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THERMOACTIVATION OF *PSEUDOMONAS AERUGINOSA* PYOCINS

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The aim of work *was the investigation of influence of different temperatures on activity of Pseudomonas aeruginosa S-type pyocins.* ***Methods.*** *The objects of investigation were S-type pyocins from eleven P. aeruginosa strains. These killer agents were subjected to the temperature influence in the range of 20 to 100° C for 10 minutes. Quantitative indices of killer activity of treated bacteriocins were compared with those ones of untreated analogs. For a more detailed study of 30 and 40° C effect on lysates, different durations of temperature treatment were used: 5, 15 and 25 minutes.* ***Results.*** *It was shown that 60° C caused the decrease in the activity of most bacteriocins to the minimum values, and 70° C – entailed the absolute loss of activity of all investigated lysates. Temperature treatment at 30 and 40° C increased the killer activity of bacteriocins. This effect was revealed with different rates depending on the used indicator cultures. The activity of the first group lysates increased after 30° C influence, the lysates from the second group – 40° C, and the third group – under the action of several temperatures, including 50° C or not changed. The 30° C effect on most bacteriocins stimulated 2-fold activity increase, and 40° C caused 4-fold rise in activity. But activity indices of some lysates extended more: 8 times – for PAE-24 and 16 times – for PAE-6. The investigation of influence of 30 and 40° C on PAE-19 and PAE-22 activity revealed no change in killer activity of bacteriocins caused by different duration of temperature impact.* ***Conclusions.*** *Temperature treatment of P. aeruginosa S-type pyocins at 30-40° C during 10 minutes makes possible to increase their activity in 2 – 4 times and in some cases, reach 8 – 16 times growth of activity indices. The extension of duration of temperature impact doesn't stimulate the additional thermoactivation of bacteriocins.*

Keywords: pyocins, thermoactivation, influence of temperature, increase of killer activity, Pseudomonas aeruginosa.

Natural ecological niches are inhabited by a large amount of different species of microorganisms [1]. During competition for nutrients, bacteria use different survival strategies. One of them consists in the synthesis of antimicrobial agents, in particular bacteriocins [2]. These substances are active against of closely related bacteria that populate the certain ecological niche [3]. Previously we have shown that *Pseudomonas aeruginosa* strains can produce bacteriocins, active against not only other *P. aeruginosa* strains [4, 5], but all investigated plant pathogenic *Pseudomonas syringae* strains too [6]. Potential application of bacteriocins for regulation of phytopathogenic bacteria quantity was proposed by a number of researchers [7, 8]. It is supposed that the use of strictly specific killer factors can become an effective strategy for the pointed control of bacterial diseases, in particular diseases of agricultural crops against which even chemical pesticides are ineffective [9].

It is obvious that one of the criteria of perspectivity of bacteriocin use is high indices of their killer activity in the composition of potential preparation. We revealed that the increase in concentration of *P. aeruginosa* S-type bacteriocin (pyocins) makes possible to expand the spectrum of lysate activity against *P. syringae* strains significantly [6]. Analyzing possible methods for further increase of bacteriocin activity we paid attention to the results obtained during the investigation of the properties of induced killer factors, namely data of their thermostability testing [10].

Therefore, the purpose of this work was to study the influence of different temperatures on the activity of *Pseudomonas aeruginosa* S-type bacteriocins.

Materials and methods. The objects of investigation were S-type bacteriocins (pyocins) from eleven collection strains *P. aeruginosa* maintained in the Ukrainian collection of microorganisms (UCM, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine).

To obtain *P. aeruginosa* pyocins, nalidixic acid was added to suspension of strain-producer 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 [5]. In the earlier studies we have shown that the properties of antimicrobial substances in the obtained lysates allowed classified them as S-type pyocins [6, 11].

The killer activity of lysates was determined by the method of "double layer agar". For this purpose 24-h culture of indicator strain was added to melted and cooled to 50 – 55° C semifluid (0,7 %) agar, then this agar was layered on MPA in Petri dishes. After hardening of upper agar, 5 μl of obtained lysates were applied on the lawn of the indicator culture. Dishes were incubated for 18 – 24 hours at 28°C. The formation of impediment zones on the lawn with indicator culture indicated the presence of substances with killer properties against the used indicator strain. To evaluate the antimicrobial activity of pyocins, *P. aeruginosa* UCM B-3 and UCM B-10 were used [4].

Quantitative activity indices of the certain lysates were determined by double serial dilutions method. The aliquots of each dilution (5 μl) were applied to a lawn with indicator culture, then the maximal dilution with impediment zone was determined. This dilution indicated the activity of the test substance. The obtained indices were counted for 1 ml of lysate and expressed in AU/mL or for convenience in thousand AU/mL or million AU/mL [5].

