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ANTAGONISTIC ACTIVITY OF ENDOSPOREFORMING BACTERIA OF DEEP WATER THE BLACK SEA SEDIMENTS

Aim. *The purpose of the work was to study the antagonistic activity of strains of the facultatively anaerobic endosporeforming bacteria, isolated from deep-sea sediments of the Black Sea.* ***Materials and methods.*** *In this work, 250 strains of facultative anaerobic endosporeforming bacteria, isolated from the samples of deep-sea sediments of the Black Sea, were used. Antagonistic activity was determined by the method of radial streaks. As indicators for screening there were used next strains of opportunistic human pathogens – Proteus vulgaris ATCC 6896, Pseudomonas aeruginosa B-329, Staphylococcus aureus ATCC 6538P, Bacillus cereus ATCC 14579, Candida albicans ATCC 10231, Escherichia coli ATCC 25922, Salmonella enterica NCTC 6017, Klebsiella pneumoniae ATCC 10031, Bacillus subtilis ATCC 10774.* ***Results.*** *It is revealed that 32% of all isolated Black Sea strains of facultative anaerobic spore-forming bacteria have been shown to exhibit antagonistic activity of varying degrees of severity in relation to opportunistic microorganisms. There is a certain relationship between species strain, horizon of its origin and its antagonistic activity.* ***Conclusions.*** *The obtained results testify to the promising nature of the strains of endospore-forming bacteria in the Black Sea sediments for the screening of the producers of antimicrobial compounds with activity directed against opportunistic human pathogens.*

Key words: endosporoferming bacteria, antimicrobial activity, the Black sea.

Most studies which focus on the antagonistic interactions of microorganisms relate to microorganisms in soil origin. The marine environment is much less explored in this aspect, and it can serve as a promising source for the search for new producers for biotechnological research. This is especially true of such specific part of the World Ocean as the Black sea, in which water from the depth of 100–150 m is saturated with hydrogen sulfide and characterized by anaerobic conditions, which makes this biotop unique [10]. Therefore, we can expect to discover in this environment new types of antagonistic activity and microorganisms – the producers of new antibiotics. The search for and development of new antimicrobial drugs is important for the control of poliresistant pathogens [3, 6, 7, 8].

The study of antagonistic activity in strains of bacteria of natural origin is interesting not only from the applied, but also from the fundamental point of

view – it gives an idea of the nature and extent of the potency of the competitive relationships in the biotope from which the bacteria-antagonists are isolated [5, 6].

For the first time in the bottom deposits of anaerobic, saturated hydrogen sulfide and methane, batialy of the Black sea at depths 888–2080 m, we identified facultative anaerobic endosporeforming bacteria, their taxonomic diversity was determined, and assumptions were made about their alochtonic origin [2]. It was established that the isolated strains belong to 18 species of 4 genera *Bacillus, Paenibacillus, Lysinibacillus* and *Brevibacillus.* Proceeding from the hypothesis of the alochtonic origin of these bacteria in the bottom sediments of the Black sea, we can assume the correlation of the antagonistic profiles of the strains obtained with such soils for bacteria of soil origin.

The aim of the work was to study the antimicrobial activity of the strains of the facultatively anaerobic endosporeforming bacteria, isolated from the deep sea sediments of the Black sea and to find strains promising for the search for new antibiotics.

Materials and methods

250 strains of facultatively anaerobic endosporeforming bacteria of genera *Bacillus, Paenibacillus, Lysinibacillus, Brevibacillus,* isolated from samples of deep-sea bottom sediments of the Black Sea, taken from the depths up to 2080 meters during the M84 / 2 expeditions of the University of Bremen on the research vessel "METEOR" (stations 233, 242, 258, 269) and on the research vessel "MARE NIGRUM" Romania (station 116) [2].

Isolated strains were previously identified by defining the spectrum of fatty acids on a BioRad gas chromatograph using the standard method [4] using an automatic identification system of MIDI Sherlock microorganisms based on a gas chromatograph with Agilent 7890 flame ionization detector (Agilent Technologies, USA).

Screening of antagonists was carried out using the radial streaks method on the Hauze-2 medium [1]. As indicators for pre-screening there were used the strains *Proteus vulgaris* ATCC 6896, *Pseudomonas aeruginosa* B-329, *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 14579, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Salmonella enterica* NCTC 6017, *Klebsiella pneumoniae* ATCC 10031, *Bacillus subtilis* ATCC 10774.

