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ALGA-LYSING PROPERTIES OF *BACILLUS* SP.

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Cyanobacterial blooms become a serious environmental threat to the freshwater ecosystem, and several physical and chemical methods have been developed for controlling the blooms. In order to develop a biocontrol agents for controlling the blooms, we carried out screening of the alga-lysing bacteria among the genus *Bacillus*. The alga-lysing characteristics bacteria isolated from various sources have been studied. Six strains of *Bacillus*: B.0.2.2.2., 57D, M2, M2a, 10.1, M18Г, with strong algicidal activity against the species of *Anabaena hassalii*, *Microcystis aeruginosa*, *M. pulverae* were selected. Wide algicidal activity ranged from 40 to 60 % was shown. It was established that the studied *Bacillus* strains did not possess marked lytic activity against *Chlorella* spp. and their activity did not reach beyond 15 %. Thus, investigated bacterial strains of the *Bacillus* genus have algicidal effect towards cyanobacteria and do not such effect towards green algae. The lytic effect of different parts of the bacterial cultures indicated that the algal cells were lysed by the algicidal active compounds in cell-free filtrate. Our results suggest that the studied strains of *Bacillus* sp. may have a potential for a use in controlling growth of harmful algal species.

Keywords: algicidal activity, *Bacillus* sp., cyanobacteria.

INTRODUCTION

In recent years water blooms of freshwater environments and the occurrence of cyanobacterial and algal blooms become a serious problem all over the world [11, 13, 14]. These blooms cause a wide range of social, environmental and economic problems, such as deterioration of water quality, damage of aquaculture industries, recreational activities and human health [1]. Cyanobacteria can produce some toxins that cause the death of fish, shellfish and other organisms in the natural environments.

Many chemical and physical methods have been proposed to mitigate or control harmful algal blooms (HABs) [2, 6]. However, these methods have strong inhibitory effects on the growth of harmful algae, received much criticism because of the disadvantages of high costs, frequent reappearance and secondary pollution. Moreover, such approaches cannot resolve the problems completely and water blooms are still very serious problem.

Recently, biological methods have gained increasing an attention of researchers as a control mean that have some preference to chemical and physical ones [5, 12]. Biological control has been seen as an economical and environment-friendly solution due to the low treatment cost and no secondary pollution. Biological agents, including bacteria, actinomycete, viruses, protozoa, macrophytes and microalgae are considered as potential suppressors in controlling the outbreak and control of HABs. Algicidal bacteria, as an important fraction of biological species in hydrophytic ecosystems, play important roles in controlling water blooms and limiting algal biomass. Most known algicidal bacteria are classified within either *Alteromonas*, *Bacillus*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Vibrio* and are prevalent in both marine and freshwater bacterial communities [3, 4].

The aim of our study was to carry out the screening of the alga-lysing bacteria of the *Bacillus* genus and to study their alga-lysing characteristics.

MATERIALS AND METHODS

Bacterial and algal cultures. One hundred thirty-five strains of bacteria isolated from different natural sources (soil, fresh and sea water, cotton, gastrointestinal tract of human). All isolates are maintained in the Ukrainian Collection of Microorganisms in Zabotny Institute of Microbiology and Virology, National Academy of Science of Ukraine.

The bacterial strains were cultivated at 37 °C for 24 h in Luria-Bertani (LB) medium [9]. Then bacteria were incubated at 37 °C for 24 h in 20 ml liquid LB medium with rotary shaking with a frequency of 250 revolutions per minute (rpm) and then the bacterial cultures were concentrated by centrifugation (6 000 g for 10 min) [3].

Cyanobacterial species of *Microcystis aeruginosa*, *M. pulvereae*, *Anabaena hassalii*, *A. cylindrica*, *Nostoc linekea*; and two green algae *Chlorella vulgaris*, *C. Kesleri* were obtained from the Algal Culture Collection, Institute of Hydrobiology, National Academy of Science of Ukraine. They were cultivated in an BG11 medium at 25 °C under the 12L:12D light-dark cycle [10].