In the study of bacteriocin resistance to temperature there were used eleven *P. aeruginosa* lysates, marked according to their species as PAE. In this case, the lysates obtained from *P. aeruginosa* UCM B-1 were labeled as PAE-1 from UCM B-6 – as PAE-5, UCM B-7 – PAE-6, UCM B-9 – PAE-8, UCM B-13 – PAE-14, UCM B-330 – PAE-19, UCM B-332 – PAE-21, UCM B-333 – PAE-22, UCM B-335 – PAE-24, UCM B-349 – PAE-38, UCM B-353 – PAE-41. The obtained lysates stability to the temperature influence were estimated at 20, 30, 40, 50, 60, 70, 80, 100° C in the water bath using standard method. With this purpose 200 μl of lysate was put into Ependorf tube and exposed to the appointed temperature for 10 min. Immediately after thermal

inactivation, for the stopping of temperature impact, the test tubes with lysates were carried into the "ice bath" for 5 minutes. For more detailed investigation of 30 i 40° C influence on bacteriocin activity tubes with lysates were kept at these temperatures during 5, 15 and 25 minutes. After lysate exposition to certain temperature killer activity indices were determined for treated lysates. Obtained results were compared with analogous ones for untreated lysates.

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. In our previous studies we have shown, that investigated antimicrobial substances in *P. aeruginosa* lysates were not transferred from the formed lysis zones to the clear lawn of indicator strain. They didn't influence own producer culture, were not visualized under an electronic microscope, not precipitated by ultracentrifugation, not split by DNAase and RNAase. Also these substances lost their activity after thermal inactivation at 80° C and trypsin treatment. The investigated substances were characterized by a narrow spectrum of action, since they influenced exclusively bacteria of *Pseudomonas* genus and did not inhibit the growth of other microorganisms. According to the given data, the killer factors in the lysates were referred to colic-like bacteriocins – S-type pycins [6, 11].

Now it was shown, that killer activity indices didn't decrease after lysate treatment by 20, 30 and 40° C temperatures for 10 minutes. Partial loss of activity was noted after 50° C, whereas 60° C caused a sharp decrease in these parameters. At the same time, PAE-5, PAE-22 and PAE-24 lysates were characterized by higher resistance to temperature action and after treatment affected both indicating strains. Whereas PAE-6, PAE-8, PAE-14, PAE-19, PAE-21 and PAE-41 lysate activity was maintained only against *P. aeruginosa* UCM B-10. After 70° C treatment all investigated lysates lost their activity.

Some unexpected results were obtained at slightly lower temperatures. After 30 and 40° C treatment, an increase in killer activity level was observed. It should be noted that this effect varied for each individual lysate and indicator strain. All studied substances can be divided into 3 groups according to their activation temperature (Table 1).

Additionally, depending on the indicator culture on which this increase was noted, the groups were divided into two subgroups: PAE-6, PAE-12, PAE-14 and PAE-41 lysates were included in 1.1 group and were characterized by increase of killer activity against *P. aeruginosa* UCM B-3 after 30° C treatment; the substances of PAE-5, PAE-19, PAE-22, PAE-24, PAE-38 lysates heightened the activity against this indicator culture after 40° C and were referred to 2.1 group. PAE-8 and PAE-21 lysates differed from other substances by their properties and were included in 3.1. group. The differentiation of lysates into the groups according to the temperature that caused the increase of their activity against *P. aeruginosa* UCM B-10 was somewhat different. So, the activity of PAE-6 and PAE-14 lysates against *P. aeruginosa* UCM B-10 increased after 30° C treatment. So, their substances were included to 1.2 group. The substances of PAE-5, PAE-12, PAE-24, PAE-38 and PAE-41 lysates were referred

to 2.2. group, since their activity against *P. aeruginosa* UCM B-10 was increased under 40°C effect. Killer properties of PAE-8, PAE-19, PAE-21 and PAE-22 against *P. aeruginosa* UCM B-10 differed from described earlier lysates and these lysates were classified as 3.2. group.

Table 1

Activation of *P. aeruginosa* bacteriocins (PAE) at different temperatures against used indicator strains

Groups	Activation temperature	Indicator strain	Lysates
1.1.	30°C	UCM B-3	PAE-6, PAE-12, PAE-14, PAE-41
2.1.	40°C	UCM B-3	PAE-5, PAE-19, PAE-22, PAE-24, PAE-38
3.1.	50°C*	UCM B-3	PAE-8, PAE-21
1.2.	30°C	UCM B-10	PAE-6, PAE-14
2.2.	40°C	UCM B-10	PAE-5, PAE-12, PAE-24, PAE-38, PAE-41
3.2.	40-50°C*	UCM B-10	PAE-8, PAE-19, PAE-21, PAE-22

Annotation: * - increase of lysate activity didn't observe at mentioned temperature or activation didn't occur.

