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Short Communication

Physiological properties of a new strain of Saccharomyces cerevisiae Dag-1 isolated from the Caspian Sea, Russia

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Abstract: This study reports the isolation of the yeast *Saccharomyces cerevisiae* Dag-1 (OQ107063.2) from the seawater of the Caspian Sea, specifically from the coastal territory of the Samursky Reserve, Republic of Dagestan, Russia. The strain's diagnostic and genetic characteristics are provided. Morphological features of the *S. cerevisiae* Dag-1 include round cells measuring 5.0×5.0 and 1.0×1.0 μkm in size. On Sabouraud's media, it forms round, convex, opaque, creamy, glossy colonies with a diameter of 3-5 mm. The isolated strain demonstrates the ability to thrive in seawater with a salinity ranging from 12.8-13.0‰, across various temperature ranges (0 –+10, 23-28, 35-40, 30-37°C), and under different pH conditions (5.6; 8.4-8.5). Additionally, it utilizes monomeric sugars (L-glucose, D-mannose, D-sucrose, D-arabinose, D-cellobiose, and D-xylose) as a source of carbon and energy, also metabolizing alcohols such as D-sorbitol, D-mannitol, and D-inositol (vitamin B8). The strain does not absorb the amino acids lysine and ornithine, and it is catalase-, amylase-, and β-glucosidase-positive while being urease-, oxidase-, and β-galactosidase-negative. Moreover, the strain exhibits high sensitivity to the antibiotics of ketoconazole, nystatin, clotrimazole, fluconazole, and itraconazole. Resistance is observed against the inhibitor potassium tellurite and pathogenic bacteria *Escherichia coli, Staphylococcus aurens,* and *Klebsiella oxitoka*. These findings contribute to expanding our understanding of the ecological distribution of marine yeasts and the isolation of an *S. cerevisiae* strain possessing characteristics of industrial microorganisms.

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Introduction

In recent decades, extensive phenotypic and genomic characterizations of both wild and domesticated strains of *Saccharomyces cerevisiae* have been conducted (Bai et al., 2022). These investigations have provided valuable insights into various aspects of the species, including ecology, adaptation to the environment, population structure, biogeography, and evolutionary processes. Currently, the genus *Saccharomyces* encompasses approximately 2000 species, with *S. cerevisiae* serving as the type species due to its complete nucleotide genome sequence. This species has been instrumental in diverse research areas, including genetic engineering (Cap et al., 2009), chronological lifespan studies (Ayer et al., 2014), cancer pharmacogenomics (Cap et al., 2012), identification of drug metabolism pathways (Nislow et al., 2015), marine environmental monitoring (Monapathi et al., 2020), and applications in the biotechnology of baking and winemaking.

In recent years, research on the yeast *S. cerevisiae*, focusing on its geographic distribution, population structure, and genetic diversity (Peris et al., 2023), holds the potential to yield the isolation of new strains with desirable phenotypes for biotechnological applications. Consequently, microorganisms residing in marine environments have garnered attention from both scientists and industries due to their diverse and abundant bioactive properties (Kwon et al., 2020). The marine yeast *S. cerevisiae* exhibits a broad distribution, encompassing the Indian, Atlantic, and Pacific Oceans, as well as the East China Sea, South

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China Sea, Bohai Sea, and Ariake Gulf (Saravanakumar et al., 2013; Zaky et al., 2014, 2016, 2020; Obara et al., 2015; Kaewkrajay et al., 2020; Tian et al., 2021; Baba et al., 2022). Cultures of this species have been obtained from various sources, including seawater, bottom sediments, marine animals, invertebrate corals, sponges, algae, plankton, mangroves, sand, and wood. However, representatives of the genus *Saccharomyces*, including *S. cerevisiae*, have not been discovered in deep-sea areas (Libkind et al., 2017). This species is known to be limited to warmer marine regions. Importantly, up to the present moment, as indicated in the work of N.A. Kopytina (Kopytina, 2018), *S. cerevisiae* from the *Ascomycota* division has not been identified in the Caspian Sea. This paper presents the findings of investigations into the physiological, biochemical properties, and taxonomic positioning of the marine strain *S. cerevisiae* Dag-1, isolated from the waters of the Caspian Sea.

