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MICROBIAL DIVERSITY IN TERRESTRIAL ANTARCTIC BIOTOPES

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Abstract. It is established, that the total number of chemoorganotrophic aerobic microorganisms in a soil, sludge of reservoirs, on a grass, mosses and lichens on islands Galindez, Skua, Corner, Barchans, Irizar, Uruguay, Jalour, Petermann, Berthelot, Cruls, Three little pigs, King-George made 10^5-10^8 cells/g of a sample that is less on 2-3 order, than in regions with a temperate climate. From samples of moss, lichen, grass, soil are isolated methylotrophic bacteria (10^2-10^4 cells/g of a sample). It is shown, that in the Antarctic lichens frequency of an occurence of coal-black yeast and also their total number is significantly higher (10^2-10^4 cells/g of sample), than in other Antarctic biotopes. Coal-black pigments are extracted from Antarctic yeast *Exophiala nigra*. These pigments are identical to melanin according to results of studying of specific chemical tests, UV spectra and other properties. For the first time presence on vertical rocks in Antarctic Region of bacteria and yeast, resistant to high dozes UV radiations is shown. The lethal doze of UV radiation for Antarctic pink pigmented strains *Methylobacterium* exceeded 200–250 J/m^2, for coal-black yeast – 500–800 J/m^2, for red-pigmented yeast – 1200–1500 J/m^2. In whole, in the Antarctic Region representatives of several phylogenetic lines are found out: *Proteobacteria*, *Firmicutes, Actinobacteria,* and also yeast. Obtained results indicate to a taxonomic diversity of microorganisms in terrestrial biotopes of Antarctic Region.
Key words. Antarctic Region, the Antarctic microorganisms, the Antarctic yeast, UV irradiation.

1. Introduction

Separate geographical location of islands of Antarctic Region, ozone "hole" as a result of which the high level UV radiation is present in Antarctica, and also low temperature have formed a unique microcosm in Antarctic Region. Despite of existence of numerous publications about microorganisms in Antarctic Region, they do not give an opportunity to estimate to the full a microbial diversity in this unique geographical zone, in particular, in its terrestrial biotopes. The purpose of work - to estimate a diversity chemoorganotrophic aerobic microorganisms in terrestrial biotopes of Antarctic Region.

2. Methods

Samples for microbiological researches collected by standard methods in Antarctic terrestrial biotopes. Microorganisms isolated from native samples (storage at +5°C, 10 days) and frozen samples (storage at –20°C, 30 days). Total number of microorganisms in samples defined by crop of tenfold dilutions of samples on media: NA – «Nutrient Agar» (company HiMedia Laboratories Pvt. Ltd.), WA – a wort agar and mineral medium of MM [Romanovskaya et al, 1996] with a methanol (0,5%). Pure cultures were isolated by standard methods. Identification of microorganisms up to a genus carried out on the basis of studying their morphological and cultural and some physiological properties by methods resulted in a manual [*Methods* of the general bacteriology, 1983], and also using the sequence-analysis of genes 16S rRNA as it is described by us earlier [Romanovskaya et al, 2004]. Production microbial melanins from cellular biomass carried out by alkaline extraction [Ruban, 1969]. Spectra of pigments determined on Specord UV VIS. Survival of microorganisms after UV

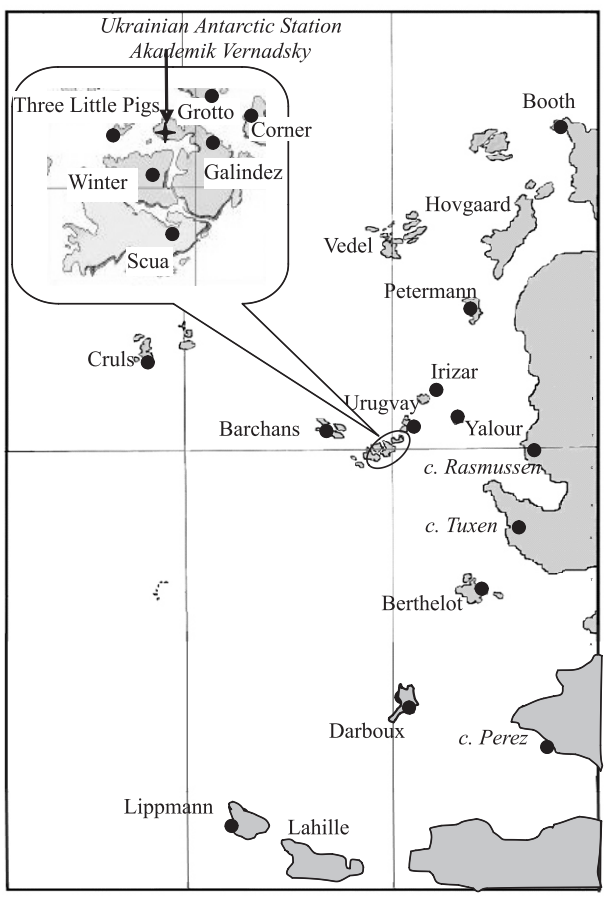
irradiations, and also a lethal doze UV, determined, how is described by us earlier [Romanovskaya et al, 1999].