For to the provided indicator strains, grateful thanks to the Head of the Department of physiology and taxonomy of micromycetes, Kurchenko I. M., and the head of the department of antibiotics in IMV NASU Avdeeva L. V.

Results and discussion

Among 250 tested strains, 79 were antagonistic, representing about 32%. It can be seen from the table that the most active studied strains were against *Candida albicans* ATCC 10231 (average growth inhibition zones 21.52 mm), *Proteus vulgaris* ATCC 6896 (average growth inhibition zones 19.88 mm), and *Staphylococcus aureus* ATCC 6538P (the average size of growth inhibition zones is 16.03 mm). The least-susceptible were *Pseudomonas aeruginosa* B-329 (average size of growth inhibition zones – 6.65 mm), *Escherichia coli* ATCC 25922 (average

growth inhibition zone 8.05 mm), and *Klebsiella pneumoniae* ATCC 10031 (average size of inhibition zones growth – 8.25 mm) (Table).

Table

Antimicrobial activity of endosporeforming bacteria isolated from deep water sediments of the Black sea

Species	Strain, station, horizon	1*	2	3	4	5	6	7	8	9
B. licheniformis	055 (233, 0-5)	3,0	4,0	4,0	5,0	0,0	2,0	3,0	3,0	3,0
B. megaterium	955 (233, 0-5)	24,0	0,0	34,0	7,0	29,0	0,0	6,0	0,0	0,0
B. subtilis	219 (233, 5-10)	24,0	0,0	32,0	13,5	27,0	5,0	6,5	0,0	0,0
B. subtilis	211 (233, 5-10)	32,5	0,0	35,0	17,0	35,0	6,0	17,5	8,5	0,0
B. subtilis	203 (233, 5-10)	29,0	0,0	35,0	13,5	25,0	8,0	13,0	8,0	0,0
B. subtilis	204 (233, 5-10)	32,0	0,0	35,0	15,0	30,0	11,0	11,5	2,0	2,5
B. atrophaeus	200 (233, 5-10)	35,0	2,5	35,0	19,0	35,0	7,0	21,0	9,0	0,0
B. cereus	213 (233, 5-10)	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0
B. subtilis	212 (233, 5-10)	32,0	0,0	35,0	14,5	35,0	7,5	15,0	4,0	0,0
B. atrophaeus	010 (233, 10-15)	5,0	3,5	4,0	10,0	5,0	5,0	2,5	1,0	0,0
B. licheniformis	043 (233, 15-20)	0,0	0,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0
B. licheniformis	014 (233, 15-20)	0,0	0,0	4,5	0,0	0,0	0,0	0,0	0,0	0,0
B. pimilus	002 (233, 15-20)	0,0	0,0	8,0	6,5	0,0	0,0	0,0	0,0	0,0
B. pimilus	004 (233, 15-20)	3,5	3,5	5,0	9,5	0,0	4,0	3,0	2,0	2,0
B. megaterium	003 (233, 15-20)	0,0	0,0	7,0	2,0	0,0	0,0	0,0	0,0	1,0
B. licheniformis	012 (233, 15-20)	0,0	0,0	6,5	5,0	0,0	0,0	0,0	0,0	0,0
B. licheniformis	99B (233, 20-25)	0,0	0,0	2,5	10,5	5,0	0,0	0,0	0,0	6,5
B. atrophaeus	99A (233, 20-25)	3,5	0,0	0,0	14,0	0,0	0,0	0,0	0,0	6,0
B. licheniformis	005 (233, 25-30)	0,0	0,0	10,0	5,0	0,0	0,0	0,0	0,0	0,0
B. licheniformis	001 (233, 30-35)	0,0	0,0	2,5	0,0	0,0	0,0	0,0	0,0	0,0
B. subtilis	920 (233, 0-5)	-**	0,0	0,0	-	0,0	0,0	-	-	9,0
B. amyloliquefaciens	921 (233, 0-5)	-	11,0	10,0	-	11,0	11,0	-	-	12,0
B. pimilus	99A (258, 0-5)	35,0	0,0	35,0	14,0	35,0	5,0	18,5	10,0	0,0
B. pimilus	232(258, 5-10)	0,0	0,0	5,0	3,5	7,0	0,0	0,0	0,0	0,0
P. larvae	018 (258, 5-10)	25,5	0,0	30,0	15,5	26,5	4,0	7,5	8,5	5,0
-	019 (258, 5-10)	5,5	0,0	0,0	14,5	0,0	3,5	0,0	0,0	6,5
B. subtilis	231 (258, 5-10)	35,0	5,0	35,0	16,0	28,0	7,5	18,0	9,0	8,0
B. subtilis	217 (258, 5-10)	27,5	3,0	35,0	16,5	35,0	9,0	10,0	7,5	0,0
B. subtilis	247 (258, 10-15)	19,5	2,0	28,0	15,0	22,0	9,5	10,5	10,0	0,0
B. atrophaeus	246 (258, 10-15	0,0	0,0	3,5	6,5	0,0	0,0	0,0	0,0	0,0
B. subtilis	021 (258, 10-15)	6,0	3,0	11,0	20,0	35,0	12,5	12,5	14,0	0,0
B. subtilis	239 (258, 30-35)	30,0	0,0	35,0	14,5	30,0	7,0	17,0	13,0	0,0
B. megaterium	035 (258, 30-35)	34,0	0,0	35,0	20,0	35,0	13,0	21,5	15,0	0,0

