NON-PIGMENTED ANTARCTIC YEASTS AND THEIR RESISTANCE TO TOXIC METALS

Despite the key role in biogeochemical processes and in the functioning of terrestrial ecosystems, yeasts of Antarctic regions still remain insufficiently studied. The study and analysis of the composition of Antarctic microbial communities remains relevant and is carried out using molecular biological approaches. The investigation of their resistance to toxic metal ions is essential to select industrially promising strains that can contribute to the development of new methods of metals detoxification via microorganisms. **Aim.** To determine the taxonomic position of non-pigmented Antarctic yeasts and investigate their resistance to toxic metal ions. **Methods.** The objects of the research are yeasts isolated from Antarctic phytocenoses. They were grown on malt wort (pH 5.0—5.5, temperature 18—20 °C). Isolation of genomic DNA was performed via the commercial DNA-sorb kit. Amplification of DNA was carried out using primers NL1 and NL4. Phylogenetic analysis was conducted by construction of trees (dendrograms) showing the position of the studied strains among closely related and typical species. The resistance of yeasts to toxic metal ions was established by cultivation in the concentration gradient of Ni²⁺, Co²⁺, CrO₄²⁻, and Cu²⁺. The ecophysiological traits of the isolated yeast strains including psychro- and halotolerance were determined. **Results.** Phylogenetic analysis showed a high percentage of similarity (99.5—99.6%) of sequences of 18S rRNA genes of Antarctic yeast strains with the yeast sequences from the GenBank database. Psychrotolerant and halotolerant Antarctic yeast strains S11 and S12 were identified as Leucosporidium scottii and Debaryomyces hansenii, respectively. The studied yeast strains were found to be the most resistant to metal ions Ni²⁺ and Co²⁺. Strain of L. scottii S11 grew at 800 mg/L of Co²⁺, and D. hansenii S12 — at 750 mg/L of Ni²⁺. The yeasts were the least resistant to CrO₄²⁻: the L. scottii S11 and D. hansenii S12 strains grew at concentrations of 25 mg/L and 150 mg/L, respectively. In the presence of Cu²⁺, they grew at the same concentration — 600 mg/L. The combined action of toxic metal ions resulted in the increased toxic effects on the studied yeasts. **Conclusions.** The nucleotide sequences of 18S rRNA gene fragment of yeast strains S11 and S12 were included in the GenBank database under the numbers.
The study of microorganisms of Antarctic ecosystems has become especially relevant due to global climate changes. Antarctica is a geographically isolated polar region. It is a remote and complex territory of the Earth (Tian et al., 2017) with low temperature, repeated freeze-thaw cycles, low nutrient availability, dehydration, high UV radiation, and the absence of flowering plants (Singh et al., 2018; Schultz & Rosado, 2019). Such climatic conditions led to the formation of bioce- noses with the dominance of certain groups of microorganisms in the unique geographical area. Microorganisms able to exist in harsh or stressful natural conditions are called extremophiles. Most microorganisms are highly sensitive to various stress factors (low temperatures, UV radiation, presence of various pollutants, etc.) (Gupta, 2015). Therefore, the selection and characterization of promising extremophilic microorganisms is of great interest for both science and industry.

The presence of microorganisms associated with plants is common for Antarctica like for the other climatic regions of the Earth. They form the basis for various groups (bacteria, fungi, and yeasts) in the phyllo-, rhizo- and endosphere of plants (Vasileva-Tonkova et al., 2014). Mosses are an important component of Antarctic terrestrial vegetation. They create microniches providing a stable thermal and hydrothermal regime for microorganisms (Kachalkin et al., 2008). Lichens are the most abundant organisms in the Antarctic regions, whereas mosses, liverworts, and two vascular plants Deschampsia antarctica and Colobanthus quitensis are less frequent (Hebel et al., 2012; Robinson et al., 2003).