So, PAE-6 lysate activity at 20° C was characterized by not high activity index – 800 AU/mL (Fig. 1A). Under 30° C influence killer activity of this lysate increased in 16 times and amounted to 12.8 thousand AU/mL. The same killer activity indices were observed after 40 and 50° C treatment. The influence of 60° C caused the loss of killer properties of the test substance. PAE-12 lysate was characterized by increase of activity index in 2 times under 30°C impact. In this case after 20° C influence killer activity index was 25.6 thousand AU/mL, but under 30°C processing it increased to 51.2 thousand AU/mL 40°C and higher temperature caused decrease of killer activity level. Complete loss of PAE-12 lysate activity was observed at 60° C. For PAE-14 lysate the increase of activity index in 2 times was discovered after 30° C treatment. Thus, the activity of initial substances was 12.8 thousand AU/mL, then after 30° C impact it grew to 25600 AU/mL. Under further elevation of temperature to 40° C slight decrease in the lysate killer activity was observed. Influence killer activity of this lysate was lost absolutely after 60° C impact.

Some higher indices of initial activity were noted for PAE-41 lysate – they were equal to 204.8 thousand AU/mL 30° C treatment resulted in a 2-fold increase in these killer properties with an achievement of 409.6 thousand AU/mL. Also a sharp decrease and total loss of killer activity were revealed under 50 and 60° C impact, respectively.

Substances activated against *P. aeruginosa* UCM B–3 by 40° C were referred to 2.1 group. (Fig. 1B). PAE-5 lysate was characterized by an insignificant level of killer activity at 20 and 30° C. Its indices were equal to 3.2 thousand AU/mL. After 40° C treatment their activity increased to 12.8 thousand AU/mL, i.e. 4 times. The killer activity at 60° C was only 200 AU/mL and completely lost under the influence of 70° C. PAE-19 lysate revealed significantly higher killer activity indices, which at 20 and 30° C were 204.8 thousand AU/mL. In this case the activity of lysate at 40° C increased 2-fold and reached 409.6 thousand AU/mL. The decrease of activity level was observed after the influence of 50°C, under 60°C there was it total loss. Similar results were obtained for lysate PAE-22. Under 40°C influence the increase of activity

level in 2 times to 819.2 thousand AU/mL was observed. The decrease of activity level was noted under 50 and 60° C impact, 70° C resulted in it total loss. The killer activity of PAE-24 lysate reached the maximum values after 40° C treatment. So, at 20° C and 30° C lysate activity was 25.6 thousand AU/mL, but after 40°C treatment it increased in 4 times to 102.4 thousand AU/mL. Gradual decrease of this lysate activity was observed under further temperature elevation and it total loss occurred at 70° C. PAE-38 lysates were characterized