Materials and Methods

Objects of research and experimental area: The strain was isolated from a water sample obtained from the Caspian Sea, specifically from the coastal territory of the Samursky Reserve in the Republic of Dagestan, in July 2022. The sampling location was approximately 1 km from the coast (41°48′57″ N, 48°34′43″ E). The environmental conditions at the time of isolation included a water salinity of 12.8-13.0‰ and a temperature range of 21.9-25.5°C. Three samples were collected, resulting in the isolation of seven fungal cultures, with the abundance of the species *S. cerevisiae* found to be minimal.

Chemical composition of the seawater samples was determined utilizing an atomic absorption spectrometer, "MGA-915" (Russia), and a luminescent photometric liquid analyzer, "Fluorat-02" (Russia). The analysis followed established methods for measuring mass concentrations of compounds.

Physiological and biochemical research: Substrates for yeast cultivation media were weighed using an analytical balance (DV215CD, Ohaus Discovery, Switzerland). Agar (Difco, Spain) was utilized to prepare solid media. The medium's acidity, adjusted to pH 4.5, was achieved using 1N HCl or 4M KOH (Russia) and monitored with a Hanna Instrumentals pH 211 pH meter (Germany). Sabouraud's nutrient medium, employed for yeast cultivation, antibiotic sensitivity testing, and potassium tellurite assessment, was prepared from industrially produced reagents following the manufacturer's guidelines (Federal Budgetary Institution of Science, State Scientific Center for Applied Microbiology and Biotechnology, Russia). For Sabouraud's medium with potassium tellurite (K_2TeO_3), a ready-made medium was supplemented with 5 ml of a 2% salt solution per liter of medium. Primary isolation and culture incubation were conducted on Sabouraud's medium in a microbiological incubator (Binder-115, USA) at temperatures of 30 and 37°C for 1-7 days. Morphological analysis of cells and photography were performed using a CX21 light microscope (Olympus, Japan) and a Canon PowerShot A640 digital camera (Japan).

Phenotypic properties, including cell and colony size, individual biochemical parameters, oxygen tolerance, substrate utilization, temperature, and pH preferences, were studied using standard methods (Kurtzman et al., 2011; Kachmazov et al., 2021). Sensitivity to antifungal drugs was assessed using the disk diffusion method with standard disks ("Paper indicator systems for identifying microorganisms," Russia) containing 10-30 μg of antimicrobial agents. Zones of growth inhibition were measured following Methodological instructions MUK 4.2.1890-04. The ability to produce enzymes was evaluated using rapid tests for the enzymatic activity of microorganisms (MERCK, Germany). Tests included β-galactosidase (EC 3.2.1.23) using indicator disks with ortho-nitrophenyl-β-d-galactopyranoside (Conda, Spain), urease (EC 3.5.3.1) via the CLO test, catalase (EC 1.11.1.6) with 3% H_2O_2 substrate, and oxidase (EC 1.4.3.3) using Kovacs reagent. All tests were conducted following the manufacturer's instructions. Yeast incubation for enzymatic activity screening was performed at 30°C for 2 days in three replicates.

