DOI: https://doi.org/10.18524/2307-4663.2024.2(61).311670

UDC: 579.262

A. G. Merlich, O. Iu. Kalnytska, M. V. Shutylo, O. O. Kovtun, V. O. Ivanytsia

Odesa I. I. Mechnikov National University,
2 Dvorianska St, Odesa, 65082, Ukraine
tel.: +38 096 4367403, e-mail: andriymerlich@gmail.com

CHARACTERIZATION OF LACTOBACTERIA FROM THE BLACK SEA WATER AND MUSSELS WITH THE POTENTIAL TO PRODUCE ANTIBACTERIAL COMPOUNDS

Aim. *Characterization of lactic acid bacteria (LAB) from water and mussels of the Black Sea with the potential to produce antibacterial compounds.* ***Methods.*** *The isolation of lactobacteria from water and mussels, their enumeration were performed by standard microbiological methods. Determination of their morphological, tinctorial, cultural, and biochemical characteristics were performed by Gram staining with immersion microscopy, describing the growth in liquid MRS media with and without 6.5% NaCl, catalase test. Genus-specific classic PCR was used for identification of isolated bacteria. To study the antagonistic interactions of the strains the perpendicular streak method was used.* ***Results.*** *In water of the Black Sea from Odesa bay, collected in winter period, $2\times10–4.8\times10^2$ CFU/mL of lactobacteria were found, while only in three mussels from seven (42.9%) LAB were detected ($1.7\times10\pm0.7$ CFU/mL). Thirteen new strains of lactobacteria were isolated and characterized – eight strains from water and five from mussels. Among them, nine strains were identified as Enterococcus sp., one strain – as Lactobacillus sp., and three LAB strains remained unidentified. The new strains Enterococcus W1.1, Enterococcus W1.2, Enterococcus W1.3, Enterococcus W2.3, and Enterococcus M7.1 exhibited antagonistic activity against other closely related strains and Lactobacillus sakei subsp. sakei JCM1157.* ***Conclusions.*** *The Black Sea water and mussels tissue liquor in winter period contain $2\times10–4.8\times10^2$ CFU/mL and $1.7\times10\pm0.7$ CFU/mL of LAB, respectively. The strains Enterococcus W1.1, Enterococcus W1.2, Enterococcus W1.3, Enterococcus W2.3, and Enterococcus M7.1 are potential producers of antibacterial compounds.*

Key words: lactobacteria, the Black Sea water, Enterococcus, Lactobacillus, mussels, bacteriocins.

The group of lactic acid bacteria (LAB) includes Gram positive, catalase- and oxidase negative, non-spore-forming microorganisms producing lactic acid as the main compound as well as other organic acids. The majority of lactobacteria are safe and they have huge biotechnological potential. Recently, lactobacteria of marine origin especially gained attention because it is supposed that these microorganisms, as well as their metabolic products, are attractive for pharmaceutical, cosmetic, and

food industry, for obtaining of biopolymers and application in aquaculture [6, 11, 14].

Despite that a lot of scientific information on terrestrial lactobacteria is available, data on representatives of this group from water sources are scarce. Moreover, there are only several scientific papers about lactobacteria namely from Odesa bay of the Black Sea. Ukrainian scientists have showed presence of lactobacteria in such hydrobionts as sponges, sea bass, cod, mackerel, dolphins, mussels, algae [1, 3, 4]. Thus, it was established that in sponges of *Haliclona* genus only representatives of *Lactobacillus* were observed: *L. bifermentans, L. parabuchneri,* and *L. vaccinostercus* [3]. It was established by Yamborko et al. that LAB of *Lactobacillus*, *Streptococcus*, and *Enterococcus* genera are present in intestine of bottlenose dolphins (*Tursiops truncatus*), mackerel (*Scomber scomber*), sea bass (*Perca fluviatilis*) and cod (*Gadys morhya morhya)*. Representatives of *Enterococcus* genus were isolated only from intestine of dolphins in amount 10^2–10^3 CFU/sm^3 [4]. Vasylieva et al. have showed the presence of LAB of the genera *Enterococcus, Streptococcus, Pediococcus, Lactococcus*, and *Leuconostoc* in Black Sea mussels (*Mytilus galloprovincialis*) and on surface of algae *Enteromorpha, Ulva, Cladophora, Porphyra*, and *Polysiphonia*. By using fatty acids composition analysis, *Enterococcus faecalis*, *Streptococcus bovis*, *Pediococus pentosaceous*, and *Leuconostoc mesenteroides* species were identified [1].