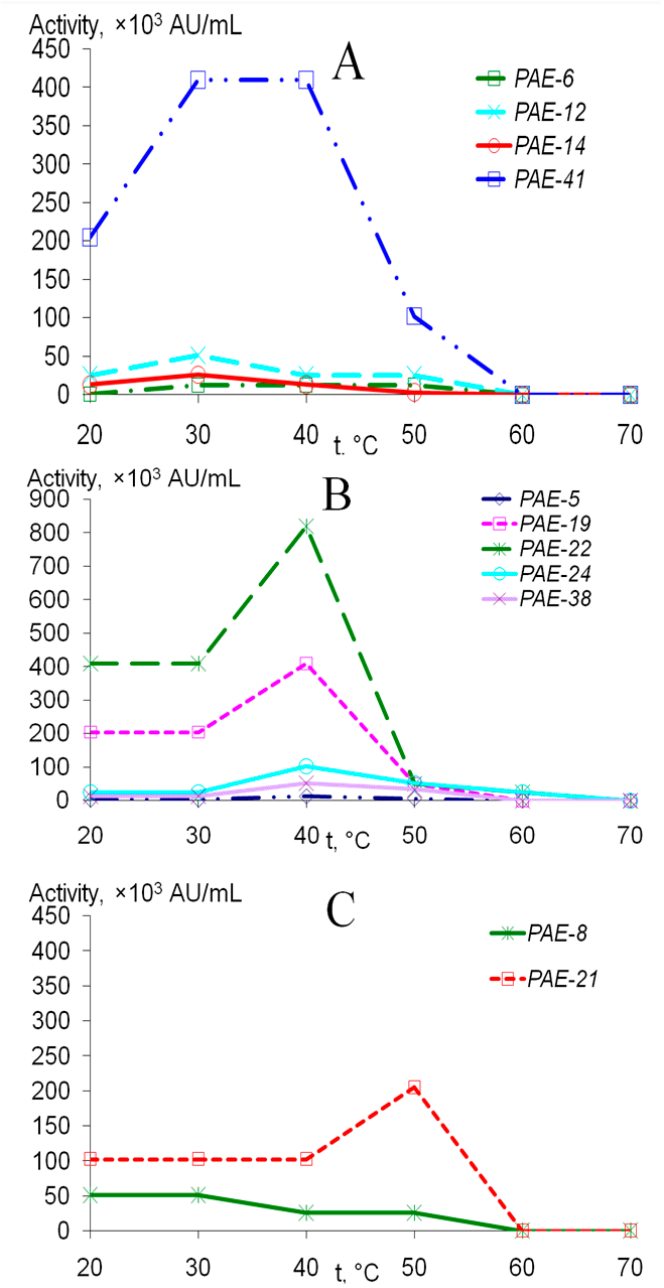

Fig. 1. The bacteriocin activity against indicator strain *Pseudomonas aeruginosa* UCM B-3 after temperature treatment. Bacteriocin groups: 1.1. (A), 2.1. (B) and 3.1. (C)

There and in next figures the error of the received data did not exceed 5 %.

by 4-fold increase of activity level at 40° C to 51.2 thousand AU/mL. Complete loss of killer activity under 60° C impact.

PAE-8 and PAE-21 lysates were referred to 3.1 group, since their substances differ from other lysates in their properties after thermal activation (Fig. 1C). Thus, the killer activity of PAE-8 lysate did not change at 20 and 30°C, but it decreased by half under 40° C influence and completely disappeared after 60° C processing. PAE-21 lysate was characterized by some other peculiarities. In this case the killer activity at 30° C and 40° C corresponded to the initial activity – 102.4 thousand AU/mL. Under the influence of 50° C the partial regeneration of activity was observed, but 60° C caused its loss.

According to influence on *P. aeruginosa* UCM B-10 the belonging of the studied lysates to the described thermoactivation groups was somewhat different. In that case 1.2 group included PAE-6 and PAE-14, that were activated under 30° C (Fig. 2A). Thus, the killer activity of PAE-6 at 20° C was 12.8 thousand AU/mL and after 30° C impact, a 4-fold increase of activity indices occurred up to 51.2 thousand AU/mL. Then under 40 and 50° C influence the killer activity level decreased by half to 25.6 thousand AU/mL. Under the influence of 60° C the activity decreased to 800 AU/mL. Complete activity loss was observed at 60° C. The same regularity was found for PAE-14 lysate, but with slightly lower killer activity indices. Thus, at 20° C the level of activity was 12.8 thousand AU/mL and under 30° C influence it increased twice and reached 25.6 thousand AU/mL. Subsequently, after 40 and 50° C processing a decrease of killer activity indices to the original values was observed. Under the influence of 60°C the activity decreased to 800 AU/mL. Full activity loss took place at 70° C.

2.2 group included substances that were activated against *P. aeruginosa* UCM B–10 by 40° C influence (Fig. 2B). PAE-5 lysate was characterized by not high killer activity indices. Thus, at 20° C and 30° C the activity of this substance was equal to 25.6 thousand AU/mL, whereas at 40° C the activity level doubled and reached 51.2 thousand AU/mL. Then, under higher temperature impact the activity level decreased gradually and at 60° C the lysate activity was only 200 AU/mL. Complete activity loss was detected after 70° C treatment. The results obtained for PAE-12 and PAE-38 lysates after their treatment by different temperatures were similar. These substances were characterized by 4-fold increase of activity level at 40° C to 12.8 thousand AU/mL and complete loss of killer activity under 60° C impact. PAE-24 and PAE-41 lysates proved to be the most active against *P. aeruginosa* UCM B-10. These substances were characterized by the highest killer activity growth at 40° C.

Thus, at 20° C the activity of PAE-24 lysate was equal to 25.6 thousand AU/mL, whereas under 30° C influence it increased in 4 times and reached 102.4 thousand AU/mL. After 40° C impact, the activity increased in 8 times as compared with the initial value and amounted to 409.6 thousand AU/mL. A decrease of activity level to 51.2 thousand AU/mL was observed at 60° with it full loss at 70° C. Results obtained for PAE-41 were more definite. Substances of this lysate didn't change activity indices under 30° C. At 40° C this value increased in 4 times and reached 819.2 thousand AU/mL. Subsequently, under 60° C impact the decrease of activity to 200 AU/mL was noted and after 70° C – it full loss.