Analysis of algicidal mode and activity. Cyanobacterium and algicidal bacterium were cocultivated in LB medium and optical density was measured to estimate algicidal activity [8]. The data of optical density absorption at 580 nm were monitored. All the cell-free filtrate of each bacterial culture were transferred separately into each well microplate, added of cyanobacterial culture (1:1 = ml:ml), followed by cocultivation at 37 °C for 24 h. LB broth served as a control. Algicidal rate, representing the algicidal activity, was calculated using the following equation:

$$\text{Algicidal rate (\%)} = (N_c - N_t) / N_c \times 100,$$

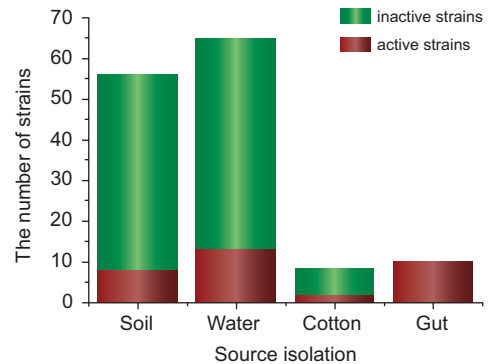
where N_c represents the number of algal cells in the control group and N_t represents the number of algal cells in the treatment group. All operations were performed in triplicate.

The lytic ability of the bacterium was regarded as strong when the data of optical density in the treated cyanobacterium/alga was more than or equal to 50 % compared to the control. The lysis phenomenon was ascribed to the ordinary when the reduction in treated cyanobacterium/alga was less than 50 %.

All experiments were done in triplicate and the results were presented as mean \pm standard deviation. Statistical analysis was performed using the software package for PCs Microsoft Excel. Differences between groups were determined by Student *t* tests.

RESULTS AND DISCUSSION

Algicidal mode and activity of *Bacillus* sp. We found that the 20 % of all studied *Bacillus* strains (135 strains) with algicidal activity were isolated from aquatic ecosystems (see Figure). In addition, a great number of algicidal bacterium *Bacillus* sp. were isolated from soil – eight strains (14 %). Active strains were not isolated from the gastrointestinal tract.



The distribution of the *Bacillus* strains isolated from different sources with activity towards cyanobacteria
Розподіл штамів бацил, виділених із різних джерел за активністю щодо ціанобактерій

Our results correlate with those of other researchers, according to which the highest number of actinomycetes with antagonism to *Microcystis* sp. were isolated from soil and freshwater environment [12]. This phenomenon is quite natural, because these ecosystems are most common for cyanobacteria.

According to literature data, diverse *Microcystis* and *Anabaena* species are most common types of cyanobacteria that cause algal blooms in natural ecosystems. Their population composes almost 90 % of the total biomass of phytoplankton. Therefore, we investigated algicidal activity of 22 *Bacillus* strains with high algicidal activity against a wide range of cyanobacterial *Anabaena hassalii*, *Microcystis aeruginosa*, *M. pulvereae* and low activity against green algae *Chlorella vulgaris* and *C. kesleri*. To determine the possible toxic effects of microbial strains to higher plants we used *Chlorella* as a convenient object like other green algae.

It was found that the level of lysis of the test cultures of cyanobacteria by these *Bacillus* strains ranged 25.6–61.6 % (table 1). Six strains of B. 0.2.2.2., 57D, M2, M2a, 10.1, M18Г were the most active then others (tab. 1). Their lytic activity against cyanobacteria *Anabaena hassabi*, *Microcystis aeruginosa*, *M. pulvereae* ranged at 44.2–61.6 %. All other studied strains showed low lytic activity, which was 23.5–56.4 % against *M. aeruginosa*, 12.8–47.3 % to *M. pulvereae* and 12.7–44.4 % to *A. hassalli*.

It should be noted that algicidal activity of the investigated bacterial strains was determined by cocultivated of cyanobacteria and cell-free filtrate of each bacterial culture and optical density was measured. Therefore, the reduction in the optical density does not fully reflect the algicidal activity level of *Bacillus*. The remains of dead cells of cyanobacteria can increase the optical density of the mixture and leads to false-negative results.

