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G.V. GLADKA[1]*, N.V. BORZOVA[1], O.V. GUDZENKO[1], V.M. HOVORUKHA[1], O.A. HAVRYLIUK[1], O.V. SHABLII[1], L.S. YASTREMSKA[2], O.B. TASHYREV[1]

[1] Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, 154 Akademik Zabolotny Str., Kyiv, 03143, Ukraine

[2] Department of Biotechnology, Faculty of Environmental Safety, Engineering and Technologies, National Aviation University, Kyiv, 03058, Ukraine

* Author for correspondence; e-mail: gladkagv@ukr.net

ECOPHYSIOLOGICAL PROPERTIES AND HYDROLYTIC ACTIVITY OF CHEMOORGANOTROPHIC BACTERIA FROM HOLOSIIVSKYI NATIONAL NATURE PARK

Any natural ecosystem contains a specific range of microorganisms. The anthropogenic impact can cause a change in the growth conditions of soil and rhizospheric microbiome and affect the number and the physiological properties of microorganisms. ***The aim*** *of the study was to isolate the representative microorganisms from terrestrial ecosystems of Holosiivskyi National Nature Park (Ukraine) that are not exposed to extreme factors, to study their ecophysiological properties (resistance to UV radiation, dehydration, hypersalinity, temperature), and to study the extracellular glycoside and proteolytic activities.* ***Methods.*** *Aerobic chemoorganotrophic bacteria isolated at 30 °C from soil and phytocenoses of Holosiivskyi National Nature Park were studied. Meat-peptone agar was used to cultivate bacteria. Bacterial UV irradiation was performed with a BUF-15 lamp (λ = 254 nm) in the range of 30—1350 J/m^2. The temperature range of growth and halotolerance of microorganisms was determined in the range of 1—42 °C and 0.1—150 g NaCl/L, respectively. Bacterial isolates were cultivated in submerged conditions at 28 °C for 4 days. Synthetic p-nitrophenyl substrates, soluble starch, and guar galactomannan were used to determine glycosidase activity. To study proteolytic activity, casein, elastin, and gelatin were used.* ***Results.*** *The study of 14 soil and plant samples revealed the number of bacteria detected from $9.3 \cdot 10^4$ to $4.8 \cdot 10^5$ CFU/g in winter, and $4.8 \cdot 10^5$ to $4.2 \cdot 10^6$ CFU/g in summer. The microorganisms were represented by 1—4 morphotypes. There were isolated 37 isolates of aerobic chemoorganotrophic microorganisms, and 69% of them were represented by gram-positive rods. The dominance of pigmented isolates was not detected. Most of the microorganisms studied were psychrotolerant and moderate halophiles. The isolates 3g3, 8g1, 8g2, 8g3 from chornozem and dark gray soil showed high resistance to UV radiation. The $LD_{99.99}$ ranged from 800 to 1100 J/m^2. The isolates from chornozem, birch moss, green moss with sand and soil, and green moss from oak (1g, 4g2, 9g1, 14g2) were moderately resistant. The $LD_{99.99}$ was 280—650 J/m^2. The UV resistance was shown to be independent of pigmentation. It correlated with dehydration. The phenom-*

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enon of resistance to such UV radiation and dehydration may indicate the presence of active reparation mechanisms of DNA damage. All isolates showed cellulose and hemicellulose degrading activities as well as caseinolytic activity. Isolate 9g1 showed high β-xylosidase activity. ***Conclusions.*** *The high resistance to UV radiation and dehydration of non-adapted microorganisms as well as the wide range of exohydrolase activity indicate the wide adaptive capacity of microorganisms from natural ecosystems, which goes beyond the influence of surrounding factors. No data existed in the available literature defining hydrolytic activity and resistance of microorganisms of the temperate region of Ukraine to extreme factors. The obtained experimental data will allow for a better understanding of the resistance level of microorganisms of temperate regions to extreme factors. As a result of the work, new bacteria with high degrading activity were isolated. The studied isolates require further characterization and analysis for biotechnological applications.*

Keywords: *chemoorganotrophic bacteria, UV resistance, psychrotolerance, halophilicity, glycosidase and proteolytic activities.*

In natural ecosystems, microorganisms are constantly affected by various physical factors: temperature, UV and electromagnetic radiation, pressure, electric pulses, magnetic fields, etc. The change of any factor might cause the suppression of biological processes. On the globe, temperature changes so fast that leaves have little chance for species and ecosystems to adapt to such rapid variability of climate system parameters. As a result, climate change can cause the extinction of 30—40% of species [1, 2]. Currently, natural ecosystems are affected by external extreme factors that have been absent in the environment yet. It is due to the explosive development of technologies, as well as significant climatic changes. These changes have contributed to the emergence of ozone holes, increased UV radiation, and new arid areas in the temperate climate zone. Currently, the arid zones make up about 10% of the Earth's landmass and continue to grow [3, 4]. As a result of ozone layer depletion, elevated levels of UV radiation can have a serious effect. Microorganisms have developed mechanisms to protect against damage caused by UV radiation and other abiotic factors that can change even in a stable environment. In addition, microorganisms preferably grow under shelters protecting them from abiotic factors (niches, pores, and cracks in rocky surfaces) [5].