Despite the key role in biogeochemical processes and in the functioning of terrestrial ecosystems, yeasts of Antarctic regions still remain insufficiently studied. Antarctic microorganisms attract attention because they have unique properties and adaptation mechanisms (evolutionary, physiological, biochemical). They provide resistance of microorganisms to the influence of low temperatures, freeze-thaw cycles, high ultraviolet radiation, low nutrient content, and other factors (Tian et al., 2017; Santiago et al., 2015). Though, yeasts are a versatile group of eukaryotic microorganisms that can survive in a wide range of ecosystems and geographic regions. The research on yeasts that colonize terrestrial ecosystems in Antarctica has received much less attention than that on bacteria (Buzzini et al., 2012; Carrasco et al., 2012; Gomes et al., 2019). Yeasts were isolated from samples of ornithogenic (penguin guano) soil, Deschampsia antarctica rhizosphere, sea water, ice, snow, sea and lake sediments as well as fresh water and lakes. They were assigned to genera Bullera, Exophiala, Cryptococcus, Rhodotorula, Debaryomyces, Leucosporidium, Candida, Dioszegia, Mrakia, Sporobolomyces, Glaciozyma, Malassezia, Saccharomyces, Clavispora, etc. (Rosa et al., 2019; Białkowska et al., 2017; de Menezes et al., 2019; Zhang et al., 2013).

Though Antarctica is one of the planet’s most untapped extreme regions, there are reports on the presence of contaminants including heavy metals, pesticides, and antibiotics that may have been transported by natural air and water flows, and improper waste disposal at research stations (Tomova et al., 2015; Corsolini, 2009). Metals in trace concentrations are important elements for living organisms. When their concentration increases, metals negatively affect metabolism of microorganisms. Researchers isolated and characterized various genera of yeasts resistant to toxic metals: Rhodotorula, Debaryomyces, Candida, Cryptococcus, Saccharomyces, Hansenula, Pichia, etc. (Kan et al., 2019; Gumá-Cintrón et...
In general, Ascomycota yeast species were more tolerant to heavy metals than Basidiomycota ones (Vadkertiová & Sláviková, 2006). However, the resistance of Antarctic yeasts to several metals present in the environment simultaneously has not been fully researched. This paper presents the results of determining the species affiliation of two non-pigmented yeast strains that colonize the terrestrial ecosystems of Antarctic phytocenoses. Also, the work shows their resistance to the most spread toxic metals (Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$, CrO$_4^{2-}$).

**Materials and Methods.** Two yeast strains (S11 and S12) isolated from moss samples collected during the Antarctic research expedition were the objects of the research. Moss samples were hermetically packed in sterile plastic containers and transported to the laboratory where they were stored at -20 °C. Malt wort agar (MW A) was used for yeast cultivation. Incubation was performed at 18—20 °C. The isolated strains are stored in the Collection of Extremophilic Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, on MW A (pH 5.0—5.5) at a temperature of 10 °C.

The PCR amplification of 18S rRNA. Yeast DNA was extracted using the kit Dr. GenTLE TAKARA BIO INC. according to the manufacturer’s instructions. The purity of DNA was determined spectrophotometrically via absorption at a wavelength of 260, 230, and 280 nm as well as using horizontal agarose gel electrophoresis. Visualization of electrophoresis results was carried out by a Fisher Bioblock Scientific UV transilluminator. Amplification of 18S rRNA genes was carried out using primers NS3 (5’—3’: GCAAGTCTGGTGCCAGCAGCC) and NS6 (5’—3’: GCATCACAGACCTGTTATTGCCTC) (George et al., 2016). The following PCR mode was used: in the first cycle, denaturation at 95 °C for 5 min, then 30 cycles: denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 90 s. In the last cycle, the elongation time was increased to 7 min. Amplification was carried out in a thermal cycler BIS-H. Nucleotide sequences were determined on an automated sequencer ABI310A (ABI PRISM 310 Genetic Analyzer).

