UCM B-330 (B), UCM B-332 (C), UCM B-333 (D), UCM B-335 (E) and UCM B-353 (F) against indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10

The obtained results indicate that all used producer strains are characterized by the production of low molecular weight substances. However, the duration of their production and the activity of these substances were different for each strain.

To establish the kind of the isolated low molecular weight substances of *P. aeruginosa*, it was taken into account that these killer factors penetrated through dialysis membrane with MWCO 15 kDa. The results of repeated dialysis of the investigated substances through dialysis membrane with 6–8 kDa MWCO showed the absence of substances with antimicrobial activity in dialysates. Therefore it was concluded that the molecular weight of the detected substances was in the range of 6–15 kDa. Such molecular weight is characteristic for antibiotic-like substances and bacteriocins. To test the belonging of the detected killer factors to antibiotics, their activity against their own producer strains was investigated (Table 1). It was determined that dialysates inhibited the growth of other microorganisms, but they didn't cause the formation of growth inhibition zones in their own producer strains in any cases.

Table 1

The activity of *P. aeruginosa* low molecular weight bacteriocins against their own producer strains

Dialysates	Producer strains					
	B-9	B-330	B-332	B-333	B-335	B-353
B-9	–	–	–	–	–	–
B-330	+	–	+	+	–	+
B-332	+	–	–	–	–	+
B-333	–	–	–	–	–	–
B-335	+	–	–	–	–	+
B-353	–	–	+	–	–	–

Annotation: + growth inhibition, – absence of impact.

This fact indicated that studied substances didn't belong to antibiotics. Treatment of low molecular weight substances by trypsin at the concentration of 1 mg/ml led to loss of their activity. It proved that the protein component was responsible for antimicrobial activity. Determination of absorption spectra of investigated dialysates detected peaks in the near UV range (λ = 205–210 nm), that are characteristic for peptide groups of proteins, which confirmed the identity of killer factors to substances with protein nature (Fig. 3). Based on the data above, the received substances were classified as bacteriocins.

The content of low molecular weight killer factors in received dialysates was low. For the further study of the nature and biological properties of these substances, we need to increase their concentration. For this, the precipitation by ammonium sulphate was carried out, adding it to 20, 40, 60 and 80% saturation.

The possibility to concentrate low molecular weight bacteriocins by ammonium sulphate was tested on the substances of *P. aeruginosa* UCM B-353 as the most active among the investigated dialysates. The initial killer activity of the investigated dialysate against indicator strain *P. aeruginosa* UCM B-3 was 12.8×10^3 AU/ml, and concerning UCM B-10 – 25.6×10^3 AU/ml. After

centrifugation of the samples, precipitated by ammonium sulphate, supernatants and sediments were obtained, and then their killer activities were determined. The results of supernatant investigation indicated that under precipitation by ammonium sulfate to 20 and 40% saturation, not all bacteriocins passed into the sediment. Their activity against indicator strain UCM B-3 was 1.6 and 0.4×10^3 AU/ml, and concerning UCM B-10 – 25.6 and 2.6×10^3 AU/ml, respectively. Under increase of ammonium sulphate concentration up to 60 and 80%, the activities in supernatants against both indicator strains weren't detected. The investigation of killer activity of obtained sediments showed that their killer indices grew with increase of ammonium sulphate concentration. Thus, after dialysate saturation with ammonium sulphate to 20%, the killer activity of sediments against indicator strain UCM B-3 was 32×10^3 AU/ml, and concerning the indicator strain UCM B-10 – 256×10^3 AU/ml. Under the increase of salt content to 40%, the activity against UCM B-3 grew up in 4 times, and concerning UCM B-10 – in 2 times. The maximum killer activity values against both indicator strains were obtained by saturation of dialysate with ammonium sulphate up to 60%. In this case, the killer activity against UCM B-3 amounted to 512×10^3 AU/ml, and concerning UCM B-10 – 2048×10^3 AU/ml. Further increase in the content of ammonium sulphate to 80% saturation led to a sharp decrease in killer activity of dialysates, detecting at a minimum level. Based on the obtained results, it was concluded that ammonium sulphate can be used to increase the activity of *P. aeruginosa* low molecular weight substances. The addition of ammonium sulphate to 60% saturation was the most optimal and allowed to increase the activity of bacteriocins in 40 and 80 times against indicator cultures UCM B-3 and UCM B-10, respectively. The ability to be concentrated by ammonium sulfate additionally confirms the protein nature of these low molecular weight bacteriocins.