In this work, we studied microorganisms of the ecosystems of the Holosiivskyi National Nature Park. The park is located in the northern part of the forest-steppe zone on the right bank of the Dnipro river. It is also a big scientific and botanical center [6]. Scientific research in the park is conducted to determine the composition of flora and fauna. Numerous investigations of the Holosiivskyi National Nature Park are devoted to the current state and features of the study of the park flora [7]. Thus, according to Onyshchenko [8], 752 species of higher vascular plants, 155 species of mosses, and more than 90 species of fungi were found in the flora of the forest-steppe part of the park in 2016. However, no publications were found that address the study of microbial diversity, composition, ecophysiology as well as hydrolytic activity.

Our previous works have shown the influence of extreme factors on bacteria from different extreme ecosystems and regions such as phytocenoses and ornithogenic soil (Antarctica), littoral and deep samples of the lake Baikal (Russia), phytocenoses and volcanic emissions from the highlands (Ecuador). **The aim** of this work is to isolate the representative microorganisms from terrestrial ecosystems of the Holosiivskyi National Nature Park that are not exposed to extreme factors, to study their ecophysiological properties (resistance to UV radiation, dehydration, hypersalinity, temperature), and to study their extracellular glycoside and proteolytic activities.

Materials and methods. Bacteria isolated from the ecosystems of the Holosiivskyi National Nature Park in 2016 and 2017 were the objects of the research.

The samples were selected in two periods: winter and summer. In winter, the samples were selected from chornozem soil (different depths

of sampling), rotten plant residues, and moss. The samples of soil, mosses, and lichens were collected in summer. Freshly selected samples were used for microbiological studies.

To detect aerobic chemoorganotrophic bacteria, ten-fold dilutions (0.1 mL) were inoculated on a meat-peptone agar medium (MPA). Microorganisms were cultured at 30 °C (up to 4 days). The number of microorganisms was expressed in colony-forming units (CFU) per 1 g of an absolutely dry sample.

Cell morphology was studied by microscopy of live and Gram-stained preparations via standard methods. Spores were detected by the negative staining method and by the Peshkov method [9]. The cells were examined by light microscopy via a microscope Mikmed-2 (LOMO, RF) (×1500).

The temperature range of microbial growth was determined by the cultivation at different temperatures (1 °C, 5 °C, 10 °C, 18 °C, 30 °C, 42 °C) on the MPA nutrient medium.

The halotolerance of microorganisms was determined via the cultivation on an MPA medium containing 1, 25, 50, 100, and 150 g/L of NaCl.

Ultraviolet (UV) resistance was determined as described previously [10].

The resistance to dehydration was detected according to the method described in [11].

To study the glycosidase activity, the cultivation of bacteria was carried out in submerged conditions for 2—4 days in a medium of the following composition, g/L: KH_2PO_4 — 1.6; $MgSO_4 \cdot 7H_2O$ — 0.75; $ZnSO_4 \cdot 7H_2O$ — 0.25; $(NH_4)_2SO_4$ — 0.5; yeast autolysate — 0.15; maltose — 1.0; soy flour — 20.0; pH — 6.0. Xylose (5 g/L) was also used as a carbon source . To reveal the proteolytic activity, the cultivation was carried out under the same conditions on the medium, g/L: KH_2PO_4 — 1.6; $MgSO_4 \cdot 7H_2O$ — 0.75; $ZnSO_4 \cdot 7H_2O$ — 0.25; $(NH_4)_2SO_4$ — 0.5; yeast autolysate — 0.15; maltose — 10.0; gelatin — 10.0; pH — 6.0. The cultivation temperature was 28 °C. After the fermentation, the biomass was separated via centrifugation at 5000 g for 10 min. Enzymatic activities were determined in the culture liquid supernatant.