However, there is a possibility that algicidal compounds produced by the *Bacillus* strains could have negative affect to other organism in aquatic ecosystems, in particular on higher plants. Thus, it is necessary to investigate lytic activity of the *Bacillus* against green algae *Chlorella* spp. It was established that the studied *Bacillus* strains did not

show magnificent lytic activity against *Chlorella* spp. – their activity does not reach beyond 15.6 %. Thus, investigated bacterial strains of the *Bacillus* genus have algicidal effect on cyanobacteria and have not on green algae.

Table 1. Algicidal activities of *Bacillus* sp. against different cyanobacteria and green algae species

Таблиця 1. Альгіцидна активність бактерій роду *Bacillus* щодо різних видів ціанобактерій і зелених водоростей

N	<i>Bacillus</i> sp.	The value of OD % mixed of cyanobacteria / <i>Bacillus</i> and green algae / <i>Bacillus</i>				
		<i>M. aeruginosa</i>	<i>M. pulvereae</i>	<i>A. hassalii</i>	<i>C. vulgaris</i>	<i>C. kesleri</i>
1	III a	56.4±2.2	37.7±2.3	29.6±0.9	10.2±1.1	8.4±0.8
2	16.1	25.9±1.7	14.8±1.1	23.9±2.0	8.5±0.8	6.5±0.5
3	B 0.2.2.2.	54.6±2.9	45.2±1.5	44.2±2.1	15.5±1.2	12.8±1.4
4	78/6ч	46.6±1.9	43.6±2.6	29.7±1.4	11.8±1.9	9.3±1.4
5	SVP1	35.4±2.1	44.4±1.9	44.4±2.0	13.1±0.9	10.4±1.2
6	6/4	29.6±2.0	28.4±1.6	12.7±1.1	8.4±0.8	6.8±0.8
7	30/2ч	44.2±2.4	42.1±1.1	23.6±1.8	12.2±1.3	10.4±1.2
8	36/6D	38.4±1.4	32.8±2.0	15.5±1.2	10.4±1.2	8.4±0.8
9	KM1	50.7±1.9	37.5±0.7	35.1±2.0	12.8±1.4	11.8±1.9
10	57D	61.6±2.5	47.6±1.9	50.3±2.4	14.4±1.0	12.8±1.4
11	18/5ч	46.6±1.9	44.8±1.5	29.7±1.5	9.3±1.4	6.8±0.8
12	KM2	42.4±2.1	12.8±2.7	23.3±1.6	7.8±0.9	9.3±1.4
13	M2	50.6±1.7	47.7±1.9	44.9±2.2	11.1±1.0	10.4±1.2
14	M2a	58.5±2.4	48.4±1.7	47.1±2.0	15.6±1.3	10.2±1.1
15	26D	46.6±1.9	44.2±2.2	32.5±1.7	12.2±1.1	9.3±1.4
16	10.1	60.8±2.2	55.7±1.8	44.4±2.0	13.9±1.6	10.4±1.2
17	78/11ч	44.4±2.0	34.4±2.1	32.7±1.9	9.2±1.3	6.8±0.8
18	CS33	25.9±1.4	34.8±2.2	23.5±1.7	7.8±0.9	6.8±0.8
19	M18 Г	60.5±2.4	45.9±1.8	47.3±2.0	9.3±1.4	8.4±0.8
20	M6Г	48.6±2.6	47.3±2.0	35.1±1.8	10.4±1.2	9.3±1.4
21	56/1ч	23.5±2.1	30.5±1.4	18.7±0.7	8.4±0.8	6.5±0.5
22	56/2ч	25.6±1.8	34.4±2.1	19.4±1.0	7.8±0.9	8.4±0.8

Cyanobacteria are capable to photosynthesis use of solar energy due to their pigment system. Pigment apparatus of cyanobacteria consists of three groups of pigments: chlorophyll A – green pigment, phycobilins – blue pigment, carotenoids – a reddish yellow pigments. The content of pigments and their amount could be changed depending on lighting and cultivation conditions. Chlorophyll A is the main pigment in cyanobacterial cells. All investigated cultures of cyanobacteria were colored green, which means they contain chlorophyll A. It gave us reason to rate algicidal activity also visually by a colour change of the incubation mixtures of planktonic forms of cyanobacteria and bacilli. Also in our experiment we observed a discoloration of the mixture, indicating the

full lysis of cyanobacteria. The color of the mixture changed from green to light-yellow, which can indicate about chlorophyll destruction.