Table continued

B. megaterium	069 (242, 0-5)	0,0	0,0	1,5	16,0	0,0	0,0	0,0	0,0	0,0
P. macerans	953 (242,0-5)	-	9,0	15,5	-	16,5	15,5	-	-	7,0
Br. reuszeri	031 (242, 0-5)	2,0	0,0	4,0	19,0	0,0	0,0	0,0	0,0	0,0
B. megaterium	957(242,0-5)	-	0,0	0,0	-	0,0	0,0	-	-	9,0
B. megaterium	067 (242, 0-5)	4,5	0,0	1,5	15,0	0,0	0,0	0,0	0,0	0,0
B. subtilis	959(242,0-5)	-	0,0	10,0	-	0,0	8,0	-	-	0,0
B. pumilus	229 (242, 0-5)	21,5	0,0	32,5	5,0	21,5	0,0	0,0	5,0	0,0
B. agaradhaerens	960(242,0-5)	-	14,0	13,5	-	13,5	15,5	-	-	7,0
B. subtilis	923 (242, 0-5)	30,0	2,5	35,0	13,5	35,0	5,5	9,0	3,5	0,0
B. subtilis	961(242,0-5)	-	0,0	9,0	-	0,0	0,0	-	-	0,0
B. subtilis	967(242,0-5)	-	0,0	6,0	-	0,0	5,5	-	-	0,0
B. subtilis	968(242,0-5)	-	0,0	0,0	-	0,0	0,0	-	-	13,0
B. megaterium	032 (242, 0-5)	0,0	0,0	4,5	0,0	0,0	0,0	0,0	0,0	0,0
B. subtilis	970(242,0-5)	-	11,0	13,0	-	0,0	12,0	-	-	0,0
B. megaterium	980(242,0-5)	-	7,0	7,5	-	0,0	0,0	-	-	0,0
B. licheniformis	986(242,0-5)	-	0,0	0,0	-	0,0	10	-	-	0,0
B. pumilus	989(242,0-5)	-	9,0	0,0	-	0,0	0,0	-	-	0,0
B. subtilis	001 (242, 5-10)	35,0	0,0	16,0	12,5	35,0	2,0	3,0	13,0	3,5
B. subtilis	013 (242,10-15)	35,0	0,0	35,0	16,0	35,0	3,5	17,0	8,0	0,0
B. pumilus	041 (242, 10-15)	7,0	0,0	20,5	0,0	0,0	0,0	0,0	0,0	0,0
B. atrophaeus	008 (242, 10-15)	0,5	3,0	1,5	5,0	0,0	1,5	1,0	0,0	2,0
B. megaterium	040 (242, 10-15)	35,0	0,0	16,0	7,5	27,0	3,0	4,5	11,0	0,0
B. licheniformis	249 (242, 15-20)	0,0	0,0	0,0	7,5	2,5	1,0	0,0	0,0	0,0
B. subtilis	251 (242, 15-20)	0,5	1,5	0,0	4,0	0,0	0,0	0,0	0,0	4,5
B. subtilis	248 (242, 15-20)	0,0	2,0	6,0	6,0	0,0	0,5	0,0	0,0	0,0
B. licheniformis	245 (242, 25-30)	0,0	0,0	7,5	2,0	0,0	0,0	0,0	0,0	0,0
B. subtilis	033 (242, 25-30)	0,0	1,5	1,5	5,0	0,0	0,0	0,0	0,0	0,0
B. pumilus	932 (269, 0-05)	-	5,0	5,0	-	0,0	13,0	-	-	5,0
B. megaterium	063 (269, 0-05)	12,0	0,0	23,5	0,0	13,0	0,0	0,0	0,0	0,0
B. pumilus	049 (269, 45-50)	1,5	0,0	17,5	10,5	35,0	2,0	4,5	10,0	0,0
B. megaterium	051 (269, 45-50)	9,0	0,0	20,5	0,0	10,0	0,0	0,0	0,0	0,0
B. subtilis	053 (269, 45-50)	35,0	0,0	35,0	19,0	35,0	12,5	19,5	8,0	0,0
B. megaterium	054 (269, 45-50)	28,0	0,0	35,0	15,0	29,5	3,5	16,5	14,0	0,0
B. licheniformis	048 (269, 45-50)	18,0	0,0	22,5	6,5	27,5	2,0	8,0	8,0	0,0
B. atrophaeus	993 (249, 0-16)	-	15,0	16,0	-	15,0	0,0	-	-	14,0
B. subtilis	997 (249, 0-16)	-	0,0	27,0	-	15,0	10,0	-	-	28,0
B. subtilis	941 (116, 0-02)	-	9,0	15,0	-	13,0	12,0	-	-	13,0
B. subtilis	943(116, 0-02)	-	15,0	11,0	-	18,0	14,0	-	-	13,0
B. amyloliquefaciens	946(116, 0-02)	-	7,5	7,5	-	8,5	14,0	-	-	19,5