Despite the conducted research on isolation, characterization, and identification of LAB from hydrobionts from Odesa bay of the Black Sea, there is not enough information on lactobacteria strains isolated specifically from marine water in winter time. Biotechnological potential of the Black Sea strains of LAB, in particular their ability to produce antimicrobial compounds including bacteriocins, is even less researched.

The aim of this work was to characterize LAB from water and mussels of Black Sea with potential to produce antibacterial compounds.

Materials and methods

Samples of water and mussels were collected by Dr. Kovtun O.O. in January 2023 from the Odesa Bay of the Black Sea near the hydrobiology station of the Odesa I. I. Mechnikov National University. A total of nine samples were analyzed – two water samples and seven samples of mussels.

For the isolation of lactobacteria, medium-sized mussels with closed, intact shells were selected and processed to collect tissue liquor according to the literature [10]. Undiluted samples as well as tenfold serial dilutions were inoculated in a volume of 100 μL on MRS-agar with pH 5.7 (MERCK, Germany) and on such the medium with neutral pH, which was prepared according to [8]. The inoculated plates were incubated in a thermostat at 37 °C and microanaerobic conditions for 48 hours.

At the same time, in order to determine the total microbial number (TMN) for calculation of the percentage of LAB among the total microbiota, inoculation was carried out on nutrient agar (NA) provided by the Himedia company (India) and the plates were incubated at 37 °C for 48 hours.

The colonies number was counted and calculated per 1 mL in a standard manner. Statistical data processing was carried out in the Microsoft Office Excel program. The mean value, standard deviation, and confidence were calculated.

To isolate pure cultures of lactobacteria, colonies similar in morphology to LAB were selected and inoculated again on new plate using the streak method. Cell morphology, tinctorial properties, and absence of spore formation were determined by Gram staining followed by microscopy using a MICROmed microscope (Ukraine) with a total magnification of 1600X. The study of cultural properties involved determining the morphology of colonies on MRS agar, the growth in MRS broth, gas formation, and the presence of growth in liquid MRS medium containing 6.5% NaCl. Among the biochemical properties, the presence of catalase enzyme was determined using 3% hydrogen peroxide [18].

Classical polymerase chain reaction (PCR) was carried out in order to identify the isolated LAB to the genus level. The heat lysis method using a solution of 1% Triton X-100 and 0.25% sodium azide was used to isolate DNA from the studied marine bacteria [21]. The presence and concentration of the obtained DNA was determined by gel electrophoresis and spectrophotometry using the UV5Nano device (Mettler Toledo, USA).

In order to identify *Enterococcus* bacteria, PCR was performed with primers E1 (5'-TCAACCGGGGAGGGT-3') and E2 (5'-ATTACTAGCGATTCCGG-3') [7]. The reaction mixture for PCR was of the following composition: 5 units of Taq DNA polymerase – 0.45 μL, primer 1 – 1.25 μL, primer 2 – 1.25 μL, 8 mM mixture of deoxynucleotide triphosphates – 0.56 μL, 10X Taq buffer + NH4 – 2.25 μL, DNA – 1 μL, 25 mM $MgCl_2$ aqueous solution – 2.7 μL, deionized water – 13 μL. The final volume of the mixture was 21.46 μL [7, 13, 16, 21]. DNA of the strain *Enterococcus italicus* ONU547 was used as a positive control. Amplification parameters are listed in Table 1.

Table 1

Parameters of amplification which were used for genus specific PCR in order to identify *Enterococcus* and *Lactobacillus* according to the literature data [9, 13]

For *Enterococcus*			**For *Lactobacillus***		
Initial denaturation	95 °C for 4 min		Initial denaturation	95 °C for 5 min	
Denaturation	95 °C for 30 sec	30 cycles	Denaturation	95 °C for 30 sec	30 cycles
Primer annealing	55 °C for 1 min		Primer annealing	55 °C for 30 sec	
Elongation	72 °C for 1 min		Elongation	72 °C for 30 sec	
Final elongation	72 °C for 7 min		Final elongation	72 °C for 7 min	

For identification of microorganisms belonging to *Lactobacillus* genus the following primers were used: LbLMA1 (5'-CTCAAAACTAAACAAAGTTTC-3') and R16-1 (5'-CTTGTACACACCGCCCGTTCA-3') [9].

The reaction mixture contained the following components: 5 units of Taq DNA polymerase – 1.16 μL, primer 1 – 2.77 μL, primer 2 – 2.77 μL, 10X PCR buf-

fer – 5 μL, 10 mM mixture of deoxynucleotide triphosphates – 1 μL, DNA– 2 μL, 25 mM $MgCl_2$ aqueous solution – 6 μL, deionized water – 25.3 μl. The total volume of the mixture was 50 μL [9, 13, 15, 21]. The DNA of the *E. italicus* ONU547 strain was used as a negative control. Amplification was carried out according to the parameters listed in Table 1.