Phylogenetic analysis. Obtained nucleotide sequences of yeast isolates were compared with the sequences of 18S rRNA genes deposited in the GenBank database using software packages BLASTN 2.2.18+ [http://blast.ncbi.nlm.nih.gov/Blast.cgi] and ClustalX 2.1 [http://www.clustal.org]. Alignment of 18S rRNA gene sequences was performed using the BioEdit [http://bioedit.software.informer.com/]. The construction of phylogenetic dendrograms based on the comparison of 18S rRNA genes was carried out via various algorithms implemented in the TreeView and ClustalX 2.1 software packages. The statistical reliability of the branching was evaluated by the bootstrap analysis of 1000 alternative trees.

Toxic metal resistance tests. The effect of toxic metals was studied on malt wort agar (MWA). Metals salts were dissolved in distilled water and sterilized in a boiling water bath for 10 min. The solutions were added to the melted and cooled to 45 °C medium. The concentration of stock solutions of metals was 40 g/L cation (calculated per metal ion).

The resistance of yeast to toxic metals ions was determined via two variants of the experiment. In the first variant, only one metal was added into the nutrient medium. In the second variant, several toxic metals were simultaneously present in the medium. In the first case, toxic metals were added to the medium in the following concentration range counting per metal ions (mg/L): Cu$^{2+}$ — 100—1000; Ni$^{2+}$ — 100—800; Co$^{2+}$ — 100—800; CrO$_4^{2-}$ — 25—150. Copper was used in the form of sulfate (CuSO$_4$) without chelators. Solutions of CrO$_4^{2-}$, Co$^{2+}$, and Ni$^{2+}$ were obtained by dissolution of K$_2$CrO$_4$, CoCl$_2$×6H$_2$O, and NiCl$_2$×6H$_2$O in distilled water, respectively.
In the second variant, the total concentration of toxic metals in the medium was chosen according to the maximum concentration of each metal. The total maximum concentration of metals was equal to the sum of maximum concentrations of metals of the first variant of the experiment with the following proportional reduction by 10% from the initial one. Experiments were carried out in triplicates.

The effect of temperature and NaCl. The effect of temperature on the growth of Antarctic yeasts was examined on wort agar at 1, 5, 10 (incubation up to 30 days), 18, 25, 30, and 42 ºC (incubation up to 10 days). Halotolerant yeasts were studied using standard microbiological methods by growing isolates on MWA medium containing 1, 25, 50, 100, 150, and 200 g NaCl/L.

Results. Morphological properties of two non-pigmented yeast strains isolated from Antarctic moss were studied. Strains S11 and S12 were cultivated on MWA at 20 ºC for 2—3 days. Colonies of strain S11 were not pigmented, convex, shiny, paste-like, with a diameter of up to 12 mm. Their consistency was soft. With aging, the colonies acquired a whitish shade and became wrinkled. The colonies of strain S12 were white, rounded, convex, dull, paste-like with a diameter of up to 7 mm and soft consistency. A brownish tint appeared during aging.

Identification of Antarctic yeast. To determine closely related species, a comparative analysis of nucleotide sequences of 18S rRNA genes fragments for strains S11 and S12 was carried out. The size of fragments was 1375 and 1373 bp, respectively. The obtained sequences were compared with those deposited in the GenBank database. Based on the obtained data, closely related species were identified (Table 1).

We were guided by the following criteria for the interpretation of sequences from the GenBank database and identification of investigated strains: for sequence identities ≥ 99%, the genus and species were accepted; for sequence identities of 98%, the genus and species were accepted, but the term ‘cf.’ (Latin for confer = compares with) was used to indicate that the specimen resembles but has minor features not found in the reference species; for sequence identities between 95% and 97%, only the genus was accepted; for sequence identities ≤ 95%, the isolates were labelled with the order or family name or as «unknown fungi» (Godinho et al., 2013).