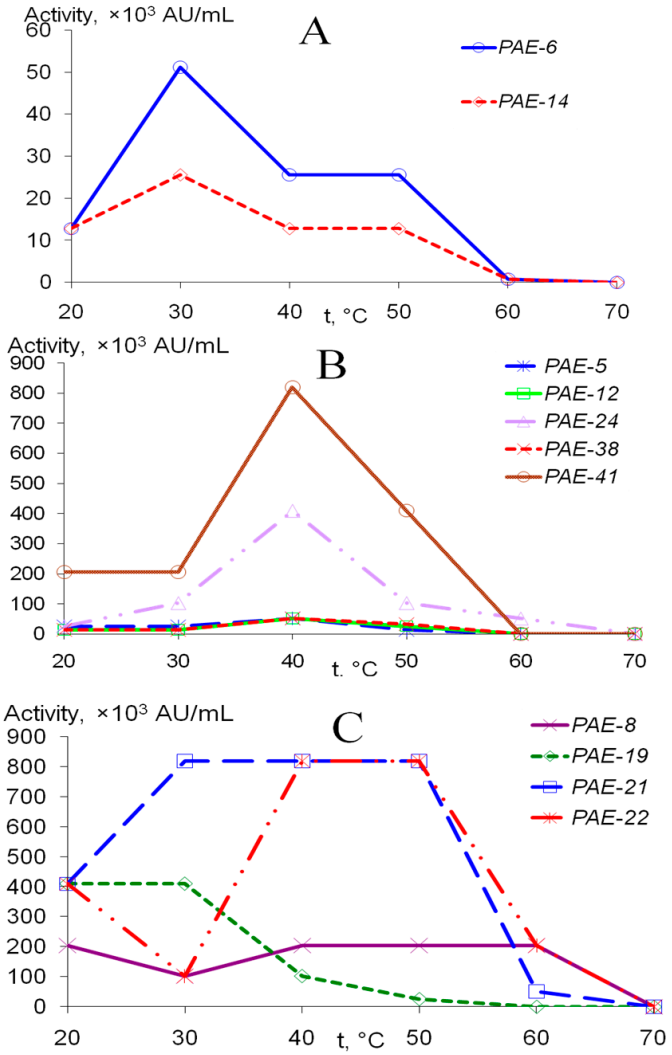

Fig. 2. The bacteriocin activity against indicator strain *Pseudomonas aeruginosa* UCM B-10 after temperature treatment. Bacteriocin groups: 1.2. (A), 2.2. (B) and 3.2. (C)

3.2 group included PAE-8, PAE-19, PAE-21 and PAE-22 lysates. Their substances were characterized by other properties then mentioned above (Fig. 2C). The peculiarity of PAE-8 and PAE-22 lysates was lowering of killer activity at 30° C with its further recovery to initial level under 40°C influence. Thus, at 30° C activity level of PAE-8 decreased twice to 102.4 thousand AU/mL, while for PAE-22 the 4-fold reduction to the same level was observed. After 50° C and 60° C influence activity indices of PAE-8 was the same as at 40° C, but activity of PAE-22 at 60° C was reduced to 204.8 thousand AU/mL. Activity loss of both lysates was noted under 70° C impact. PAE-19 was characterized by significant sensitivity to temperature effects. The killer activity level of this lysate remained stable at 30°C, but went down sharply after 40° C impact. Complete activity loss was observed under 70° C influence. At the same time, PAE-21 showed a high stability of killer activity, which was at the level of 819.2 thousand AU/mL after 30, 40 and 50° C treatment. Sharp

decrease to 51.2 thousand AU/mL and complete loss of activity were observed only under the influence of 60 and 70° C respectively.

For more thorough examination of this thermoactivation phenomenon the change of lysate killer activity was researched after their treatment by 30 – 40° C for different periods. PAE-19 and PAE-22 were used because their activity against *P. aeruginosa* UCM B-3 increased at 40° C only 2-fold. The influence of these substances on UCM B-10 was ambiguous too, which created an opportunity for a more detailed study of thermoactivation peculiarities.

It was determined that killer activity of PAE-19 didn't change at 30° C for different time period and amounted to 3.2 thousand AU/mL (Fig. 3A).