3. Results

The microbiological analysis of 120 samples of terrestrial biotopes (a soil, a grass, mosses, lichens and lake sludge) the western coast of Antarctic peninsula (cape Rasmussen, cape Tuxen, mountain Waugh), islands of the Argentina archipelago and also some other islands (Galindez, Skua, Corner, Barchans, Irizar, Uruguay, Jalour, Petermann, Berthelot, Cruls, Three little pigs, King-Georg) has been carried out (Fig. 1).

3.1. Taxonomic diversity of microorganisms in terrestrial biotopes of Antarctic Region

It is established, that the total number of chemoorganotrophic microorganisms made 10^5-10^9 cells/g of sample that is less on 2-3 order, than in regions with a temperate climate. The tendency of decrease of quantity chemoorganotrophic microorganisms in Antarctic biotopes in such order is observed (cells/g of sample): soil ($1x10^7$ - $8x10^8$), underground part of moss ($1x10^6$ - $5x10^7$), grass *Deschampsia antarctica* (10^6-10^8), lake sludge (10^5-10^7), ground part of moss (10^3-10^6), lichens (10^3-10^6). On the basis of studying diagnostic criteria of the Antarctic microorganisms it is shown, that some from them concern to genera *Bacillus, Actinomyces, Streptomyces, Pseudomonas, Methylobacterium, Enterobacter, Staphylococcus, Brevibacterium*. So, in the Antarctic samples have been found out representatives of several phylogenetic lines: *Proteobacteria*, *Firmicutes, Actinobacteria*.



Obtained results indicate to a taxonomic diversity of microorganisms in terrestrial biotopes of Antarctic Region. It is not revealed significant distinctions concerning quantitative structure of a microflora in the investigated islands. On a tentative estimation, despite of geographical disconnetion of islands of Antarctic Region and specific climatic conditions, the majority of isolated microorganisms concern to traditional classical taxons, widespread in various regions of the Earth characteried by temperate climate conditions.

Fig. 1. The map-scheme of the western coast of Antarctica with adjoining islands. In the left top part of the map-scheme increased in several times islands of the Argentina archipe-lago (in particular, island Galindez on which Ukrainian Antarctic st. "Acad. Vernadsky" and biogeographical polygon is located) is shown. Circles show places of sampling.

3.2. Occurrence of methylotrophic bacteria in terrestrial Antarctic biotopes

Practically there are no data on existence in Antarctic Region of methylotrophic bacteria (methylotrophs) which are capable to use a methanol as a unique source of carbon and energy. These researches are small and do not give complete representation about distribution methylotrophs in such unique geographical zone, as Antarctic Region. Therefore we studied their occurrence to the given region. From samples of moss, lichen, grass, soil in Pacific sector of Antarctic Region are isolated methylotrophic bacteria (Tab. 1), their total number made 10^2-10^4 cells /g of sample.

On the basis of diagnostic properties the isolated pink pigmented strains, facultatively using a methanol and realizing a serine cycle of an assimilation of single-carbon compounds, are identified as strains of genus *Methylobacterium.*

Table 1

Quantity of samples from Antarctic terrestrial biotopesin which methylotrophic bacteria of genus *Methylobacterium* (% from quantity of the studied samples of each type of an ecosystem) have been revealed

Biotope	Number of samples		
	Total number	In which are revealed methylotrophs	
		Number	% from number
Moss	25	22	88
Lichen	15	10	66
Grass	10	8	80
Soil under plants	8	7	87
Soil without plants	12	2	16
Sludge	10	3	30

Strains *Methylobacterium* often live on mosses, grass *Deschampsia antarctica* and lichens, and less often in a soil and lake sludge. Some Antarctic islands are comparable on number of cells *Methylobacterium* to regions characteried by temperate climate conditions. The analysis sequences of genes 16S rRNA of Antarctic methylotrophic bacteria with those of other bacteria in database GenBank has shown a high level of similarity to species *Methylobacterium extorquens* (99.4-99.7%).