After the PCR, the presence of amplification products was determined by electrophoresis in a 1% agarose gel with 1X Tris-acetic buffer [9]. After the electrophoresis, the staining with ethidium bromide was performed and photographed using the Gel Doc video system (Bio-Rad, USA) in the "Trans UV" mode.

Study of antagonistic interactions of lactobacteria was carried out by the perpendicular streak method according to [19]. Five strains of isolated bacteria (W1.1, W1.2, W1.3, M4.1, M5.2) were used as test cultures, as well as the indicator strain *Lactobacillus sakei* subsp. *sakei* JCM1157. The presence of zones of growth inhibition from 2 mm to 30 mm was noted indicating the production of antimicrobial compounds [19].

Results and discussion

Isolation of lactobacteria from the marine sources

As a result of the inoculation of nine samples collected in the Odessa bay of Black Sea (two water and seven mussel samples) on nutrient media, microorganisms growing on MRS with an acidic or neutral pH value were isolated from most of them (Table 2). No LAB-like colonies were isolated from Mussel №1 and Mussel №3 samples. The number of microorganisms on these media after inoculation of other samples ranged from 1×10 CFU/mL to 3×10^3 CFU/mL.

In average, the TMN of seawater was $1.7\times10^6\pm0.3$ CFU/mL, which is in agreement with the data of other scientists, who found 1.1×10^6 CFU/ml of microorganisms in the Black Sea water sampled in October when cultured on the NA [2].

In contrast to the water samples, the TMN of the tissue liquor of mussels fluctuated in a wide range depending on the studied mussel – from 4.11×10^3 to 1.085×10^7 CFU/mL.

Table 2

Results of inoculation of samples of marine water and mussels collected in winter time on solid media

Sample	CFU/mL		
	MRS with pH 5.7	MRS with pH 7	NA (TMN)
Water №1	4.8×10^2	3×10^3	1.6×10^6
Water №2	3×10	1.1×10^2	1.89×10^6
Mussel №1	0	0	5.5×10^5
Mussel №2	0	1×10	1×10^5
Mussel №3	0	0	2.52×10^6
Mussel №4	0	1×10^2	1.08×10^6
Mussel №5	0	9×10	5.23×10^3
Mussel №6	0	7×10	4.11×10^3
Mussel №7	0	2×10	1.085×10^7

All colonies that grew on MRS media and visually resembled LAB were transferred to new Petri dishes to obtain pure cultures and were subsequently characterized by a complex of tinctorial, morphological, cultural, and biochemical features.

By the Gram staining, determining the absence of spore formation and catalase activity, the presence of a sour smell [7, 18] it was established that part of the cultures we isolated belonged to the LAB group. After that, we recalculated their content in 1 mL of test samples and calculated the percentage of lactobacteria from the TMN (Table 3). It was established that LAB were isolated from both samples of sea water, but in different numbers: in the first sample, 4.8×10^2 CFU/mL of lactobacteria were found, and in the second one – only 2×10 CFU/mL. They composed only 0.03% and 0.001% of the TMN, respectively.

Table 3

LAB bacteria number in samples of marine water and mussels

Sample	CFU/mL of lactobacteria	% of LAB among other microbiota representatives
Water №1	4.8×10^2	0.03
Water №2	2×10	0.001
Mussel №1	0	-
Mussel №2	0	-
Mussel №3	0	-
Mussel №4	1×10	0.0009
Mussel №5	2×10	0.4
Mussel №6	0	-
Mussel №7	2×10	0.0002

And even smaller number of lactobacteria was found in the Black Sea mussels collected in winter. Indeed, LAB were found in only three mussels out of seven (42.9%). The average number of LAB in the studied hydrobionts, among those where they were detected, was 1.7×10±0.7 CFU/mL. The detected lactobacteria composed a very low percentage of the total microbiota of mussels – from 0.0002 to 0.4%. To our knowledge, this is the first report on the composition of lactic acid microbiota of the water and mussels of the Odessa Bay of the Black Sea in winter.

The number of LAB in seawater and mussels in our study was significantly lower when compared to studies of Kranga et al., which showed that in October the number of lactobacteria in the Black Sea water was 1.42×10^3 CFU/mL, and inside mussels – 1.33×10^4 CFU/mL [2]. This difference can be explained by the lower water temperature in January, when our samples were collected, compared to October. As for the number of lactobacteria in mussels, it was also significantly lower than that shown in other works. Thus, in the publication of Bulgarian scientists who also isolated LAB from Black Sea mussels, but from the Bulgarian water area, it was reported that 83×10^8 CFU/mL of lactobacteria were isolated [12]. This difference can also be explained by the different seasonality of sampling.