The of glycosidase activities of the isolates were determined using synthetic nitrophenyl substrates such as *n*-nitrophenyl-a-L-rhamnopyranoside, *n*-nitrophenyl-a- and b-D-glucopyranoside; *n*-nitrophenyl-a- and b-D-galactopyranoside; *n*-nitrophenyl-N-acetyl-a- and b-D-glucosaminide; *n*-nitrophenyl-N-acetyl-b-D-galactosaminide; *n*-nitrophenyl-b-D-glucuronide; *n*-nitrophenyl-a- and b-D-xylopyranoside; *n*-nitrophenyl-a-D-mannopyranoside; p-nitrophenyl-a-D-fucopyranoside (Sigma-Aldrich, United States) [12]. The number of enzymes that hydrolyzes 1 μmol of the substrate per 1 min under the experimental conditions was taken as a unit of enzyme activity.

The β-mannanase activity was assessed by the amount of mannose formed as a result of hydrolysis of the substrate, which was determined by the dinitrosalicylic method. Guar galactomannan was used as a substrate [13]. Mannose was used as a standard. A unit of activity was determined as the amount of the enzyme that promotes the formation of 1 μmol of mannose per 1 min under the experimental conditions.

The α-amylase activity of microorganisms was detected using a potato agar medium. It was assessed via the ratio of the hydrolysis zone diameter to the colony diameter. The level of α-amylase activity was determined by the dinitrosalicylic acid method as the amount of glucose formed during soluble starch (1 %) hydrolysis [13].

Screening for the proteolytic activity was carried out by applying a 1 % casein agar medium. The activity was assessed by the size of casein hydrolysis zones after 24—48 hrs. The total proteolytic activity was quantified by the Anson method modified by Petrova [14]. The gelatinase activity was assessed by the ability to liquefy gelatin. The diameter of the hydrolysis zones was estimated after for 24 hrs incubation on Petri plates with the medium containing 120 g of gelatin/L at 20 °C.

The elastase activity was evaluated by the splitting of elastin congo [15]. A unit of activity was

determined as the amount of enzyme hydrolyzing 1 mg of elastin per 1 hr.

Protein concentration in the supernatant of culture liquid was measured by the method of Lowry [16].

All experiments were performed in at least 3—5 replicates. The statistical analysis of the results was carried out by standard methods using Student's t-test with a 95% confidence level.

Results. The samples to study were selected in two periods: winter (samples 1—6); summer (samples 7—14) (Table 1). There were isolated 37 isolates of aerobic chemoorganotrophic bacteria from them.

The study revealed that the number of isolated bacteria in the summer period by an order outweighed this index for isolates in the winter period. The number of aerobic chemoorganotrophic microorganisms was from $n\times10^4$ to $n\times10^6$ CFU/g of terrestrial biotopes at 30 °C.

From 1 to 4 different morphotypes of colonies were detected at this temperature. The vast majority of isolates (69%) were represented by gram-positive rods. Gram-negative bacteria reached 31%. The proportion of pigmented isolates was 13%, while that of non-pigmented ones was 87%. All isolated bacteria were aerobic and chemoorganotrophic. They did not form mycelium, however, some of them (spore-forming) were filamentous. Some isolates emitted pigments into the medium. It should be noted that filamentous microorganisms have not yet been isolated from any ecosystem before.

For further work, 16 dominating isolates were selected from the isolates (Table 2).

The ecophysiological properties of the selected isolates were investigated. The results revealed 44% of the studied isolates to be capable to grow

Table 1. **Quantitative characteristics of aerobic chemoorganotrophic microorganisms in samples from the Holosiivskyi National Nature Park**

No. of sample	Sample description	Number of morphotypes	CFU/g of sample
	The sampling was conducted in the winter period		
1	Evenly colored chornozem, without roots, collected at a depth of 30-35 cm. Park	1	$(4.8\pm0.2)\times10^5$
2	Chornozem, top layer. Park	2	$(3.8\pm0.1)\times10^5$
3	Chornozem evenly colored, with small roots, collected under a birch at a depth of 4 cm. Park	3	$(1.3\pm0.06)\times10^5$
4	Green moss from a birch, collected at a height of 18 cm. Park	2	$(2.6\pm0.1)\times10^5$
5	Rotten plant residues collected under oak leaves. Park	3	$(4\pm0.3)\times10^5$
6	Green moss from oak collected at a height of 25 cm. Park	2	$(9.3\pm0.6)\times10^4$
	The sampling was conducted in the summer period		
7	Gray soil with lobes of small roots of plants. Meadow	3	$(5.8\pm0.2)\times10^5$
8	Dark gray soil with lobes of small roots of plants. Meadow	4	$(1.6\pm0.05)\times10^6$
9	Green moss with sand and soil. Park	4	$(1.2\pm0.03)\times10^6$
10	Soil with sand under a pear. Park	4	$(1.8\pm0.2)\times10^6$
11	Lichen with sand. The outskirts of the park	3	$(5.5\pm0.3)\times10^5$
12	Moss and lichen with wood particles of acacia. The outskirts of the park	2	$(1.2\pm0.07)\times10^6$
13	Black soil is with sand under nettles. The outskirts of the park	2	$(4.8\pm0.2)\times10^5$
14	Green moss from oak with wood particles. Park	2	$(4.2\pm0.1)\times10^6$

at 1°C, 63% of them grew at 5 °C, and 19% were detected to grow at 42 °C. All isolates grew in the temperature range from 10 to 30 °C (Table 2).