Using the BLAST 2.3.0+ algorithm, a high similarity of the studied sequences (99.5—99.6%) with the deposited nucleotide sequences of various strains in the GenBank database was shown. According to the results of the comparative analysis, there were several strains to be the closest homologues of strain S11 *L. scottii* (AY707092, X53499, KF036682, KR336838) with a sequence similarity of 99.3—99.6% (Table 1, Fig. 1).

Phylogenetic dendrograms (trees) were constructed for non-pigmented psychrotolerant Antarctic yeasts isolated from the moss of Galin-

<table>
<thead>
<tr>
<th>Strain No/Number of nucleotide pairs</th>
<th>Yeast species that are the closest to the studied strains in BLASTN 2.3.0+</th>
<th>Similarity, %</th>
<th>Taxonomic position</th>
</tr>
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<tr>
<td>S11/1375</td>
<td>Leucosporidium scottii, Leucosporidium scottii, Leucosporidium scottii</td>
<td>99.6, 99.5, 99.3</td>
<td>Basidiomycota, Pucciniomycotina, Microbotryomycetes, Leucosporidiales, Leucosporidium</td>
</tr>
<tr>
<td>S12/1373</td>
<td>Debaryomyces Hansenii, Debaryomyces Hansenii, Debaryomyces Hansenii</td>
<td>99.5, 99.5, 99.5</td>
<td>Ascomycota, Saccharomycotina, Saccharomycetes, Saccharomycetales, Debaryomyceaceae, Debaryomyces</td>
</tr>
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</table>
dez Island at 1 °C. According to the phylogenetic analysis (Fig. 1), yeast strain S11 was identified as *Leucosporidium scottii*, which is consistent with the comparative analysis results (Table 1). It belongs to the phylum *Basidiomycota*, subphylum *Pucciniomycotina*.

Several strains *Debaryomyces hansenii* with a sequence similarity of 99.5% (Table 1), as well as strain AY520242 *Candida* sp. (99%) were the closest homologues of yeast strain S12. According to the phylogenetic analysis, the sequence of strain S12 is reliably clustered with the sequence of the typical strain *D. hansenii*, as well as other strains of this species isolated from various ecosystems, including Antarctica (KR336844). It can be identified as *D. hansenii* S12 (Fig. 2). Nucleotide sequences of 18S rRNA genes of yeast strains S11 and S12 are registered in the GenBank database (accession numbers LT220858 and LT220859).

Therefore, according to the series of molecular biological characteristics, the studied Antarctic yeasts S11 and S12 were identified as *L. scottii* and *D. hansenii*.

**Resistance to toxic metal ions.** The Antarctic yeasts were investigated for resistance to four toxic metals. The toxicity of metals cobalt and nickel is determined by irreversible replacement of divalent cations in the active centres of enzymes and cellular structures of microorganisms causing the growth inhibition or cell death (Silver & Phung, 2005). As a result of our research, it has been established that the maximum resistance to cobalt for the studied strain of *D. hansenii* S12 constituted 600 mg/L, while for *L. scottii* S11 — 800 mg/L (Table 2). The resistance to Ni²⁺ for *D. hansenii* S12 and *L. scottii* S11 was 750 mg/L and 600 mg/L respectively.

Cr (VI) also has a toxic effect on microorganisms and is one of the most common environmental contaminants and frequently non-biodegradable in nature. It is a strong oxidant and has a high standard redox potential ($E'_0 = +555$ mV). It causes irreversible oxidation of enzymes and structural components of microbial cells (Sharma et al., 2022). Obtained results showed chromate to be one of the most toxic metals for the studied yeast strains. The strains *L. scottii* S11
Non-Pigmented Antarctic Yeasts and Their Resistance to Toxic Metals

Fig. 2. The phylogenetic dendrogram based on the analysis of nucleotide sequences of 18S rRNA genes, which shows the formation of clusters of different species of the genus *Debaryomyces* and position of investigated yeast strain S12 among closely related representatives of the genus *Debaryomyces*. The scale of 0.002 corresponds to 2 substitutions per 1000 bp.