Study of morphological, tinctorial, cultural properties and identification of the isolated strains

As a result of the conducted staining and microscopy of the preparations, it was established that the studied bacteria of all the strains were stained positively by Gram. Most of them had a rounded shape, except for W2.4, which was rod-shaped. The cells in the preparations were placed singly, in pairs, in clusters, in chains, or in tetrads. No spores was observed in the cells of the studied microorganisms.

Among the cultural characteristics, the morphology of colonies on the solid MRS medium, growth in MRS broth without and with 6.5% NaCl were studied. All the isolated strains on MRS medium with neutral pH formed round, small colonies with smooth surfaces, convex profiles, and regular edges. They were white or white-gray in color, shiny and opaque, had a uniform structure and a soft or slimy consistency. None of the strains produced a pigment.

As a result of the conducted research, it was found that bacteria of all the strains, with the exception of M 5.1, gave turbidity when growing in MRS broth. None of the strains formed films and was capable of gas formation. All samples had a sediment. Most cultures, with the exception of M 5.1, had a pronounced sour smell.

Intensive growth of the majority of bacterial isolates was also observed in MRS broth in the presence of 6.5% NaCl, which can indicate that they belong to the genus *Enterococcus* [18]. Only M5.2 strain showed weak growth, while M5.1, M4.1, and W2.4 showed no growth at all. Probably, these bacteria belong to other taxonomic groups. The morphology of cells of the strain W2.4 indicates the possibility of their belonging to the genus *Lactobacillus*.

As a result of the PCR with E1/E2 primers, we found that in the case of nine strains, namely W1.1, W1.2, W1.3, W1.4, W1.5, W1.dc, M5.2, M7.1, and W2.3, amplification products with a size of approximately 700 base pairs (b. p.) were formed (Fig. 1), which indicates that the studied strains belong to the genus *Enterococcus* [13].

Bacteria of the genus *Enterococcus* in mussels of the Odesa Bay of the Black Sea were also detected by Vasilyeva et al., however, the characteristics of their strains, besides of sensitivity to antibiotics, were not published [1]. In the works of other scientists LAB, which were isolated from the Black Sea mussels of the Bulgarian coast, belonged to the genera *Lactobacillus, Sporolactobacillus,* and *Streptococcus* (*L. plantarum, L. sakei, L. brevis, Sporolactobacillus kofuensis, Streptococcus gallolyticus ss gallolyticus*) and enterococci were not found among them [10, 11].

In order to identify the rest of the strains that showed a negative reaction with the E1/E2 primers, the PCR was performed with primers for the genus *Lactobacillus*. As a result of the PCR, we found the presence of amplification products with a size of approximately 250 b. p. in the case of only one strain – W2.4 (Fig. 2) indicating its belonging to the *Lactobacillus* genus [9].

Thus, among the microbiota of seawater and mussels, collected in winter time, we found cultivable *Enterococcus* and *Lactobacillus* bacteria. However, for a more detailed study of the composition of lactobacteria – representatives of the microbiota of water and mussels of the Black Sea, further research is needed. It is

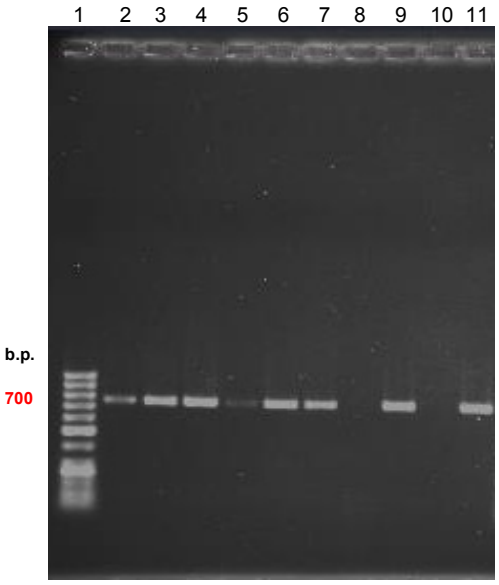

Fig. 1. Electrophoregram of the amplification products obtained by the classic PCR with the primers E1 and E2
Footnote: 1 – molecular weight marker GeneRuler 50 bp DNA Ladder (Thermo Scientific, USA), 2 – W1.1, 3 – W1.2, 4 – W1.3, 5 – W1.4, 6 – W1.5, 7 – W1.dc, 8 – M5.1, 9 – M5.2, 10 – negative control (solution for isolation DNA), 11 – positive control (*E. italicus* ONU547)