All isolates were shown to grow at NaCl concentration from 0.1 to 25 g/L of the medium. 56% of bacterial isolates grew at NaCl concentration of 50 g/L. No isolates were observed to survive at 75 g/L of NaCl (Table 2).

The effect of UV radiation on the microorganisms of the Holosiivskyi National Nature Park was also studied. 50% of the isolates were shown to remain viable in the range of UV doses from 0 to 1350 J/m^2. Isolates 3g3, 8g1, 8g2, and 8g3 from chornozem and dark gray soil were highly resistant to UV. Their $LD_{99.99}$ ranged from 800 to 1100 J/m^2 (Fig. 1A, Table 2).

Isolates 1g, 4g2, 9g1, and 14g2 from chornozem, collected at a depth of 30-35 cm, birch moss, green moss with sand and soil, and green moss from oak were moderately resistant to UV. The lethal doses of UV ($LD_{99.99}$) ranged from 280 to 650 J/m^2 (Fig. 1A, Table 2). Highly sensitive isolates (50%) were also found. The $LD_{99.99}$ for them was 37—100 J/m^2 (Fig. 1B, Table 2).

Moreover, the resistance of the studied isolates to dehydration was investigated. The founders of the method to study the resistance of bacteria to dehydration V. Mattimore and J.R. Battista [11] suggested that microorganisms are considered to be resistant if the number of survived cells after dehydration is ≥10%.

Both highly resistant and highly sensitive microorganisms were observed (Table 2). The gram-positive isolates (1g, 3g3, 8g1, 8g2, 8g3) were shown to possess the highest resistance to dehydration (from 11.1 to 17.6%). Isolates 8g4, 9g1, and 14g2 showed a resistance close to 10% (from 8.3 to 9.6%). A thin cell wall of gram-negative bacteria could be related to the highest sensitivity of such isolates to dehydration.

The extracellular hydrolytic activity of the UV-resistant isolates was studied as well. The soy flour was shown to promote the induction of the synthesis of glycosidases of bacteria and micro-

Table 2. **Ecophysiological properties of the studied microorganisms**

No. of isolate	Cell staining	Description of the sample	Temperature, °C	NaCl, g/L	% of cells survived after dehydration	$LD_{99.99}$ UV, J/m^2
1g	Gr+	Chornozem	10—42	0.1—50	11.1 ± 0.5	550
3g1	Gr+	Chornozem	5—30	0.1—50	4.5 ± 0.1	55
3g2	Gr+	Chornozem	5—30	0.1—50	1.8 ± 0.006	48
3g3	Gr+	Chornozem	10—30	0.1—50	12.2 ± 0.7	800
4g1	Gr-	Moss from birch	1—30	0.1—25	2.2 ± 0.06	60
*4g2	Gr+	Moss from birch	1—30	0.1—25	6.5 ± 0.2	450
6g1	Gr-	Moss	1—30	0.1—50	1.1 ± 0.002	70
*8g1	Gr+	Dark gray soil	10—30	0.1—25	12.7 ± 0.4	800
8g2	Gr+	Dark gray soil	10—42	0.1—50	16.1 ± 0.7	1100
8g3	Gr+	Dark gray soil	10—42	0.1—50	17.6 ± 0.5	900
8g4	Gr-	Dark gray soil	1—30	0.1—50	8.5 ± 0.4	50
*9g1	Gr+	Moss with sand	5—30	0.1—25	9.6 ± 0.3	650
9g2	Gr+	Moss with sand	1—30	0.1—25	1.0 ± 0.005	100
9g4	Gr-	Moss with sand	1—30	0.1—25	0.7 ± 0.003	58
12g2	Gr-	Moss and lichen	1—30	0.1—25	0.9 ± 0.002	37
14g2	Gr+	Moss	10—30	0.1—50	8.3 ± 0.4	280

* Spore-forming isolates

mycetes [17, 18]. Therefore, it was added to the medium in all treatments. The studied isolates showed a wide range of glycosidase activity on maltose (Fig. 2A). Xylose contributed to the appearance of glycosidase activity of only two isolates (Fig. 2B). However, they revealed its lower level as compared to that on maltose. It should be noted that the replacement of the carbon source in the medium caused changes in the enzyme activity. All isolates showed from 5 to 7 glycosidase activities. Six of seven isolates showed the α-amylase activity (Fig. 2A). Isolates 8g3 and 9g1 secreted a complex of four cellulose-degrading enzymes. In addition, isolate 9g1 showed high β-xylosidase activity on maltose.