Table 2. The resistance of Antarctic yeasts to toxic metal ions

<table>
<thead>
<tr>
<th>Species, Strain No</th>
<th>Maximum concentration of toxic metal ions in the nutrient medium, mg/L</th>
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<tr>
<td></td>
<td>Cu^{2+}</td>
</tr>
<tr>
<td><em>Leucosporidium scottii</em> S11</td>
<td>600</td>
</tr>
<tr>
<td><em>Debaryomyces hansenii</em> S12</td>
<td>600</td>
</tr>
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and *D. hansenii* S12 were resistant to 25 mg/L and 150 mg/L of CrO$_4^{2-}$ respectively.

The interaction of yeast strains with toxic metal ions Cu$^{2+}$ was also studied. The resistance to Cu$^{2+}$ for studied strains was 600 mg/L.

The stability range according to the resistance value of toxic metals for Antarctic yeasts can be depicted as follows. For *Leucosporidium scottii* S11, it is Co$^{2+} > $ Ni$^{2+} = $ CrO$_4^{2-}$ (800 > 600 = 600 > 25) and for *D. hansenii* S12: Ni$^{2+} > $ Cu$^{2+} = $ Co$^{2+} > $ CrO$_4^{2-}$ (750 > 600 = 600 > 150).

The multiple tolerance to several metal ions is reported for different yeast species. An increase in the number of metals in the environment leads to a sharp increase in their toxicity (Havryliuk et al., 2018). The resistance to the simultaneous presence of ions of four toxic metals at different concentrations was investigated (Fig. 3).

For the studied yeast strain *D. hansenii* S12, the resistance to toxic metal ions simultaneously present in medium was 600 mg/L (Co$^{2+} - $ 200 mg/L, Cu$^{2+} - $ 200 mg/L, Ni$^{2+} - $ 150 mg/L, and CrO$_4^{2-} - $ 50 mg/L). For *L. scottii* S11, only 240 mg/L (Cu$^{2+} - $ 80 mg/L, Co$^{2+} - $ 70 mg/L, Ni$^{2+} - $ 70 mg/L, and CrO$_4^{2-} - $ 20 mg/L). Thus, the simultaneous presence of toxic metal ions resulted in the increased toxic effects on the studied yeasts. The resistance of Antarctic non-pigmented yeast strains to toxic metal ions has been determined as well. It was shown that *D. hansenii* S12 was resistant to all tested toxic metal ions, and *L. scottii* S11 was resistant to Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$ and less to CrO$_4^{2-}$. Due to the simultaneous presence of ions of 4 toxic metals, their resistance decreases by 2—3 times.

**Discussion.** Today, molecular biological methods are intensively used to study the taxonomic diversity of Antarctic organisms. Published data indicate a high diversity of living organisms, particularly microorganisms, in Antarctic biotopes. Compared to bacteria, yeasts from Antarctic terrestrial ecosystems have been little studied (Carrasco et al., 2012; Gomes et al., 2019). However, they were suggested to be better adapted to the existence in low-temperature ecosystems than bacteria (Buzzini et al., 2012). Yeasts are a versatile group of eukaryotic microorganisms that have different trophic levels and can survive in a wide range of ecosystems and geographic regions (Buzzini et al., 2012; Margesin & Mitre, 2011). In addition, they are the most important in the carbon cycle in low-temperature ecosystems (Gupta et al., 2017).