In general, utilization of a methanol in ecosystems of Antarctic Region, undoubtedly, has great ecological importance as protects an atmosphere from pollution by this toxic compound. Moreover, as we have shown, strains *Methylobacterium* are capable to decrease temperature of freezing of water, i.e., in the nature they influence process of ice crystallization. These properties of strains *Methylobacterium* are of great importance for a survival of biota in Antarctic Region.

3.3. Screening of yeast-producers of melanin in terrestrial Antarctic biotopes

In recent times it is considered, that melanin it is perspective for application in medicine and pharmacology. In connection with that earlier black yeast have been found out in polar regions, we carry out screening a yeast-producers of melanin in Antarctic biotopes. Dark pigmented microorganisms are revealed in 20 % of samples of Antarctic terrestrial biotopes (Tab. 2).

Coal-black yeast are found out on crustose and bushy lichens on vertical rocks, also in soil less often, their quantity made $1x10^2$ - $6x10^4$ cells/g of sample. This Antarctic yeast are close to species

Exophiala nigra (Issatsch.) Haase et de Hoog 1999. From a biomass of two strains of black yeast we have extracted coal-black pigments.

These pigments are identical to melanin according to results of studying of specific chemical tests, UV spectra (220-230 nm) and spectra of absorption in seen (400-800 nm). The quantity of a synthesized melanin /g of yeast biomass made more than 10 %. So, we have revealed in terrestrial Antarctic biotopes the yeast intensively synthesizing melanin.

3.4. Resistance to UV radiation of the microorganisms isolated from rocky biotopes of Antarctic Region

We have assumed, that vertical rocks on islands of the Antarctic Region, practically always open for the Sun, should be characterized by the microorganisms, resistant against UV radiation. Therefore we have studied resistance of rocky Antarctic microorganisms to UV irradiation.

Table 2

Quantity of the Antarctic samples in which darkly pigmented microorganisms (% from quantity of the studied samples of the certain биотопа) have been revealed

Biotope	Number of samples		
	Total number	In which are revealed darkly pigmented microorganisms	
		Number	%
[1] Black lichens	15	5	33
[2] Mixture of lichens	10	2	20
[3] Grey and green lichens	10	2	20
Moss	10	2	20
Grass *Deschampsia antarctica*	10	1	10
Soil	10	1	10
Silt	5	0	0

Note.
[1] Black crustose lichens on a vertical rock (island Galindez).
[2] Several species of lichens: a villous filamentous lichen, a bushy lichen on a rock (island Galindez).
[3] Different ecological groups of lichens of the grey and green color, located on mosses and stones (islands Lippmann, Darboux, Three little pigs).

Study of rocky Antarctic samples has shown that the pigmented microorganisms are widely distributed there. Frequency of their occurence, and also total number and a biodiversity significantly higher, than in others Antarctic biotopes. For the first time presence on vertical rocks in Antarctic Region of bacteria and yeast, resistant to high dozes UV radiations (Fig. 2 - 4) is shown.

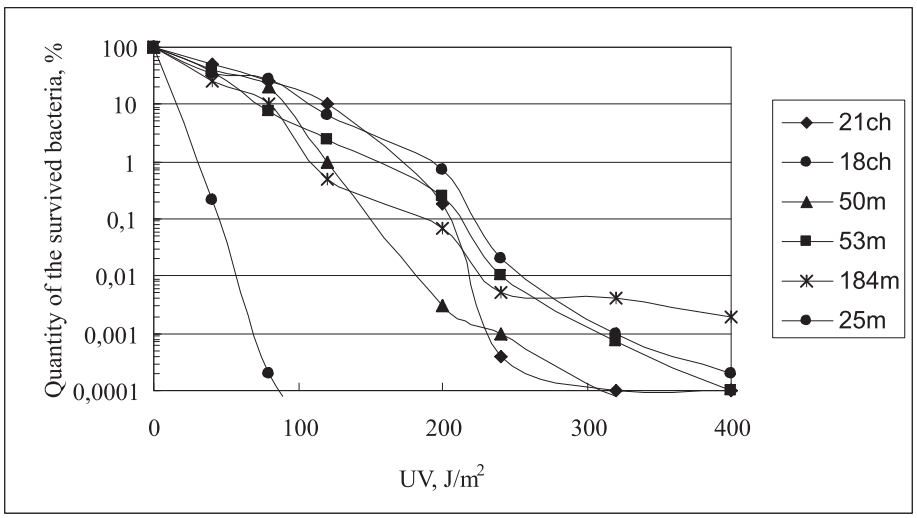


Fig. 2. Survival of strains *Methylobacterium* at various dozes UV irradiations.