The proteolytic activity of the isolates was also studied. All of them possessed the ability to hydrolyze casein growing on Petri plates as well as submerged in the medium (Fig. 3A, B). The four most active isolates also showed gelatinase activity. Elastase activity was not observed.

In general, the high hydrolase activity of freshly isolated soil bacterial isolates was noted.

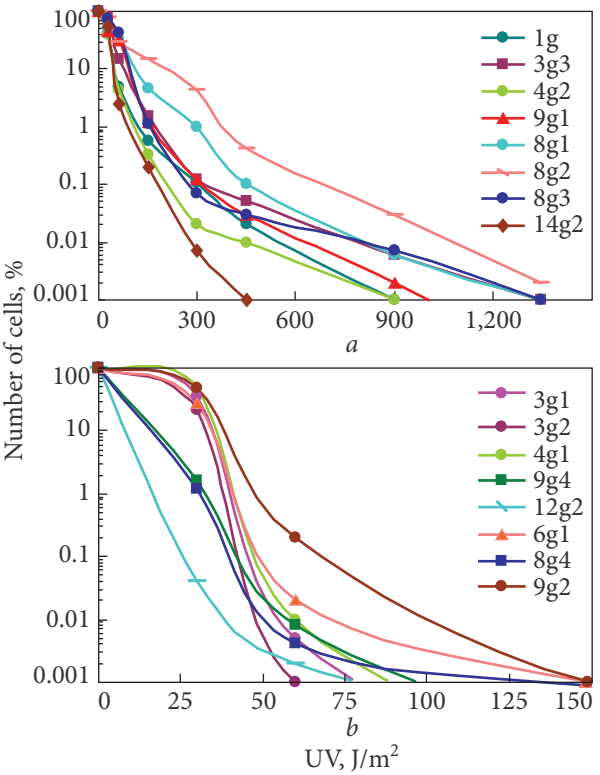


Fig. 1. The sensitivity of bacteria to UV radiation

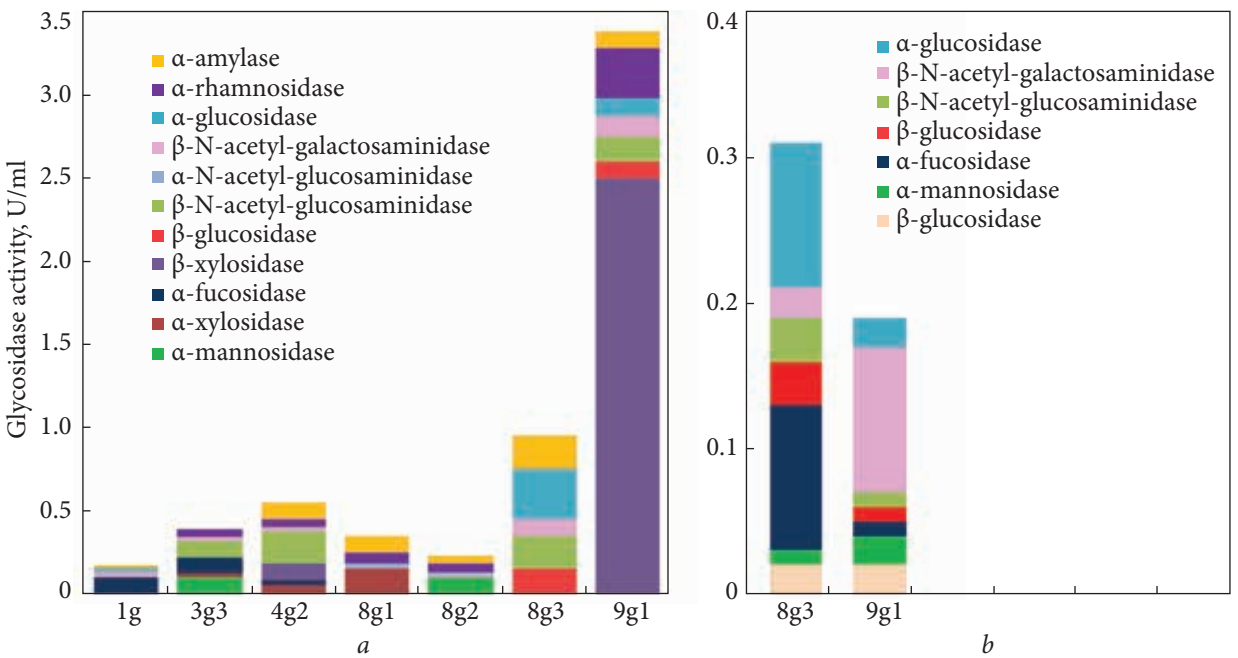
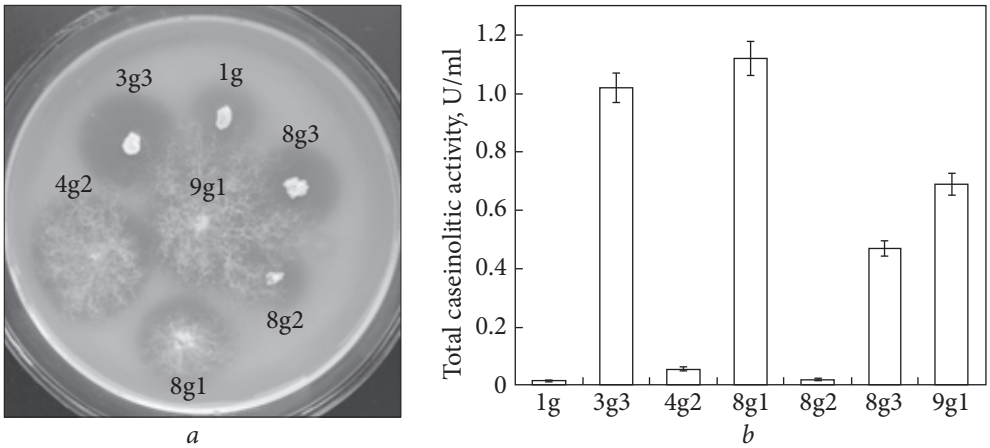