Molecular diagnosis of yeast isolates: The strain

identification procedures were conducted at the Collective Use Center "Promyshlennie Biotehnologii" of the Federal Research Center "Biotehnologii" of the Russian Academy of Sciences in Moscow. Polymerase chain reaction (PCR) was employed for strain identification using the following primers: 18S rRNA F566-18S R1200r (Hadziavdic et al., 2014) and ITS4-ITS5 (Bellemain et al., 2010; Angelov et al., 2015). PCR products were subjected to electrophoresis in a 2% agarose gel under an electric field strength of 6 V/cm. Isolation and purification of PCR products were carried out from low-melting agarose using the WizardPCRPreps reagent kit (Promega, USA) in accordance with the manufacturer's recommendations. Sequencing of the obtained PCR fragments of genes encoding 18S rRNA was performed following the Sanger method (Sanger et al., 1977) using the Big Dye Terminator v.3.1 reagents (Applied Biosystems, Inc., USA) on an ABI PRIZM 3730 genetic analyzer (Applied Biosystems, Inc., USA). Amplification and internal primers were employed for sequencing, and readings were conducted in both directions. Phylogenetic analysis of the nucleotide sequences of the 18S rRNA gene was carried out using the BLAST program in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov). Assembly and editing of the obtained sequences were performed using the BioEdit program (http://jwbrown .mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model (Tamura et al., 1993) by applying the Neighbor-Join and BioNJ algorithms to the pairwise distance matrix. The length of tree branches was measured by the number of substitutions per site. The analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+noncoding. Positions with site coverage of less than 95% were excluded, with less than 5% tolerance for missed alignments, missing data, and ambiguous bases. Evolutionary analysis was conducted in MEGA6 (Tamura et al., 2013).

Results and Discussions

The marine environment is an understudied natural source that may harbor strains displaying characteristics akin to industrial microorganisms (Zaky et al., 2016). Caspian seawater analysis revealed elevated concentrations of certain microelements, mg/dm³: Zn (0.020) > Ni (0.002) > Cu (0.0018) > Cr (0.0002) > Pb (0.0002) > Cd (0.0001). Cations (mg/dm3): Na^{+}(10180), Mg^{2+}(2457), Ca^{2+}(1056), K^{+}(171.4), and anions Cl^{-}(10200), SO_4^{2-} (4850), HCO_3^{-}(42.7) concentrations differed significantly from standard normal, indicating pollution with anomalous concentrations of pollutants.

The cells of *S. cerevisiae* strain Dag-1 are round, measuring 5.0×5.0 and 1.0×1.0 μkm, which corresponds to the morphological characteristics of the genus *Saccharomyces* (Kurtzman et al., 2011). On Sabouraud's media, round, convex, opaque, creamy, glossy colonies with a diameter of 3-5 mm were formed. The strain is catalase-, amylase- and β-glucosidase-positive; urease-, oxidase- and β-galactosidase negative (Table 1). It is generally accepted that typical strains of *S. cerevisiae* are unable to metabolize arabinose, xylose, cellobiose, mannitol, sorbitol, amylase, etc. (Kurtzman et al., 2011). However, marine strains of *S. cerevisiae* may exhibit properties atypical for the species: assimilation of arabinose (Tian et al., 2021), cellobiose (Ogawa et al., 2008; Obara et al., 2012; Zaky et al., 2016; Tian et al., al., 2021), mannitol (Greetham et al., 2019, Usha et al., 2022), xylose (Zaky et al., 2016; Greetham et al., 2019; Tian et al., 2021), sorbitol (Urano et al., 2017), amylase (Obara et al., 2015; Usha et al., 2022), and sorbitol (Urano et al., 2017, 2021). The isolated marine strain used monomeric sugars (L-glucose, D-mannose, D-sucrose, D-arabinose, D-cellobiose, D-xylose) as a source of carbon and energy; utilized D-sorbitol, D-mannitol, D-inositol (vitamin B8), (except indole); did not absorb lysine and ornithine (Table 1). Yeasts known to be capable of fermenting mannitol and inositol; pentoses (arabinose, cellobiose, xylose, etc.) may be of particular importance in ethanol production where marine yeasts, marine organisms, and water are used (Zaky et al., 2016).

Antibiotic resistance may serve as a means of combating environmental pathogens. The

Table 1. The results of biochemical tests for the strain of *Saccharomyces cerevisiae* Dag-1 isolated from the waters of the Caspian Sea.