It is not revealed distinctions concerning lethal effect UV at strains *Methylobacterium* isolated from regions with various climate. In comparison it is necessary to note, that these dozes UV significantly exceed maximum permissible dozes for many microorganisms.

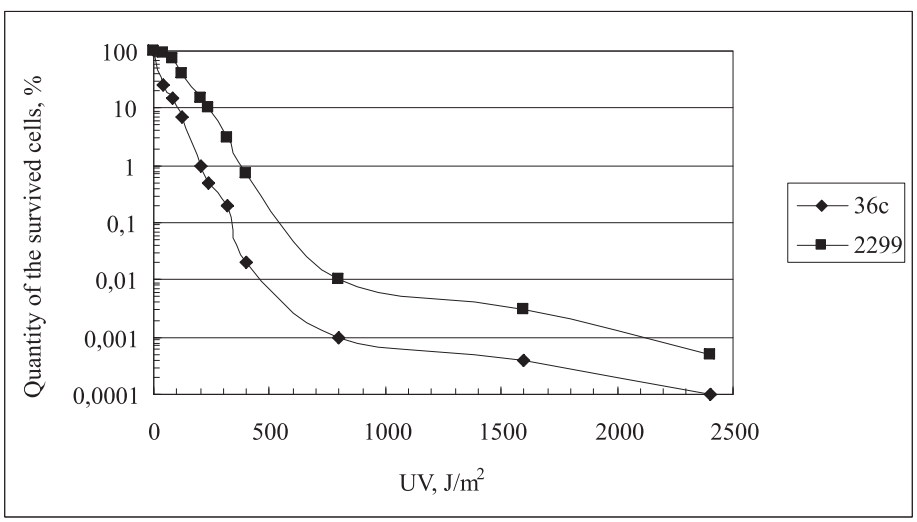


Fig. 3. Survival of Antarctic coal-black yeast *Exophiala nigra* at various dozes UV irradiations.

Lethal UV irradiation doze at which there are 99,99 % cell death, for Antarctic pink pigmented strains *Methylobacterium* exceeded 200-250 J/m^2 (Fig. 2), for coal-black yeast – 500-800 J/m^2 (Fig.3), for red yeast – 1200-1500 J/m^2 (Fig. 4).

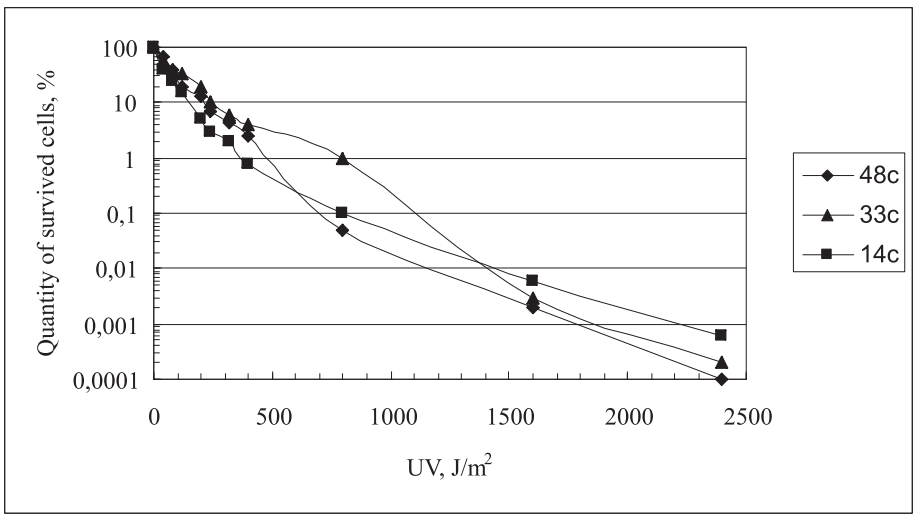


Fig. 4. Dose-dependent curve survivals of the Antarctic red yeast after UV irradiations.

Probably, adaptation of the Antarctic microcenosis to extreme factors of an environment was carried out as a result of natural selection from rocky microcenosis those microorganisms at which resistance to these factors is genetically determined. Therefore in rocky Antarctic ecosystems those microorganisms adapt to change of conditions of an environment (from number both allochthonic and autochthonic microorganisms) which are capable to realize the certain strategy of survival, for example, have mechanisms of a resistance to a high level of solar radiation and/or are protected by pigments.

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