The populations of cyanobacteria are heterogeneous at morphological and physiological-biochemical characteristics. Therefore, we can expect differences in the sensitivity diverse ages of cyanobacteria to algicidal action *Bacillus*. Different age populations of cyanobacteria were investigated: young – 2 weeks, mature – 8 weeks and old culture – 16 weeks. According to the data presented in table 2, cyanobacteria cultures of 2 and 16-week were most sensitive to algicidal actions of *Bacillus*.

Depending on the age of cyanobacteria, value of the optical density ranged from 34.5 to 70.5 % for *M. aeruginosa*, 35.4–75.9 % for *M. pulvereae* and 35.4–67.5 % for *A. hassalii*. Cyanobacteria cultures of 8-week were less sensitive to action of the studied *Bacillus* strains and the value of optical density was slightly lower compared to the values obtained for 2-and 16-week cultures: 34.5–59.2 % relative to *M. aeruginosa*, 35.4–52.7 % relative to *M. pulvereae* and 35.4–54.7 % for *A. hassalii*.

Table 2. The sensitivity of cyanobacteria of different age to lytic action of *Bacillus* bacteria

Таблиця 2. Чутливість ціанобактерій різного віку до альгіцидної дії різних штамів бактерій роду *Bacillus*

<i>Bacillus</i> sp.	The value of OD % of a mixture of cyanobacteria and <i>Bacillus</i> strains								
	<i>M. aeruginosa</i>			<i>M. pulvereae</i>			<i>Anabaena hassabi</i>		
	The age of the studied cyanobacteria, weeks								
	2	8	16	2	8	16	2	8	16
M2	70.5± 3.0	34.5± 1.4	68.2± 2.7	61.4± 2.0	35.4± 1.6	45.5± 1.8	45.5± 1.8	35.4± 1.6	58.2± 2.4
57Д	70.4± 3.1	59.2± 2.3	70.4± 3.1	65.8± 1.9	52.7± 2.4	52.7± 2.4	60.4± 2.9	50.7± 2.1	65.9± 2.0
В.о.2.2.2	68.2± 2.7	45.5± 1.8	68.2± 2.7	65.8± 1.9	39.5± 1.7	45.5± 1.8	67.5± 2.4	42.9± 2.0	60.4± 2.0
M2a	56.7± 2.5	47.6± 1.7	68.5± 2.5	73.1± 2.8	35.6± 1.5	41.2± 1.6	57.7± 2.5	42.9± 2.0	58.8± 2.6
10.1	64.6± 2.2	50.7± 2.1	70.4± 3.1	75.9± 2.1	39.5± 1.7	52.2± 2.6	63.1± 2.0	54.7± 2.1	65.9± 2.0
M18r	64.6± 2.2	59.2± 2.3	70.5± 3.0	58.8± 2.6	48.4± 1.8	52.2± 2.6	41.2± 1.6	46.9± 1.9	63.1± 2.0

In general, algicidal bacteria inhibit growth of cyanobacteria or lysis of their cells by direct or indirect attack types. Duo to indirect attack bacteria lyse algal cells through the extracellular production of algicidal substances, while another type of bacteria kill algal cells by cell-to-cell contact mechanism [7]. These compounds can be proteins, peptides, amino acids, antibiotics, etc. Only some algicidal bacteria inhibit growth of algae through direct contact or penetration into the cell [4].

We investigated lytic activity of cell-free filtrate and washed twice with physiological saline the biomass of the investigated strains of *Bacillus*. As can be seen from table 3, activity was detected only in the supernatant. It could be due to release of specific bacterial metabolites from the bacterial cells and their presence in the cell-free filtrate, which provided algicidal activity of the investigated strains.

Table 3. Algicidal activity of different parts of *Bacillus* sp.Таблиця 3. Альгіцидна активність безклітинного фільтрату і біомаси *Bacillus* sp.