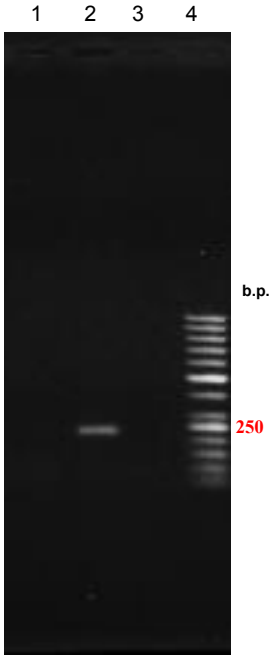

Fig. 2. Electropherogram of amplicons obtained by the PCR with the primers LbLMA1 and R16-1
Footnote: 1 – M7.2, 2 – W2.4, 3 – negative control (DNA of *E. italicus* ONU547), 4 – molecular weight marker GeneRuler 50 bp DNA Ladder (Thermo Scientific, USA)

known that the MRS medium is suitable for cultivation of limited range of lactobacteria (only *Enterococcus, Lactobacillus, Lactococcus*, and *Pediococcus*), and in order to isolate a wider spectrum of them, it is necessary to use M17 medium and others [5, 8], that is planned in our further works. Moreover, by standard microbiological methods, which consist in the use of culture media to isolate microorganisms from the environment, less than 1% of them can be detected. That is why, in order to expand our ideas about the composition of the microbiota of any hydrobiont, in addition to cultivation methods, molecular biology approaches are needed [5, 20].

Study of antagonistic interactions of the isolated strains of marine lactobacteria and their potential to produce bacteriocins

As a result of the conducted experiments, it was observed that the strains of *Enterococcus* W1.1, *Enterococcus* W1.2, *Enterococcus* W1.3 showed antagonistic activity only against the test strain *L. sakei* subsp. *sakei* JCM1157 (Table 4). Such the specificity of antimicrobial activity can indicate the production by these strains of specific factors of active antagonism, such as bacteriocins. The sensitivity of *L. sakei* subsp. *sakei* JCM1157 to bacteriocins of LAB is well known [17]. We plan further studies to confirm this hypothesis.

Table 4

Results of determination of antagonistic interactions by perpendicular streak method

Strain antagonist	Test culture	Presence of inhibitory activity (+/-)	Strain antagonist	Test culture	Presence of inhibitory activity (+/-)
Enterococcus W1.1	W1.2 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	 - - - - +	*Enterococcus* W1.5	W1.1 W1.2 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	+ + + - - +
Enterococcus W1.2	W1.1 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	 - - - - +	*Enterococcus* W1.dc	W1.1 W1.2 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	+ + + + - +
Enterococcus W1.3	W1.1 W1.2 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	 - - - - +	*Enterococcus* M7.1	W1.1 W1.2 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	+ - + - - -
Enterococcus W1.4	W1.1 W1.2 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	- - + - + +	*Enterococcus* W2.3	W1.1 W1.2 W1.3 M4.1 M5.2 *L. sakei* subsp. *sakei* JCM1157	- + + - - -

The *Enterococcus* M7.1 and *Enterococcus* W2.3 strains did not inhibit *L. sakei* subsp. *sakei* JCM1157, however, they showed inhibitory activity against other marine lactobacteria, that can also suggest the bacteriocin production. The other three producers – *Enterococcus* W1.4, *Enterococcus* W1.5, and *Enterococcus* W1.dc inhibited the growth of more than two test strains used in the work that can indicate a non-specific mechanism of antimicrobial action, such as production of organic acids (active non-specific antagonism). The *Enterococcus* M5.2 did not show an inhibitory activity against any of the test culture used in the work.

The composition of metabolic products of the studied LAB strains with antimicrobial properties will be studied by using chromatography-mass spectrometry. This is important for their further use in medical purposes or in aquaculture.

The new lactobacteria strains of the genera *Enterococcus* and *Lactobacillus* from water and mussels of the Black Sea were isolated and their basic tinctorial, morphological, and cultural properties were determined. It was established that representatives of lactobacteria group can be found in water of the Black Sea in winter time in low number that is $2\times10 – 4.8\times10^2$ CFU/mL. In tissue liquor of three mussels from seven, LAB were found in average number of $1.7\times10\pm0.7$ CFU/mL, which composed 0.0002 – 0.4% from their total microbiota. The strains *Enterococcus* W1.1, *Enterococcus* W1.2, *Enterococcus* W1.3, *Enterococcus* W2.3, *Enterococcus* M7.1 have a potential to produce antibacterial compounds.