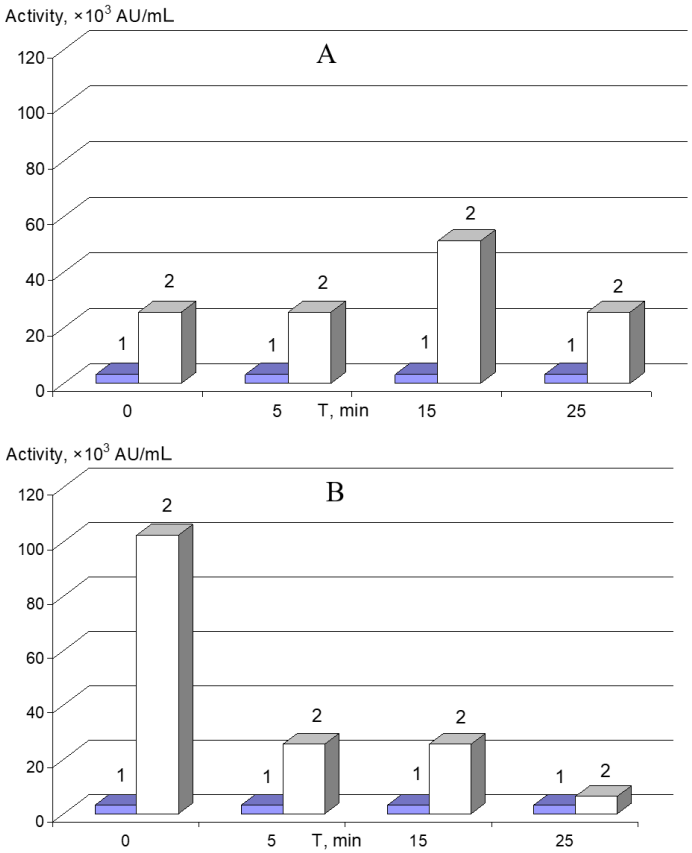

Fig. 3. The killer activity of PAE-19 (1) and PAE-22 (2) lysates after 30° C treatment during different time periods (T) against indicator cultures *Pseudomonas aeruginosa* UCM B-3 (A) and UCM B-10 (B)

The activity of PAE-22 substances, untreated and obtained after holding at 30° C during 5 and 25 minutes was 25.6 thousand AU/mL. But after 30° C treatment during 15 minutes this parameter increased twice and amounted to 51.2 thousand AU/mL. After checking of killer activity of PAE-19 it was found that this lysate didn't change its indices against *P. aeruginosa* UCM B-10 as well as against UCM B-3. (Fig. 3B). PAE-22 was characterized by decrease of killer activity under prolonged heat treatment. Thus, initial activity of this lysate was 102.4 thousand AU/mL. Under influence of 30° C during 5 and

15 minutes the activity decreased in 4 times to 25.6 thousand AU/mL. The action of this temperature factor during 25 minutes led to further 4-fold decrease in activity rates.

It was shown that after 40° C treatment killer activity of PAE-19 against *P. aeruginosa* UCM B-10 didn't change and during all time periods was 2-fold higher the initial activity – 6.4 thousand AU/mL (Fig. 4A).

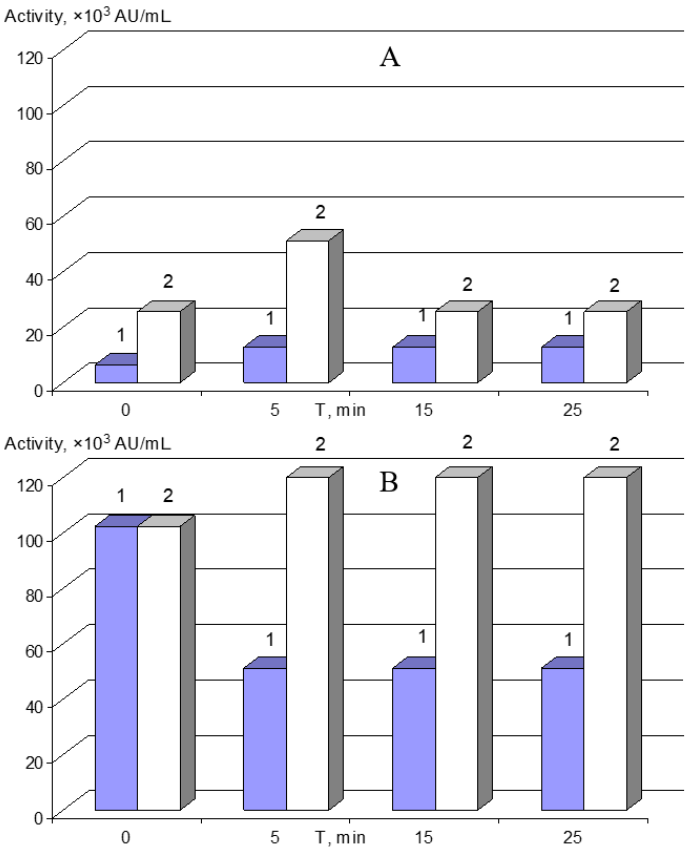

Fig. 4. The killer activity of PAE-19 (1) and PAE-22 (2) lysates after 40° C treatment during different time periods (T) against indicator cultures *Pseudomonas aeruginosa* UCM B-3 (A) and UCM B-10 (B)