<i>Bacillus</i> sp.	The value of OD %					
	<i>M. aeruginosa</i>		<i>M. pulvereae</i>		<i>Anabaena hassabi</i>	
	cell-free filtrate	bacterial culture	cell-free filtrate	bacterial culture	cell-free filtrate	bacterial culture
M2	53.2±2.7	0	37.5±1.7	0	42.2±1.6	0
57Д	61.4±2.0	0	47.4±1.9	0	48.4±1.8	0
B.o.2.2.2	47.6±1.7	0	39.6±1.9	0	48.4±1.8	0
M2a	31.4±2.0	0	18.6±2.3	0	34.5±1.4	0
10.1	57.2±2.4	0	53.2±2.8	0	52.2±2.6	0
M18r	50.7±2.1	0	47.4±1.9	0	46.5±1.8	0

Therefore, algicidal effect is not associated with direct contact of bacteria with the algae cell, and fully depends on metabolites produced by the *Bacillus*.

CONCLUSION

Our results showed strong algicidal activity of six strains of algicidal *Bacillus* sp. bacterium against cyanobacterial cells. The algicidal activity of *Bacillus* strains might come from metabolites produced by the *Bacillus*. This implied that our strains of *Bacillus* lysed the algal cells by releasing active compounds to cause the dysfunction of algal cells or inhibit the growth. The studied strains of *Bacillus* sp. could be a potential candidate for use for controlling HABs in the aquatic environment.

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АЛЬГИЦИДНІ ВЛАСТИВОСТІ *BACILLUS* SP.

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Цвітіння води становить серйозну екологічну проблему для прісноводної екосистеми. Наразі відомі різні фізичні та хімічні засоби пригнічення росту водоростей. З метою розробки біопрепарату для контролю цвітіння води нами проведено скринінг альгіцидної активності серед штамів бактерій роду *Bacillus*, виділених із різних джерел. Відібрано 6 найактивніших штамів бацил, а саме B.0.2.2.2., 57D, M2, M2a, 10.1, M18Г, альгіцидна активність яких щодо *Anabaena hassalii*, *Microcystis aeruginosa*, *M. pulvereae* коливалась у межах 45–60 %. З'ясовано, що досліджувані штами бацил не проявляли вираженої лізуючої дії щодо зелених водоростей роду *Chlorella* spp., їхня активність не перевищувала 15 %. Отже, досліджені бактерії роду *Bacillus* характеризуються вибірковою дією на ціанобактерії та на зелені водорості. Встановлено, що альгіцидний ефект проявляли екзометаболіти досліджуваних штамів бацил тільки в супернатанті, біомаса була неактивна. Наші результати свідчать про те, що досліджені бактерії роду *Bacillus* можуть бути перспективними об'єктами у подальшому дослідженні їх можливого застосування для контролю росту ціанобактерій.

Ключові слова: альгіцидна активність, бактерії роду *Bacillus*, ціанобактерії.

АЛЬГИЦИДНЫЕ СВОЙСТВА *BACILLUS* SP.

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Цветение воды является серьезной экологической проблемой для пресноводной экосистемы. Сегодня известны различные физические и химические средства

угнетения роста водорослей. С целью разработки биопрепарата для контроля цветения воды нами проведен скрининг альгицидной активности среди штаммов бактерий рода *Bacillus*, выделенных из разных источников.

Отобрано 6 наиболее активных штаммов бацилл, а именно В.0.2.2.2., 57D, M2, M2a, 10.1, M18Г, альгицидная активность которых против *Anabaena hassalii*, *Microcystis aeruginosa*, *M. pulvereae* колебалась в пределах 45–60 %. Показано, что исследованные штаммы бацилл не проявляли выраженного лизирующего действия относительно зеленых водорослей рода *Chlorella* spp., их активность не превышала 15 %. Таким образом, исследованные бактерии рода *Bacillus* характеризуются избирательным действием на цианобактерии и на зеленые водоросли. Установлено, что альгицидный эффект проявляли экзометаболиты исследованных штаммов бацилл только в супернатанте, биомасса была неактивна. Наши результаты свидетельствуют о том, что исследованные бактерии рода *Bacillus* могут быть перспективными объектами в дальнейшем исследовании их возможного применения для контроля роста цианобактерий.

Ключевые слова: альгицидная активность, бактерии рода *Bacillus*, цианобактерии.

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