А. Г. Мерліч, О. Ю. Кальницька, М. В. Шутило,
О. О. Ковтун, В. О. Іваниця
Одеський національний університет імені І. І. Мечникова,
вул. Дворянська, 2, Одеса, 65082, Україна,
тел.: +380964367403, e-mail: andriymerlich@gmail.com

ХАРАКТЕРИСТИКА ЛАКТОБАКТЕРІЙ З ВОДИ ТА МІДІЙ ЧОРНОГО МОРЯ З ПОТЕНЦІАЛОМ ДО ПРОДУКЦІЇ АНТИБАКТЕРІАЛЬНИХ СПОЛУК

Реферат

Мета. *Характеристика молочнокислих бактерій (МКБ) з води та мідій Чорного моря з потенціалом продукувати антибактеріальні сполуки.* ***Методи.*** *Виділення лактобактерій із води та мідій, їх підрахунок було виконано стандартними мікробіологічними методами. Визначення морфологічних, тинкторіальних, культуральних та біохімічних ознак проводили шляхом забарвлення за Грамом з імерсійною мікроскопією, описання росту в MRS бульйоні з 6,5% NaCl та без нього, проведення каталазного тесту. Для ідентифікації виділених бактерій використовували родоспецифічну класичну ПЛР. Для вивчення антагоністичної взаємодії штамів використовували метод перпендикулярних штрихів.* ***Результати.*** *У воді Чорного моря з Одеської затоки, зібраній у зимовий період, виявлено $2\times10 – 4,8\times10^2$ КУО/ мл лактобактерій, тоді як лише у трьох мідіях із семи (42,9%) були знайдені МКБ (1,7×10±0,7 КУО/мл). Виділено та охарактеризовано тринадцять но-*

вих штамів лактобактерій – вісім штамів з води та п'ять з мідій. Серед них дев'ять штамів були ідентифіковані як Enterococcus sp., один штам – як Lactobacillus sp. та три штами залишилися неідентифікованими. Нові штами Enterococcus B1.1, Enterococcus B1.2, Enterococcus B1.3, Enterococcus B2.3 та Enterococcus M7.1 виявили антагоністичну активність щодо інших близькоспоріднених штамів та Lactobacillus sakei subsp. sakei JCM1157. ***Висновки.*** *Чорноморська вода та тканинний ліквор мідій у зимовий період містять 2×10 – 4,8×10² КУО/мл та 1,7×10±0,7 КУО/мл МКБ, відповідно. Штами Enterococcus B1.1, Enterococcus B1.2, Enterococcus B1.3, Enterococcus B2.3 та Enterococcus M7.1 є потенційними продуцентами антибактеріальних сполук.*

Ключові слова: лактобактерії, вода Чорного моря, Enterococcus, Lactobacillus, мідії, бактеріоцини.

СПИСОК ВИКОРИСТАНОЇ ЛІТЕРАТУРИ

1. *Васильєва Н.Ю., Страшнова І.В., Басюл О.В., Ковтун І.О., Іваниця В.О.* Стійкість до антибіотиків молочнокислих коків, ізольованих з чорноморських водоростей і мідій // Мікробіологія і біотехнологія. – 2020. – № 49. – С. 8–19.
2. *Кранга К.М., Васильєва Н.Ю., Страшнова І.В.* Розподіл і мінливість чисельності гетеротрофних, коліморфних і молочнокислих бактерій у воді і гідробіонтах Чорного моря // Вісник Одеського національного університету. – 2019. – № 24. – С. 113–125.
3. *Страшнова І.В., Ковтун І.О., Коротаєва Н.В.* Характеристика молочнокислих бактерій губок Чорного моря // Мікробіологія і біотехнологія. – 2020. – № 1. – С. 79–94.
4. *Ямборко Г.В., Толпіна М.Г.* Виділення бактерій роду *Lactobacillus* від гідробіонтів Чорного моря та їх ідентифікація // Вісник ОНУ. Біологія. – 2006. – Т. 11, Вип. 6. – С. 215–220.
5. *Amin M., Adams M.B., Burke Ch.M., Bolch Ch.J.S.* Isolation and screening of lactic acid bacteria associated with the gastrointestinal tracts of abalone at various life stages for probiotic candidates // Aquaculture Reports. – 2020. – V. 17: 100378. – https://doi.org/10.1016/j.aqrep.2020.100378
6. *Bergey's manual of systematic bacteriology. The Firmicutes* / Editors: P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer, W.B. Whitman. – London; New York: Springer, 2009. – V. 3. – 1422 p.
7. *Deasy B.M., Rea M.C., Fitzgerald G.F., Cogan T.M., Baresford T.P.* A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus* // Systematic and Applied Microbiology. – 2000. – V. 23 (4). – P. 510–522.
8. *De Man J.C., Rogosa M., and Sharpe M.E.* A medium for the cultivation of *Lactobacilli* // J. Oppl. Bact. – 1960. – V. 23 (l). – P. 130–135.
9. *Dubernet S., Desmasures N., Guéguen M.* A PCR-based method for identification of lactobacilli at the genus level // FEMS Microbiology Letters. – 2002. – V. 214. – P. 271–275.