Killer activity of PAE-22 after the influence of this temperature during 15 and 25 minutes didn't change too and was equal to untreated substances index – 25.6 thousand AU/mL. But the shortening of treatment time to 15 minutes caused double increase of this parameter. The test of lysates activity against *P. aeruginosa* UCM B-10 found out the decrease of PAE-19 activity index after 5 minutes of 40° C processing (Fig. 4B). The prolongation of temperature effect didn't influence on substance activity, its index was 51.2 thousand AU/mL. Initial level of PAE-22 activity was 102.4 thousand AU/mL. The 40° C impact during different time periods caused the increase of this parameter doubly.

Discussion. We discovered that all investigated bacteriocins were thermolabile substances, since 70° C treatment caused the absolute loss of their killer activity. This property is characterized to pyocins. Thus, low molecular

weight *P. aeruginosa* S2 and AP41 bacteriocinins are also completely inactivated after 70° C treatment and pyocin S1 loses its activity after 60° C impact during 10 minutes [12].

But it was revealed that 30 and 40° C treatment increased the indices of bacteriocin killer activity. The activation of some lysates, for example PAE-6 and PAE-14 occurred at 30° C, and PAE-5, PAE-24 and PAE-38 lysates – exclusively at 40° C. However, the activity of other substances grew under the impact of both indicated temperatures. So, the indices of PAE-12 and PAE-41 against *P. aeruginosa* UCM B-3 indicator strain increased after 30° C treatment, but concerning *P. aeruginosa* UCM B-10 – both at 30°C and at 40° C. So, PAE-12 and PAE-41 activity against *P. aeruginosa* UCM B-3 increased after 30° C treatment, but these lysates indices against *P. aeruginosa* UCM B-10 grew both at 30° C and at 40° C. The activity of another lysate – PAE-22 against *P. aeruginosa* UCM B-3 increased under 40° C influence, but its index concerning *P. aeruginosa* UCM B-10 – at 40° C and even at 50° C. The absence of the influence of temperature treatment was noted only for PAE-8 lysate. Obviously the revealed regularity is connected with multiplicity of bacteriocin synthesis, the typical feature of pyocins [3]. As a result, some of the lysates contain pyocins, which killer activity increase at the same temperature, while in the composition of the lysate majority, bacteriocins are activated by different temperatures. In favor of this assumption, different intensity of thermoactivation effect was revealed depending on the used indicator cultures. Thus, the activity of the lysate majority against *P. aeruginosa* UCM B-3 increased at one temperature, while in the case of *P. aeruginosa* UCM B-10 the influence of temperatures had a more varied effect.

It should be noted that the action of 30° C on most bacteriocins stimulated 2-fold, and 40° C – 4-fold increase in activity. However, activity indices of some substances grew significantly: 8 times – for PAE-24 and 16-fold – for PAE-6. The revealed property may indicate that the synthesized bacteriocins are associated with each other or with other substances in an unstable complex. The temperature treatment, obviously, breaks these bonds, improves the availability of bacterial killer domain, exhibiting in bacteriocin activity increase. For *E. carotovora* J2/S2 carotovoricins it was shown the production of low-molecular bacteriocins with pigment-containing lipid [13] and *Xanthomonas campestris* pv. *glycines* glycinecin is synthesized in the form of a heterodimer containing two polypeptides [14]. The phenomenon of killer factor activation is the characteristic feature of some microcins, but in this case a posttranslational modification associated with the proteolytic cleavage of their molecule occurs [15]. However, similar results of the temperature influence on the killer activity of pyocins were not found in literature available to us.

Using a different duration of temperature effect on lysates PAE-19 and PAE-22 at 30 and 40° C it was shown that the obtained results correlated with the data of previous experiments. It was also noted that the indices of bacteriocin killer activity did not significantly change with the variation in the treatment duration: 5, 15 and 25 minutes. It is evidence of single-stage of temperature on the structural organization of synthesized bacteriocins and confirms the assumption about the instability of the complex.

Thus, it has been shown for the first time that the use of temperature treatment of *Pseudomonas aeruginosa* bacteriocins at 30-40° C makes possible to increase their activity in 2 – 4 times, and in some cases to achieve 8 – 16 times rise of activity parameters. The prolongation of temperature impact doesn't stimulate the additional thermoactivation of bacteriocins, since the key factor of activity increase is the temperature value not the duration of the temperature effect. The revealed regularity can be used for pyocin concentrating at the stage of their research or to increase the killer activity of bacteriocins for their application.