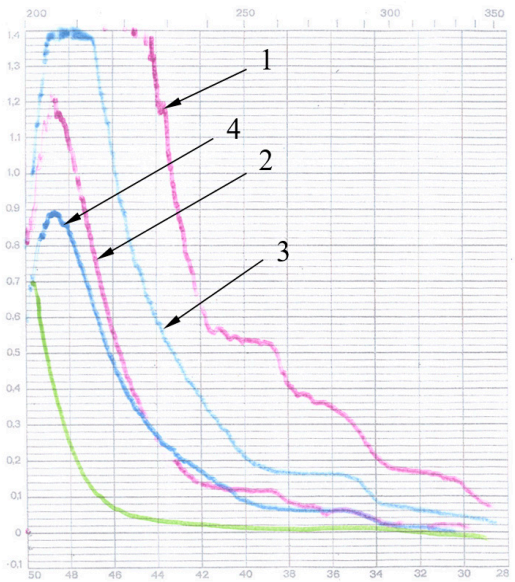

Fig. 3. Absorption spectra of *P. aeruginosa* UCM B-353 dialysate substances in the UV range: prior to dialysis (1 – dilution 1:10; 2 – dilution 1:50) and after (3 – dilution 1: 5; 4 – dilution 1:10)

The obtaining of concentrated samples of low molecular weight bacteriocins from *P. aeruginosa* UCM B-353 made possible to conduct their electrophoretic separation (Fig. 4). As a result, a single protein band with an approximate molecular weight of 9 kDa was revealed in the electrophoregram. No other additional peptides were observed. Low molecular weight bacteriocins of other producer strains *P. aeruginosa* UCM B-9, UCM B-330, UCM B-332, UCM B-333 and UCM B-335 were also concentrated by ammonium sulphate. After their SDS-PAGE, the obtained results were similar to described above.

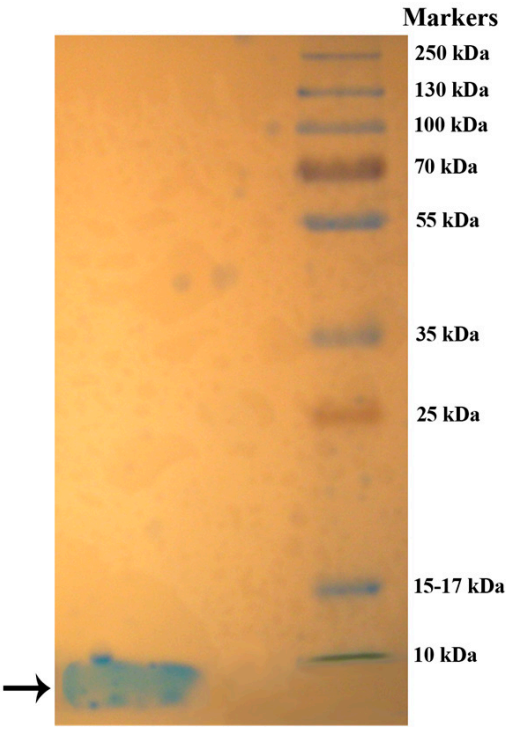

Fig. 4. The electrophoregram of low molecular weight bacteriocin from *P.aeruginosa* UCM B-353 (the peptide found is indicated by an arrow)