Antarctic yeasts are represented by the phyla *Ascomycota* and *Basidiomycota*. According to the literature, the yeasts of the Antarctic regions are mainly represented by the phyla *Basidiomycota*, while representatives of *Ascomycota* are less common (Buzzini et al., 2012; Brandao et al., 2017; Martinez & Cavello, 2016). It was suggested that the predominance of *Basidiomycota* yeasts in these extreme environments is caused by their

![Fig. 3. The resistance to the simultaneous presence of toxic metal ions in Antarctic yeasts.](image-url)
Non-Pigmented Antarctic Yeasts and Their Resistance to Toxic Metals

nutritionally versatile and high resistance to extreme environmental conditions compared to representatives of the *Ascomycota* (Brandao et al., 2011; Sampaio et al., 2004). It was reported that basidial genera *Leucosporidiella*, *Rhodotorula*, and *Mrakia* were dominant in sphagnum mosses (Kachalkin et al., 2008). Yeasts belonging to genera *Leucosporidiyum*, *Cryptococcus*, *Mrakia*, and *Sporobolomyces* were isolated from soil, moss, lichen, and sea water (Martinez & Cavello 2016; Poli et al., 2010; Turkiewicz et al., 2003). Among ascomycete yeasts, representatives of *Debaryomyces*, *Candida*, and *Meyerozyma* are often found in Antarctic environments (Carrasco et al., 2012; Martinez & Cavello, 2016; Duarte et al., 2013).

We used sequencing along with comparative and phylogenetic analysis to identify Antarctic non-pigmented yeasts isolates from Galindez Island moss samples. The studied yeast strains S11 and S12 belong to the genera *Leucosporidiium* and *Debaryomyces* (phyla *Basidiomycota* and *Ascomycota*). The 18S rRNA sequence of yeast strain S12 showed 99.5% similarity with many different sequences of *D. hansenii* from the GenBank nucleotide database and also with strain AY520242 *Candida* sp. (99%). On the phylogenetic tree (Fig. 2), the sequence of strain S12 (1373 bp) grouped in a cluster consisting of the sequences of *D. hansenii* isolates (X58053, AY940171, AB070854, and others) and one unspecified strain of *Candida* sp. BG02-6-9-1. In addition, the phylogenetic tree showed that sequences of unidentified *Candida* species were clustered within separate branches of subclusters of the *Debaryomyces* genus, which may indicate the unreliability of the primary identification of these yeasts as *Candida* sp. Note that the sequence of strain S12 was clustered with many other *D. hansenii* yeast sequences isolated from different ecosystems, including Antarctica (KR336844). Such data indicate the widespread distribution of these species in various ecosystems. Also, it was shown that *D. hansenii* is the most common (ubiquitous) species in the soil

and water of King George Island (Martinez & Cavello, 2016; Nagahama, 2006).

Considerable attention was also paid to the study of resistance of Antarctic yeasts to toxic metal ions. Currently, there is no clear quantitative criterion to determine the concepts of «resistance» or «sensitivity» to stress factors. This also applies to toxic metals. We can talk about the fact that some objects are more resistant or more sensitive in relation to other ones.

Toxic metal ions are known to suppress the growth of microorganisms, affect microbial diversity and morphology, disrupt the integrity of the cell membrane, cause mutations, etc. (Sodhi et al., 2020). Yeasts and micromycetes are more resistant to toxic metal ions than bacteria and often dominate in metal-contaminated ecosystems (Zibilski & Wagner, 1982). *Ascomycete* yeasts are more resistant to metals than basidiomycetes. In addition, differences were found between strains originating from different natural sources (Vadkertiová & Sláviková, 2006).