Table continued

B. subtilis	902 (116, 0-05)	-	0,0	6,0	-	13,0	11,0	-	-	12,5
B. subtilis	903 (116, 0-05)	-	5,5	5,5	-	11,5	0,0	-	-	7,0
B. amyloliquefaciens	905 (116, 0-05)	-	7,5	15,0	-	6,5	13,0	-	-	14,0
B. agaradhaerens	906 (116, 0-05)	-	7,5	13,5	-	6,5	15,5	-	-	14,0
B. subtilis	907 (116, 0-05)	-	6,5	7,0	-	7,0	11,5	-	-	12,5
B. amyloliquefaciens	908 (116, 0-05)	-	7,0	8,0	-	11,5	15,5	-	-	8,5
B. subtilis	909 (116, 0-05)	-	16,0	14,0	-	16,0	-		-	14,0
Average***		19,88	6,65	16,03	11,28	21,51	8,05	11,01	8,20	9,10
Portion of active strains (%)		68	38	89	85	57	60	51	47	39

Note: *– 1. *Proteus vulgaris* ATCC 6896, 2. *Pseudomonas aeruginosa* B-329, 3. *Staphylococcus aureus* ATCC 6538P, 4. *Bacillus cereus* ATCC 14579, 5. *Candida albicans* ATCC 10231, 6. *Escherichia coli* ATCC 25922, 7. *Salmonella enterica* NCTC 6017, 8. *Klebsiella pneumoniae* ATCC 10031, 9. *Bacillus subtilis* ATCC 10774;

** – Was not determined;

*** Average values of growth absence zones in millimeters without zero ones.

A high degree of antagonism is shown for gram-positive bacteria *S. aureus* and *B. cereus* (the portion of bacteria active against to them reached 89% and 85%, respectively, among all active strains). Antagonism to enterobacteria *(Proteus vulgaris, Escherichia coli, Salmonella enterica)* was 68, 60 and 51%, respectively. At the lowest level, there was antagonistic activity in relation to *Pseudomonas aeruginosa.* A characteristic feature is the significant difference between the average antagonistic activity of the museum strains in relation to the indicator strains *B. cereus* and *B. subtilis*.

The highest activity among all identified species was detected in *P. larvae, P. macerans* and *B. atrophaeus* with a corresponding index of 86%. Significant activity was noted also for species known from the literature as the producers of bacteriocins and antibiotics – *B. subtilis* with a 60% and *B. licheniformis* – 58%, *B. amyloliquefaciens* – 57%. It is interesting, that the strains of the species *B. agaradhaerens* (66%), for which there is no known ability to synthesize antibiotics or bacteriocins, have been shown to be very close. The lowest activity was observed for the strains of *B. megaterium* (26%), *B. pumilus* (19%), *B. cereus* (17%) and *Br. reuszeri* (6%).