Discussion. In our studies, the molecular weight of investigated bacteriocins was about 9 kDa. The production of similar killer factors in gram-negative bacteria is described for bacteriocins of *E. coli* – microcins, which molecular weight is lower than 10 kDa. The analogous sizes are typical for the bacteriocins of most gram-positive bacteria [10]. Nowadays about 22 subtypes of S-type pyocins have been investigated or predicted for *P. aeruginosa.* These substances belong to colicin-like or low-molecular-weight bacteriocins with molecular weight in the range of 30 to 100 kDa [1, 2]. However, a number of authors state that *Pseudomonas* genus bacteria are peculiar to production of low molecular weight bacteriocins. So, Saleem et al. showed that isolated from soil *Pseudomonas aeruginosa* strains were capable of synthesizing Pa pyocins, whose molecular weight was less than 10 kDa [11]. In other studies, conducted by Barberis et al., in pseudomonads there were found similar antibacterial substances with molecular weight less than 10 kDa [12]. In Hubert et al. work,

the molecular weight of the synthesized by *Pseudomonas* sp. R-10 bacteriocin was ascertained more precisely, it was detected in the range from 2 to 4 kDa [13]. Thus, bacteria of *Pseudomonas* genus are characterized by production of low molecular weight microcin I- and microcin II-like bacteriocins.

It is known that protein compounds absorb UV rays. The absorption peaks of our investigated bacteriocins were detected in the near-UV range at wavelengths from 205 to 210 nm. Absorption in 200–220 nm is characteristic of peptide bonds, which are the basic structure of all proteins [14]. Also it is considered, that typical feature of the most proteins is absorption in the range of 280–290 nm. This is due to the presence of amino acids with aromatic residues in their structure – tryptophan, tyrosine and, to a lesser extent, phenylalanine and histidine [14]. In our case, the absorption in this region was insignificant, which may indicate the minimum number or absence of the mentioned amino acids. Since these amino acids are hydrophobic, it can be assumed that the test substances are characterized by hydrophilic properties. This fact is also indicated by the nature and duration of their dialysis. Thus, the penetration of low molecular weight bacteriocins in most strains followed the general regularities: the maximum activity was detected in dialysates of the 1st day with a tendency to decrease the activity indices in subsequent periods of sampling. However, during dialysis for three days, none of UCM B-335 dialysates inhibited the growth of indicator cultures *P. aeruginosa* UCM B-3 and UCM B-10 (Fig. 1). In this experiment, features that were not characteristic to other bacteriocins were detected for UCM B-332 dialysates – the lack of activity in substances of the 1st day and the growth of these parameters in subsequent periods of sampling. The results of repeated dialysis for 10 days showed that UCM B-332 and UCM B-335 bacteriocins are possessed of general regularities of penetration (Fig. 2). Since in both experiments (dialysis of 3 and 10 days) the same initial lysates were used, the identified differences were connected with the peculiarities of the membrane preparation. The dialysis membrane for the first experiment (dialysis of 3 days) was used immediately after preparation and sterilization, respectively [7], whereas for the second experiment (dialysis of 10 days), after similar treatment, the membrane was additionally kept in distilled water under sterile conditions for 14 days. It is considered that keeping of dialysis membrane in water or glycerin leads to its swelling, which can increase the diffusion coefficient of low molecular weight substances [15]. Comparing the activity of bacteriocins from 3 (Figure 1) and 10-days dialysis (Figure 2), it should be noted, that in the last all bacteriocins were released according to the general regularities, the duration of their period of penetration through the membrane was prolonged, and the activity of most dialysates increased by 8 times. The mentioned fact may be an additional evidence of the hydrophilicity of the investigated low molecular weight bacteriocins.

Thus, the studied *P. aeruginosa* strains are characterized by the production of low molecular weight microcin II-like bacteriocins with molecular weight about 9 kDa. Based on the results of this study and the previous investigation [4], we can make an assumption that these substances may be associated with S-type pyocins, which are also synthesized by the same strains. But confirmation of this hypothesis requires further researches.