Fig. 2. The range of glycosidase activity of bacteria: *a* — medium with maltose, *b* — medium with xylose; cultivation for 48 hr 28 °C



a *b*

Fig. 3. Proteolytic activity: *a* — zones of hydrolysis of casein in agar; *b* — the total proteolytic activity of bacteria in the culture liquid supernatant

Discussion. Microbial diversity is a component of any ecosystem. Particularly, it is a necessary component of soil. Soil, in turn, is a natural habitat for microorganisms that participate in soil formation and provide the circulation of substances in the biosphere [19]. The diversity of microbial species and their number are determined by soil conditions: temperature, humidity, aeration, pH, availability of nutrients, the type of vegetation cover, etc. [20, 21]. The largest number of microorganisms was reported to be observed in moist soil ($4.2 \cdot 10^9$—$5.2 \cdot 10^9$ CFU/g). The smallest number was observed in forest soil and sand soil ($0.9 \cdot 10^9$—$1.2 \cdot 10^9$ CFU/g). The richest microbiome was found in the upper soil layer (2.5—15 cm), where the largest supply of organic nutrients was concentrated [20]. In addition, the total number of microorganisms in soil varies not only during the year but also for short periods of time depending on soil conditions [21]. We have isolated 37 isolates of aerobic chemoorganotrophic bacteria from various samples collected in the Holosiivskyi National Nature Park. The sampling was carried out in winter and summer to study the conditions closer to the extreme and to isolate microorganisms resistant to abiotic environmental factors. It should be noted that the sampling in summer was carried out during the extremely hot and dry months when the rainfall was significantly below normal. That could affect the number of microorganisms. In winter, the sampling was carried out when the thawing of the surface layer of soil started. The total number of aerobic chemoorganotrophic bacteria in these samples was shown to be one order higher compared to the samples collected in summer [22]. That corresponds to the data confirming that the number of bacteria decreases significantly in summer in the dry period in the southern regions on non-irrigated soils [21]. It was noted that long periods of drought caused a sharp change in the metabolic activity and the diversity of microorganisms [2, 23].

Most of the isolated bacteria of Holosiivskyi National Nature Park (62%) were shown to be psychrotolerant. Such microorganisms, as a rule, dominate in environments affected by thermal fluctuations (for example, daily or seasonal). As for mesophiles, they grow at 20—40 °C, but they are also able to withstand low temperatures [24, 25].

The studied isolates (56%) were moderately halophilic bacteria capable of withstanding 5% NaCl in the nutrient medium. In addition, most of the isolates growing in the medium with 5% NaCl were resistant to other environmental

factors (UV radiation, dehydration) (Table 2), which is typical for halophiles [26].