10. *Ibryamova S., Arhangelova N., Koynova T., Dimitrov D., Dimitrova Z., Ivanov R., Kalchev K., Chipev N., Natchev N., Ignatova-Ivanova T.* Antifungal activity of lactic acid bacteria, isolated from (*Mytilus galloprovincialis* Lam.) in the bulgarian Black sea aquatory // Journal of IMAB – Annual Proceeding (Scientific Papers). – 2020. – V. 26 (1). – P. 2875–2882.
11. *Ignatova-Ivanova T., Ibryamova S., Bachvarova D., Salim S., Valkova S., Simeonova Y., Dimitrov D., Ivanov R., Chipev N., Natchev N.* Determination of the antimicrobial activity of lactic acid bacteria isolated from the Black sea mussel *Mytilus galloprovincialis* Lamarck, 1819 // Pharmacia. – 2022. – V. 69 (3). – P. 637–644.
12. *Ignatova-Ivanova T., Ibrjmova S., Stanachkova E., Ivanov R.* Microbiological characteristic of microflora of (*Mytilus galloprovincialis* Lam.) in the Bulgarian Black Sea aquatory // Research Journal of Pharmaceutical, Biological and Chemical Sciences. – 2018. – V. 9 (1). – P. 199–205.
13. *Jackson C.R., Fedorka-Cray P.J., Barrett J.B.* Use of a genus and species-specific multiplex PCR for identification of Enterococci // Journal of Clinical Microbiology. – 2004. – V. 42(8). – P. 3558–3565.
14. *Kathiresan K., Thiruneelakandan G.* Prospects of lactic acid bacteria of marine origin // Indian Journal of Biotechnology. – 2008. – V. 7. – P. 170–177.
15. *Kwon H.S., Yang E.H., Yeon S.W., Kang B.H., Kim T.Y.* Rapid identification of probiotic *Lactobacillus* species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA // FEMS Microbiology Letters. – 2004. – V. 239. – P. 267–275.
16. *Layton B.A., Walters S.P., Lam L.H., Boehm A.B. Enterococcus* species distribution among human and animal hosts using multiplex PCR // Journal of Applied Microbiology. – 2010. – V. 109. – P. 539–547.
17. *Merlich A., Galkin M., Choiset Y., Limanska N., Vasylieva N., Ivanytsia V., Haertlé T.* Characterization of the bacteriocin produced by *Enterococcus italicus* ONU547 isolated from Thai fermented cabbage // Folia Microbiol (Praha). – 2019. – V. 64(4). – P. 535–545.
18. *Nikita Ch.*, *Hemangi D.* Isolation, identification and characterization of lactic acid bacteria from dairy sludge sample // Journal of Environmental Research and Development. – 2012. – V. 7 (1A). – P. 1–11.
19. *Singh V., Haque Sh., Singh H., Verma J., Vibha K., Singh R., Jawed A., Tripathi C.K.M.* Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications // Front Microbiol. – 2016. – V. 7: 2016. – https://doi.org/10.3389/fmicb.2016.01921
20. *Schmeisser C., Steele H., Streit W.R.* Metagenomics, biotechnology with non-culturable microbes // Appl. Microbiol. Biotechnol. – 2007. – V. 75. – P. 955–962
21. *Szegedi E., Bottka S.* Detection of *Agrobacterium vitis* by polymerase chain reaction in grapevine bleeding sap after isolation on a semiselective medium // Vitis. – 2002. – V. 41(1). – P. 37–46.