The antimicrobial activity of the investigated strains unlinearly depended on the depth of the horizon from which they were obtained. This dependence can be described as the gradual increase in the proportion of antagonistic active strains from the surface to a depth of about 15 cm, followed by gradual decrease to 40% on the horizon of 25–30 cm (Figure).

The known average rate of accumulation of bottom sediments in the Black sea, which is approximately 10 mm in 20 years [9], can help to interpret this information from paleoclimatic point of view as reflection of changes in precipitation dynamics in this region during the last millenium.

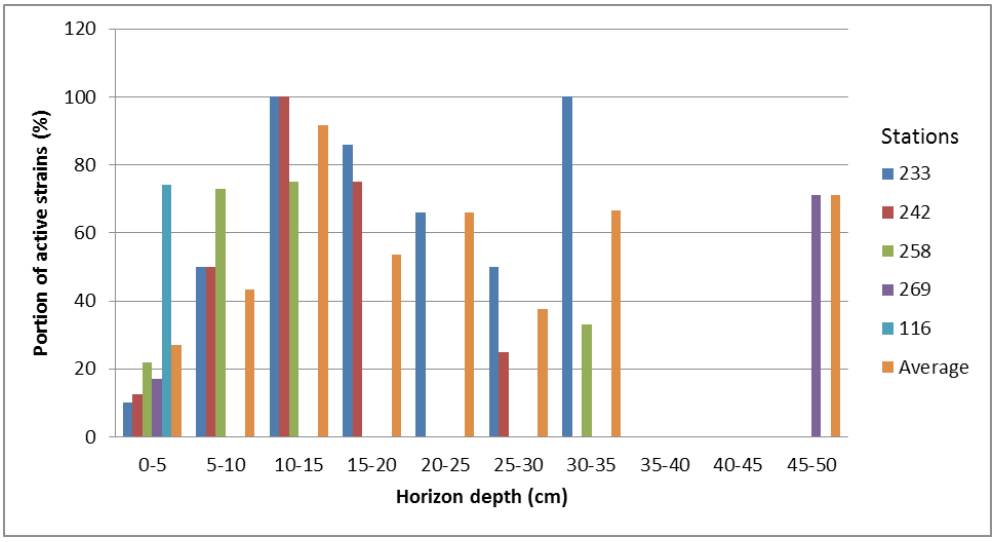

Fig. Dependence of portion of antagonistically active strains (in percentages) on the horizon depths

Thus, the obtained data indicate the perspectivness of naturally preserved marine bacteria in the bottom sediments of the Black sea for the search for the producers of new antimicrobial drugs against opportunistic human pathogens.

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АНТАГОНИСТИЧЕСКАЯ АКТИВНОСТЬ СПОРООБРАЗУЮЩИХ БАКТЕРИЙ ГЛУБОКОВОДНЫХ ДОННЫХ ОСАДКОВ ЧЕРНОГО МОРЯ

Реферат

Цель. *Целью работы было изучить антагонистическую активность штаммов факультативно-анаэробных спороутворювальних бактерий, выделенных из глубоководных отложений Черного моря.* ***Методы.*** *В работе использованы 250 штаммов факультативно-анаэробных спороутворювальних бактерий, выделенных из проб глубоководных отложений Черного моря. Антагонистическую активность определяли по методу радиальных штрихов. Как индикаторы для скрининга использовали штаммы оппортунистических патогенов человека – Proteus vulgaris ATCC 6896, Pseudomonas aeruginosa B-329, Staphylococcus aureus ATCC 6538P, Bacillus cereus ATCC 14579, Candida albicans ATCC 10231, Escherichia coli ATCC 25922, Salmonella enterica NCTC 6017, Klebsiella pneumoniae ATCC 10031, Bacillus subtilis ATCC 10774.* ***Результаты.*** *Выявлено, что 32% всех изолированных черноморских штаммов факультативно-анаэробных спорообразующих бактерий демон-*

стрируют антагонистическую активность разной степени выраженности по отношению к условно-патогенных микроорганизмов. Отмечена определенная зависимость между видовой принадлежностью штамма, горизонтом его происхождения и антагонистической активностью. ***Выводы.*** *Полученные результаты свидетельствуют о перспективности штаммов спорообразующих бактерий донных отложений Черного моря для скрининга продуцентов антимикробных соединений с активностью, направленной против оппортунистических патогенов человека.*

Ключевые слова: спорообразующие бактерии, антимикробная активность, Черное море.

References

1. *Егоров Н. С.* Основы учения об антибиотиках – М.: Высшая школа, 1986. – 448 с.