High doses of UV radiation damage DNA. Therefore, they are often lethal to most biological objects. In addition, UV radiation can cause DNA mutations, oxidative stress, and protein denaturation [27]. The tolerance to UV radiation of the studied isolates was studied by their irradiation for 1—45 min (30—1350 J/m^2). Current studies show that natural microorganisms that have not been exposed to stress have shown extreme resistance to UV radiation. Since microorganisms were not isolated from sunlight in natural conditions, it was expected that at least some of the studied isolates could be resistant to UV. It should be noted that the number of resistant microorganisms to UV radiation was 50%. As reported [28], resistance to UV radiation was observed in natural soil bacteria that were isolated from stress-free environments. These studies were conducted in the Allegheny National Forest (Pennsylvania, USA) in August. The obtained results show that gram-positive bacteria, as a rule, are more resistant to UV radiation than gram-negative, even if they have been isolated from the same type of habitats [29].

A low frequency of pigmented bacteria detection was observed in the studied samples. According to the literature data, pigmentation correlates with the survival of bacteria under the environmental stress factors. It is part of one of the mechanisms of counteraction to damage caused by UV radiation [30]. In the absence of pigments, microorganisms implement other protection systems. In our study, no significant difference between the ability of pigmented and non-pigmented bacteria to withstand UV radiation was observed. It contributed that UV resistance does not depend on pigmentation. We found that most of the highly resistant isolates were unpigmented isolates, some of which were spore-forming. The minimization of damage can also be achieved through spores. The species living within one consortium were suggested to have common mechanisms of UV resistance [31]. Comparing the resistance to dehydration and UV radiation, the correlation between the resistance of the studied bacteria and these factors was shown. Obtained results were also confirmed by the literature [32].

Resistance to intense stress can serve as an indicator of the physiological activity of a microorganism [33]. Therefore, the extracellular glycosidase and proteolytic activity of some isolated bacteria were studied. The spectrum of the studied activities showed the presence of a whole complex of hydrolyzing enzymes in bacteria. Cellulose degrading (α- and β-glucosidase, α- and β-N-acetyl-glucosaminidase, β-N-acetyl-galactosaminidase), hemicellulase (α- and β-xylosidase), α-amylase, and proteolytic activities were observed, which may indicate the active role of these bacteria as soil decomposers. In addition, the resistance of these bacteria to stress factors suggests that they have enzymes with increased stability [34, 35]. As a result of the study, a new bacterial culture, 9g1, with a high degrading and especially β-xylosidase activity, was selected. This strain can be used in demand as a biodegrading agent in technologies for processing plant materials.

Thus, the properties of the studied bacterial communities indicate that microorganisms are resistant to extreme environmental factors even in natural ecosystems with a temperate climate that was shown by the example of the Holosiivskyi National Nature Park. Studies of the ecophysiological characteristics of bacteria have shown that most of them are psychrotolerant, moderately halophilic, and resistant to high doses of UV radiation and dehydration. The resistance of microorganisms to these factors is assumed to be formed under the effect of abiotic (physicochemical) factors typical to this region.

No data were found in the available literature defining the hydrolytic activity and levels of resistance of microorganisms of the temperate regions of Ukraine to extreme factors. Obtained

experimental data provides the base to fill in the gap in this knowledge to get a deeper insight in the microbial metabolic activity and resistance to extreme factors. The effective functioning of the microbiome is one of the key factors that provide the sustainable function of the ecosystem in general. Understanding the range of microbial resistance contributes to the opportunity to save the environment. The conducted work resulted in the characterization of microbial representatives of the valuable natural complex contributing to the study of the pathways for the preservation of the natural resources of Ukraine. Moreover, new bacterial strains with high degrading activity were isolated. Their further study can contribute to the development of effective biotechnological approaches in the field of environment protection, etc.

Conclusions. The high resistance to UV radiation and dehydration of non-adapted microorganisms as well as the wide range of exohydrolase activity indicate the wide adaptive capacity of microorganisms from natural ecosystems, which goes beyond the influence of surrounding factors.