REFERENCES

1. Vasylieva NYu, Strashnova IV, Basiul OV, Kovtun IO, Ivanytsia VO. Resistance of lactic cocci isolated from Black Sea algae and mussels to antibiotic. Microbiology and Biotechnology. 2020; (49): 8-19 (In Ukrainian).
2. Kranga KM, Vasylieva NYu, Strashnova IV. Distribution and variability of the heterotrophic, colimorphic and lactic acid bacteria number in water and aquatic organisms of the Black Sea. Visnyk Odeskogo Natsionalnogo Universytetu. Biologiia. 2019; (24): 113-125 (In Ukrainian).
3. Strashnova IV, Kovtun IO, Korotaeva NV. Characteristics of lactic acid bacteria from the Black Sea sponges. Microbiology and Biotechnology. 2020; (1): 79-94 (In Ukrainian).
4. Yamborko GV, Tolpina MG. Isolation *Lactobacillus* strains from gidrobionts of the Black Sea and their identification. Visnyk Odeskogo Natsionalnogo Universytetu. Biologiia. 2006; (11/6): 215-220 (In Ukrainian).
5. Amin M, Adams MB, Burke ChM, Bolch ChJS. Isolation and screening of lactic acid bacteria associated with the gastrointestinal tracts of abalone at various life stages for probiotic candidates. Aquaculture Reports. 2020; (17): 100378. Doi: https://doi.org/10.1016/j.aqrep.2020.100378
6. Bergey's manual of systematic bacteriology. The Firmicutes / Editors: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB. Springer, London, New York, 2009:3. 1422 p.
7. Deasy BM, Rea MC, Fitzgerald GF, Cogan TM, Baresford TP. A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus*. Systematic and Applied Microbiology. 2000; (23 (4)):510-522.
8. Dubernet S, Desmasures N, Guéguen M. A PCR-based method for identification of lactobacilli at the genus level. FEMS Microbiology Letters. 2002; 214: 271-275.
9. De Man JC, Rogosa M, and Sharpe ME. A medium for the cultivation of *Lactobacilli*. J. Oppl. Bact. 1960; (23 (l)): 130-135.
10. Ibryamova S, Arhangelova N, Koynova T, Dimitrov D, Dimitrova Z, Ivanov R, Kalchev K, Chipev N, Natchev N, Ignatova-Ivanova T. Antifungal activity of lactic acid bacteria, isolated from (*Mytilus galloprovincialis* Lam.) in the bulgarian Black sea aquatory. Journal of IMAB – Annual Proceeding (Scientific Papers). 2020; (26 (1)): 2875-2882.
11. Ignatova-Ivanova T, Ibrjmova S, Stanachkova E, Ivanov R. Microbiological characteristic of microflora of (*Mytilus galloprovincialis* Lam.) in the Bulgarian Black Sea aquatory. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018; (9(1)): 199-205.
12. Ignatova-Ivanova T, Ibryamova S, Bachvarova D, Salim S, Valkova S, Simeonova Y, Dimitrov D, Ivanov R, Chipev N, Natchev N. Determination of the antimicrobial activity of lactic acid bacteria isolated from the Black sea mussel *Mytilus galloprovincialis* Lamarck, 1819. Pharmacia. 2022; (69(3)): 637-644.
13. Jackson CR, Fedorka-Cray PJ, Barrett JB. Use of a genus and species-specific multiplex PCR for identification of Enterococci. Journal of Clinical Microbiology. 2004; (42(8)): 3558-3565.

14. Kathiresan K, Thiruneelakandan G. Prospects of lactic acid bacteria of marine origin. Indian Journal of Biotechnology. 2008; 7: 170-177.
15. Kwon HS, Yang EH, Yeon SW, Kang BH, Kim TY. Rapid identification of probiotic *Lactobacillus* species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. FEMS Microbiology Letters. 2004; 239: 267-275.
16. Layton BA, Walters SP, Lam LH, Boehm AB. *Enterococcus* species distribution among human and animal hosts using multiplex PCR. Journal of Applied Microbiology. 2010; 109: 539-547.
17. Merlich A, Galkin M, Choiset Y, Limanska N, Vasylieva N, Ivanytsia V, Haertlé T. Characterization of the bacteriocin produced by *Enterococcus italicus* ONU547 isolated from Thai fermented cabbage. Folia Microbiol (Praha). 2019; (64(4)): 535-545.
18. Nikita Ch, Hemangi D. Isolation, identification and characterization of lactic acid bacteria from dairy sludge sample. Journal of Environmental Research and Development. 2012; (7(1A)): 1 – 11.
19. Schmeisser C, Steele H, Streit WR. Metagenomics, biotechnology with non-culturable microbes. Appl. Microbiol. Biotechnol. 2007; 75: 955-962
20. Singh V, Haque Sh, Singh H, Verma J, Vibha K, Singh R, Jawed A, Tripathi CKM. Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. Front Microbiol. 2016; (7): 2016. doi: https://doi.org/10.3389/fmicb.2016.01921
21. Szegedi E, Bottka S. Detection of *Agrobacterium vitis* by polymerase chain reaction in grapevine bleeding sap after isolation on a semiselective medium. Vitis. 2002; (41(1)): 37-46.

Стаття надійшла до редакції 15.08.2024 р.