Though cobalt and nickel are essential trace elements necessary for the correct biological functioning of metabolic pathways of microorganisms, they are toxic in the excessive concentration (Gumá-Cintrón et al., 2015; Forzani et al., 2001). Metals substitution was performed by cobalt and nickel capable of entering the cell via the NiCoT (nickel/cobalt transporter) system of bacteria, archaebacteria, and fungi (Rodionov et al., 2006; Degen & Eitinger, 2002). Toxic Co$^{2+}$ also inhibited the growth of *D. hansenii* J6 cells isolated from the Swedish estuary by 60% (Gumá-Cintrón et al., 2015). The indicated concentration was two times lower than the inhibitory concentration of Co$^{2+}$ for the studied yeast strain *D. hansenii* S12. In general, when comparing the resistance to toxic ions of cobalt and nickel, cobalt ions were more toxic to *D. hansenii* S12 than nickel ions, and nickel ions were more toxic to *L. scottii* S11. The resistance to CrO$_4^{2-}$ of Antarctic yeast strains *L. scottii* S11 and *D. hansenii* S12 was 25 and 150 mg/L, respectively,
which shows that the studied strains were the least resistant to chromate. These results are expected since chromium is a strong metal oxidizer. It competes for enzymes and transport systems of microorganisms and leads to oxidation of structural components of cells (Silver & Phung, 2005). Cr(VI) can enter yeast cells through the permease system that transports anions such as sulfate and phosphate (Cervantes et al., 2001). It was previously reported that 55% of yeast strains isolated from various locations of King George Island (Antarctica) were resistant to 1 mM Cr(VI) (i.e., 52 mg/L) (Fernandez et al., 2017). In another case, the chromate-reducing yeasts Candida sake and D. hansenii isolated from Antarctic soils were resistant to 2 mM (i.e., 104 mg/L) Cr(VI). That is, the studied D. hansenii S12 strain showed resistance to Cr(VI) 1.5 times higher than the yeast strain of this species isolated from the Antarctic soil (Cruz et al., 2022). The Cu²⁺ cation block reactions of dissimilatory metabolism due to the branching of the flow of electrons to it as an acceptor provoking energy depletion of cell (Rehman et al., 2019). According to the results (Table 2), the studied strains were characterized by the same high level of resistance to copper (Cu²⁺) up to 600 mg/L in the form of sulfate (CuSO₄). Fernandez and co-authors have reported that 80% (n=10³) of yeast strains were resistant to 1 mM Cu(II) (i.e., 63.5 mg/L) (Fernandez et al., 2017). Extremely high resistance to Cu²⁺ was found in the pigmented strain Rhodotorula mucilaginosa LM9 isolated from the Antarctic sea ice, which was resistant to 200 mM (i.e., 12700 mg/L) CuSO₄. Such data indicate the high adaptive capacity of yeasts with high resistance to metals in the extreme conditions of the Antarctic (Kan et al., 2019). A manifestation of the adaptation of microorganisms to toxic metals can be the synthesis of protective pigments, for example, the carotenoids and melanins. In addition, it has been reported that one of the mechanisms of microbial resistance to heavy metals is biosorption. Effective metal biosorbents are yeasts of the genera Candida, Saccharomyces, and Pichia (Podgorskiĭ & Kasatkina, 2003).

Metal-resistant yeast strains studied in this work (L. scottii S11 and D. hansenii S12) may have great potential for purification of refinery and textile effluents. According to the literature, in addition to bacteria, fungi, algae, and plants, yeasts are also an alternative for the bioremediation of metal-contaminated soils (in particular, chromium-contaminated) (Cervantes et al., 2001). The yeasts are of particular interest as a biosorbent for the removal of heavy metals from wastewater due to the high growth rate and specific cell wall structure (Ram et al., 2012). Moreover, yeast is a readily available and inexpensive source of biomass that can be easily obtained by the cultivation in bioreactors. The Saccharomyces cerevisiae yeast has shown effectiveness in removing heavy metals from aquatic environments. Removal of toxic metal ions occurred mainly by biosorption on the outer surface of yeast cells (Savastru et al., 2022). The research has shown that out of 128 Antarctic yeast strains, 24 have some degree of tolerance to the three metals studied and can use phenol as a carbon source. Strains Leucosporidium creatinivorum 276 and Mrakia frigida 190 which demonstrated a high level of resistance to metals are promising for use in low-temperature treatment of effluents containing phenol and a high level of metal ions (Cervantes et al., 2001). The ability of yeasts of the phylum Ascomycota (genera Debaryomyces, Candida, Galactomyces, Pichia, and Issatchenka) to treat wastewater containing dyes has been also shown (Sampaio et al., 2018). However, further research is needed to explore the potential applications of these yeast strains in biotechnology.