ТЕРМОАКТИВАЦІЯ ПІОЦИНІВ *PSEUDOMONAS AERUGINOSA*

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Резюме

Метою роботи було дослідження впливу різних температур на показники кілерної активності піоцинів S-типу *Pseudomonas aeruginosa*. **Методи.** Об'єктом дослідження були піоцини S-типу, отримані із 11 штамів *Pseudomonas aeruginosa*. Дані кілерні фактори піддавали дії температур в діапазоні від 20 до 100° С протягом 10 хв. Кількісні показники кілерної активності бактеріоцинів після обробки температурою порівнювали із такими у необроблених бактеріоцинів. Основну увагу приділяли впливу на лізати температур 30 і 40° С. Для цього використовували різну тривалість температурного впливу – 5, 15 та 25 хвилин. **Результати**. Вплив температури 60° С обумовлював зниження до мінімальних показників, а 70° С – викликав абсолютну втрату активності усіх досліджуваних лізатів. Температурна обробка при 30 та 40° С підвищувала показники кілерної активності бактеріоцинів. Даний ефект проявлявся на використаних індикаторних культурах з різною інтенсивністю. Активність лізатів першої групи підвищувалась після впливу 30° С, другої групи – 40° С, а третьої групи – під дією декількох температур, у т. ч. 50° С або не змінювалась. Вплив 30° С на більшість бактеріоцинів стимулювала 2-х кратне, а 40° С – 4-х кратне підвищення показників активності. Проте показники активності деяких речовин зростали значно вище: у 8 разів – для PAE-24 та у 16 разів – для PAE-6. Результати дослідження впливу 30 та 40° С на активність PAE-19 та PAE-22 показали відсутність змін кілерної активності бактеріоцинів, спричиненої різною тривалістю температурної обробки. **Висновки.** Використання температурної обробки піоцинів S-типу *Pseudomonas aeruginosa* при 30 – 40° С дозволяє підвищити їх активність у 2 – 4 рази, а в окремих випадках досягнути і 16-кратного зростання активності. Тривалість температурного впливу не призводить до додаткової термоактивації бактеріоцинів.

Ключові слова: піоцини, термоактивація, підвищення кілерної активності, *Pseudomonas aeruginosa*.

ТЕРМОАКТИВАЦИЯ ПИОЦИНОВ *PSEUDOMONAS AERUGINOSA*

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Резюме

Целью работы было изучение воздействия разных температур на показатели киллерной активности пиоцинов S-типа *Pseudomonas aeruginosa*. **Методы.** Объектом исследования были пиоцины S-типа, выделенные из 11 штаммов *Pseudomonas aeruginosa*. Данные киллерные факторы подвергали воздействию температур в диапазоне от 20 до 100° С в течение 10 мин. Количественные показатели киллерной активности бактериоцинов после температурной обработки сравнивали с аналогичными показателями необработанных бактериоцинов. Основное внимание было уделено влиянию на лизаты температур 30 и 40° С. Для этого использовали разную продолжительность температурной обработки – 5, 15 и 25 минут. **Результаты**. Влияние температуры 60° С обуславливало снижение киллерной активности к минимальным показателям, а 70° С – приводило к полной потере активности всех исследованных лизатов. Температурная обработка при 30 и 40° С повышала показатели киллерной активности бактериоцинов. На использованных индикаторных культурах данный эффект проявлялся с разной интенсивностью. Активность лизатов первой группы повышалась после влияния 30° С, второй группы – 40° С, а третьей группы – под действием нескольких температур, в том числе 50° С или не изменялась. Влияние 30° С на большинство бактериоцинов стимулировало 2-х кратное, а 40° С – 4-х кратное повышение показателей активности. Однако показатели активности некоторых веществ увеличивались значительно больше: в 8 раз – для PAE-24 и в 16 раз – для PAE-6. Результаты изучения влияния 30 и 40° С на активность PAE-19 и PAE-22 показали отсутствие изменений киллерной активности бактериоцинов при разной продолжительности температурной обработки. **Выводы.** Использование температурной обработки пиоцинов S-типа *Pseudomonas aeruginosa* при 30 – 40° С позволяет повысить их активность в 2 – 4 раза, а в отдельных случаях достигнуть и 16-кратного увеличения активности. Продолжительность температурного воздействия не влияет на дополнительную термоактивацию бактериоцинов.

Ключевые слова: пиоцины, термоактивация, повышение киллерной активности, *Pseudomonas aeruginosa*.

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