2. *Іваниця В. О., Штеніков М. Д., Остапчук А. М.* Факультативно-анаеробні спороутворювальні бактерії глибоководних відкладень Чорного моря // // Мікробіологія і біотехнологія. – 2015. – № 4). – С. 28–36.

3. *Abriouel H., Franz C.M., Ben Omar N., Gálvez A.* Diversity and applications of Bacillus bacteriocins // FEMS Microbiology Rev. – 2011. –35, N 1. – P. 201–232.

4. *Costa, da M. S., Albuquerque L., Nobre F. M., Wait R.* The Identification of Fatty Acids in Bacteria // Methods in Microbiology. – 2011. – V. 38, N 1. – P. 183–196.

5. *Fajardo-Cavazos P., Maughan H., Nicholson W. L.* Evolution in the *Bacillaceae* // Microbiol. Spectrum. – 2014. – V. 2, N 5. – Режим доступу: https:// www.ncbi.nlm.nih.gov/pubmed/26104365

6. *Mandic-Mulec I., Stefanic P., Elsas van J. D.* Ecology of *Bacillaceae.* // Microbiology Spectrum. – 2015. – V. 3, N 1. – P. 1–24.

7. *Pérez-Gutiérrez R.-A., López-Ramırez V., Islas A., Alcaraz L. D., Hernández-González I.* Antagonism influences assembly of a *Bacillus* guild in a local community and is depicted as a food-chain network // The ISME Journal. – 2013. – V. 7, N 3. – P. 487–497.

8. *Prieto M. L., O'Sullivan L., Tan S. P., McLoughlin P., Hughes H., O'Connor P. M., Cotter P. D., Lawlor P. G., Gardiner G. E.* Assessment of the Bacteriocinogenic Potential of Marine Bacteria Reveals Lichenicidin Production by Seaweed-Derived *Bacillus* spp. // Mar. Drugs. – 2012. – V.10, N 10. – P. 2280–2299.

9. *Smith L.D.S., Cato E. Clostridium durum,* sp. nov., the predominant organism in a sediment core from the Black Sea // Canadian Journal of Microbiology. – 1974. – V. 20, N 10. – P. 1393–1397.

10. *Zaitsev Y. P., Alexandrov B. G.* Black Sea Biological Diversity. – New York, United Nations Publications, 1998. – 362 p.

References

1. Egorov NS Osnovyi ucheniya ob antibiotikah – M.: Vyisshaya shkola, 1986. – 448 s.

2. Ivanitsia VO, Shtenikov MD, Ostapchuk AM. Fakultatyvno-anaerobni

sporoutvoriuvalni bakterii hlybokovodnykh vidkladen chornoho moria. Mikrobiolohiia i biotekhnolohiia. 2015;32(4):28-36.

3. Abriouel H, Franz CM, Ben Omar N, Gálvez A. Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiology Rev. 2011;35(1):201-232.

4. Costa da M.S, Albuquerque L, Nobre FM, Wait R. The Identification of Fatty Acids in Bacteria. Methods in Microbiology. 2011;38(1):183-196.

5. Fajardo-Cavazos P., Maughan H., Nicholson W.L. Evolution in the Bacillaceae // Microbiol. Spectrum. – 2014. – V. 2, N 5. – Режим доступу: https://www.ncbi.nlm.nih.gov/pubmed/26104365

6. Mandic-Mulec I, Stefanic P, Elsas van JD. Ecology of Bacillaceae. Microbiology Spectrum. 2015;3(1):1-24.

7. Pérez-Gutiérrez RA., López-Ramırez V, Islas A, Alcaraz LD, Hernández-González I Antagonism influences assembly of a Bacillus guild in a local community and is depicted as a food-chain network. The ISME Journal. 2013;7(3):487-497.

8. Prieto ML, O'Sullivan L, Tan SP, McLoughlin P, Hughes H, O'Connor PM, Cotter PD, Lawlor PG, Gardiner GE. Assessment of the Bacteriocinogenic Potential of Marine Bacteria Reveals Lichenicidin Production by Seaweed-Derived Bacillus spp. Mar. Drugs. 2012;10(10):2280-2299.

9. Smith LDS, Cato E. *Clostridium durum,* sp. nov., the predominant organism in a sediment core from the Black Sea. Canadian Journal of Microbiology. 1974;20(10)1393-1397.

10. Zaitsev YP, Alexandrov BG. Black Sea Biological Diversity. New York, United Nations Publications, 1998. 362 p.

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