БАКТЕРІОЦИНИ *PSEUDOMONAS AERUGINOSA* З НИЗЬКОЮ МОЛЕКУЛЯРНОЮ МАСОЮ

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Резюме

Метою роботи було дослідження здатності культур *Pseudomonas aeruginosa* синтезувати бактеріоцини з низькою молекулярною масою, встановлення їх природи і належності до певної групи антимікробних речовин. **Методи.** Об'єктом дослідження були речовини, синтезовані 6 штамами *Pseudomonas aeruginosa* – високоактивними продуцентами бактеріоцинів. Виділення речовин з низькою молекулярною масою здійснювали методом діалізу через напівпроникну мембрану з діаметром пор 15 кДа. Належність отриманих речовин до бактеріоцинів перевіряли за допомогою впливу на власні штами-продуценти, обробкою трипсином (1 мг/мл), виявлення спектрів їх поглинання. Досліджувані речовини концентрували висолюванням сульфатом амонію, методом білкового електрофорезу визначали їх молекулярну масу. **Результати**. Проведення діалізу лізатів *P. aeruginosa* протягом 3-х та 10-ти діб показало, що досліджувані культури здатні виділяти низькомолекулярні речовини, які проникають через пори діалізної мембрани з порогом відсічення 15 кДа. Тривалість виділення та активність даних речовин для кожного штаму відрізнялись, але в усіх випадках максимальною активністю характеризувались діалізати першої доби з тенденцією до зниження даних показників у наступні періоди відбору. Виявлені речовини не проникали через діалізну мембрану з порогом відсічення 6–8 кДа, не впливали на власні штами-продуценти, втрачали активність після обробки трипсином (1 мг/мл). Піки їх поглинання становили 205–210 нм, що є характерним для пептидних зв'язків білків. Отримані дані дозволили віднести виявлені речовини до бактеріоцинів з низькою молекулярною масою. Проведення білкового електрофорезу виявило у їх складі єдиний пептид з молекулярною масою 9 кДа. **Висновки.** Для досліджуваних культур *P. aeruginosa* характерним є виділення низькомолекулярних, мікроцинподібних бактеріоцинів молекулярною масою близько 9 кДа. Вказані речовини можуть бути асоційовані з піоцинами S-типу, які також синтезуються даними штамами.

Ключові слова: піоцини з низькою молекулярною масою, мікроцин II-подібні бактеріоцини, *Pseudomonas aeruginosa*, очистка методом діалізу, піки поглинання при 205–210 нм.

БАКТЕРИОЦИНЫ *PSEUDOMONAS AERUGINOSA* С НИЗКОЙ МОЛЕКУЛЯРНОЙ МАССОЙ

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Резюме

Целью работы было изучение способности культур *Pseudomonas aeruginosa* синтезировать бактериоцины с низкой молекулярною массой, определение их природы и принадлежности к определенной группе антимикробных веществ. **Методы.** Объектом исследования были вещества, синтезированные 6 штаммами *Pseudomonas*

aeruginosa – высокоактивными продуцентами бактериоцинов. Выделение веществ с низкой молекулярной массой осуществляли методом диализа через полупроницаемую мембрану с диаметром пор 15 кДа. Принадлежность полученных веществ к бактериоцинам проверяли с помощью влияния на собственные штаммы-продуценты, обработки трипсином (1 мг/мл), определения их спектра поглощения. Исследованные вещества концентрировали высаливанием сульфатом аммония и методом белкового электрофореза определяли их молекулярную массу. **Результаты.** Результаты проведения диализа лизатов *P. aeruginosa* в течение 3-х и 10-ти суток показали, что исследуемые культуры способны выделять низкомолекулярные вещества, которые проникают через поры диализной мембраны с порогом отсечения 15 кДа. Продолжительность выделения и активность данных веществ отличались для каждого штамма, однако во всех вариантах максимальной активностью характеризовались диализаты первых суток с тенденцией к снижению данных показателей в следующие периоды отбора. Полученные вещества не проникали через диализную мембрану с порогом отсечения 6–8 кДа, не влияли на собственные штаммы-продуценты, теряли активность после обработки трипсином (1 мг/мл). Пики их поглощения составляли 205–210 нм, что характерно для пептидных связей белков. Согласно полученным данным выявленные вещества были отнесены к бактериоцинам с низкой молекулярной массой. Результаты белкового электрофореза показали наличие в их составе единственного пептида с молекулярной массой 9 кДа. **Выводы.** Для исследованных культур *P. aeruginosa* характерным является выделение низкомолекулярных, микроцин-подобных бактериоцинов с молекулярной массой около 9 кДа. Данные вещества могут быть ассоциированы с пиоцинами S-типа, которые также синтезируются этими штаммами.

Ключевые слова: пиоцины с низкой молекулярной массой, микроцин II-подобные бактериоцины, *Pseudomonas aeruginosa,* очистка методом диализа, пики поглощения при 205–210 нм.

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