Conflict of interest. The authors declare that there are no conflicts of interest.
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НЕПІГМЕНТОВАНІ ДРІЖДЖІ АНТАРКТИКИ ТА ЇХНЯ РЕЗИСТЕНТНІСТЬ ДО ТОКСИЧНИХ МЕТАЛІВ

У даний час мікроорганізми, а саме дріжджі Антарктичних регіонів, незважаючи на їхню ключову роль у біогеохімічних процесах та функціонуванні наземних екосистем, залишаються недостатньо вивченими. Дослідження складу мікробних угруповань Антарктики залишається актуальним і здійснюється з використанням молекулярно-біологічних підходів. Визначення резистентності до іонів токсичних металів має важливе значення для відбору промислово перспективних штамів, які можуть сприяти розробці нових методів детоксикації металів за допомогою мікроорганізмів. Мета. Визначити таксономічне положення непігментованих антарктичних дріжджів та дослідити їх резистентність до іонів токсичних металів. Методи. Об’єкти дослідження — дріжджі, ізольовані з фітоценозів Антарктики. Дріжджі вирощували на солодовому суслі (рН 5.0—5.5, температура 18—20 °С). Виділення геномної ДНК проводили, використовуючи комерційний набір ДНК-сорб; ампліфікацію препаратів ДНК проводили, використовуючи праймери NL1 і NL4; філогенетичний аналіз визначали за побудовою дерев (дендрограм), які показують положення досліджуваних штамів серед близькоспоріднених і типових видів. Резистентність дріжджів до іонів токсичних металів встановлювали культивуванням у концентраційному градієнті Ni2+, Co2+, CrO42-, Сu2+. Визначені деякі екофізіологічні властивості досліджених штамів, такі як психро- та галотолерантність.

Результати. Філогенетичний аналіз показав високий відсоток подібності (99.5—99.6%) нуклеотидних послідовностей генів 18S рРНК дріжджових штамів до послідовностей антарктичних штамів та до послідовностей дріжджів з бази данних GenBank. Психротолерантні та галотолерантні антарктичні штами дріжджів S11 та S12 ідентифіковано як Leucosporidium scottii та Debaryomyces hansenii, відповідно. Досліджені штами дріжджів виявилися найбільш резистентними до іонів металів Ni2+ та Co2+. Штам L. scottii S11 ріс за 800 мг/л Co2+, а D. hansenii S12 — за 750 мг/л Ni2+. Дріжджі були найменш резистентними до іонів металу CrO42-: штам L. scottii S11 ріс за 25 мг/л, а D. hansenii S12 — за 150 мг/л. У присутності іонів металу Cu2+ вони росли за одноакової концентрації — 600 мг/л. Встановлено, що за одночасної присутності токсичних металів в середовищі їхня токсичність підвищується у 2—3 рази. Одночасна присутність кількох токсичних металів збільшувала токсичність середовища у 2—3 рази. Висновки. Нуклеотидні послідовності фрагменту гена 18S рРНК штамів дріжджів S11 та S12 внесено до бази данних GenBank під номерами LT220858 та LT220859. Психротолерантні штами дріжджів, резистентні до металів, можуть бути використані для оцінки рівнів металів в полярних регіонах, а також для біоремедіації екосистем, забруднених металами. Проте для розробки та оптимізації процесів біоремедіації необхідні подальші дослідження.

Ключові слова: Антарктика, дріжджі, 18S рРНК, філогенетичний аналіз, Leucosporidium scottii, Debaryomyces hansenii, токсичні металі.