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Г.В. Гладка[1], Н.В. Борзова[1], О.В. Гудзенко[1], В.М. Говоруха[1], О.А. Гаврилюк[1], О.В. Шаблій[1], Л.С. Ястремська[2], О.Б. Таширев[1]

[1] Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

[2] Кафедра біотехнології, факультет екологічної безпеки, інженерії та технологій, Національний авіаційний університет, Київ, 03058, Україна

ЕКОФІЗІОЛОГІЧНІ ВЛАСТИВОСТІ ТА ГІДРОЛІТИЧНА АКТИВНІСТЬ ХЕМООРГАНОТРОФНИХ БАКТЕРІЙ НАЦІОНАЛЬНОГО ПРИРОДНОГО ПАРКУ «ГОЛОСІЇВСЬКИЙ»

Мікроорганізми є складовою будь-якої екосистеми, проте різні види антропогенного впливу можуть змінювати умови ґрунтово-рослинної біоти, впливати на їх кількість та фізіологічні властивості. **Метою** дослідження було виділити типові мікроорганізми з наземних екосистем Національного природного парку «Голосіївський» (Україна), які не піддаються дії екстремальних факторів, дослідити їх екофізіологічні влас-

тивості (резистентність до УФ опромінення, дегідратації, гіперсолоності, температури), а також вивчити позаклітинну глікозидазну та протеолітичну активності. **Методи.** Об'єктами дослідження були аеробні хемоорганотрофні бактерії, ізольовані за температури 30 °С з ґрунту і фітоценозів Національного природного парку «Голосіївський». Для культивування бактерій використовували м'ясо-пептонний агар. УФ опромінення бактерій проводили лампою БУФ-15 (λ = 254 нм) в діапазоні 30—1350 Дж/м^2. Температурний діапазон росту і галотолерантність мікроорганізмів визначали відповідно в діапазоні 1—42 °С і 0.1—150 г NaCl/л. Для дослідження ензиматичної активності культури вирощували у глибинних умовах за температури 28 °С протягом 4 діб. Для визначення глікозидазної активності використовували синтетичні *n*-нітрофенільні субстрати, розчинний крохмаль, галактоманан гуару, для протеолітичної — казеїн, еластин та желатин. **Результати.** З 14 ґрунтових і рослинних зразків Національного природного парку «Голосіївський» ізольовано 37 штамів аеробних хемоорганотрофних мікроорганізмів. Кількість бактерій, виявлених у зимовий період, становила від $9.3 \cdot 10^4$ до $4.8 \cdot 10^5$ КУО, у літній період — $4.8 \cdot 10^5$ до $4.2 \cdot 10^6$ КУО/г зразків наземних біотопів, представлених 1—4 морфотипами. У даній роботі 69% штамів представлено грампозитивними паличками. Не виявлено домінування пігментованих штамів у культивованих спільнотах. Більшість досліджених мікроорганізмів є психротолерантними та помірними галофілами. Високу резистентність до УФ опромінення показали штами 3g3, 8g1, 8g2, 8g3, ізольовані з чорнозему, відібраного під березою та темно-сірого ґрунту, для яких $LD_{99,99}$ становила від 800 до 1100 Дж/м^2. Помірно резистентними були штами 1g, 4g2, 9g1, 14g2, ізольовані з чорнозему, відібраного на глибині 30—35 см, моху з берези, моху зеленого з піском і грунтом та з моху зеленого з дуба, летальні дози УФ ($ЛД_{99,99}$) для яких складали від 280 до 650 Дж/м^2. Показано, що УФ резистентність не залежить від пігментації та корелює з дегідратацією. Явище стійкості досліджених мікроорганізмів до таких факторів як УФ випромінювання та дегідратація свідчить про наявність у них активних механізмів репарації пошкоджень ДНК. Всі культури проявляли целюлозо- та геміцелюлозодеградувальну активність, а також гідролізували казеїн при вирощуванні на середовищі з мальтозою та соєвим борошном. Для штаму 9g1 показано високу β-ксилозидазну активність. **Висновки.** Показано, що природні мікроорганізми, які існують в умовах відсутності УФ-стресу, мають високу стійкість до УФ опромінення і дегідратації. Всі культури демонстрували широкий спектр екзогідролазної активності. Вивчені екофізіологічні властивості бактерій, ізольованих з ґрунтів і фітоценозів Національного природного парку «Голосіївський», вказують на широкі адаптаційні можливості цих мікроорганізмів, які виходять за межі дії оточуючих їх факторів. У доступній літературі немає даних, які б визначали гідролітичну активність та резистентність мікроорганізмів помірного регіону України до екстремальних факторів. Отримані експериментальні дані дозволять краще зрозуміти рівень резистентності мікроорганізмів помірних регіонів до екстремальних факторів. Також було виділено нові бактерії з високою деградувальною активністю. Досліджені ізоляти потребують подальшої характеристики та аналізу для застосування у біотехнологічних процесах.

Ключові слова: *хемоорганотрофні бактерії, УФ резистентність, психротолерантність, галофільність, глікозидазна та протеолітична активності.*