Phenotypic traits	*S. cerevisiae* Dag-1 (OQ107063.2)
Oxygen susceptibility	Facultative anaerobe
T, °C	0 – +10, 23-28, 35-40, 30-37 (opt. 30 °C)
pH	4.5; 5.6; 8.4-8.5 (opt. 4.5)
Urease	-
Oxidase	-
β-galactosidase	-
β-glucosidase	+
D-cellobiose	+
D-xylose	+
Amylase	+
Catalase	+
Lysine	-
Ornithine	-
D-sorbitol	+
D-mannitol	+
D-inositol	+
Indole	-
L-glucose	+
D-mannose	+
D-sucrose	+
D-arabinose	+
Antifungal drugs (area of effect, mm)	
Ketoconazolum	9
Clotrimazolum	9
Fluconazolum	8
Nystatinum	9
Itraconazolum	8
Potassium tellurite susceptibility	lack of color

Note. "–" – lack of activity or consumption; "+" – activity, consumption. The column indicates statistically significant values in triplicate.

S. cerevisiae Dag-1 strain was highly sensitive to the II-generation synthetic antibiotic ketoconazole and the naturally occurring polyene antibiotic nystatin; clotrimazole from the imidazole group; to a lesser extent – fluconazole and itraconazole from the triazole group. There is evidence of the ability of individual representatives of the yeast *S. cerevisiae* to detoxify tellurite, which determined the survival strategy of microorganisms in unfavorable conditions (Chasteen et al., 2010). It was found that the inhibitor potassium tellurite did not affect the Dag-1 strain. The *S. cerevisiae* Dag-1 showed resistance to pathogenic bacteria *Escherichia coli, Staphylococcus aurens,* and *Klebsiella oxitoka* (agar diffusion assay).

The 18S rRNA gene fragment sequence of the isolated strain *S. cerevisiae* Dag-1 is deposited in the NCBI GenBank database under number OQ107063.2. Phylogenetic analysis placed Dag-1 within the *S. cerevisiae* cluster with a confidence level of 94%, indicating its distinctiveness as a separate strain of the *S. cerevisiae* species (Figs. 1-5). This difference in the sequence of *S. cerevisiae* may be associated with their evolution aimed at adapting to the characteristics of the environment, such as pollution with aggressive organic compounds, heavy metals, and the salinity of seawater in the Caspian Sea, which could lead to genetic changes modifying the phenotype within one species (Greetham et al., 2019). Related homologous sequences belonged to isolates isolated from different geographical areas and substrates: Balinese rice wine (China), alcoholic fermentation (Japan), human gastrointestinal tract (Ukraine), and mangroves (Porto Novo estuary, Indian Ocean, India). Selected strains are recommended for traditional spirits (India) and freezing (China).

Thus, when analyzing data from marine and

Figure 1. Isolation of yeast cultures on Sabouraud medium as a result of seawater filtration.

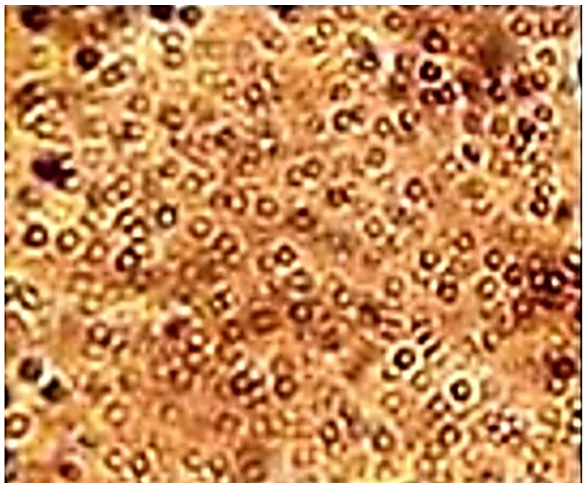

Figure 2. Cells of the isolated culture Saccharomyces cerevisiae Dag-1 on solid Sabouraud medium, 600x.

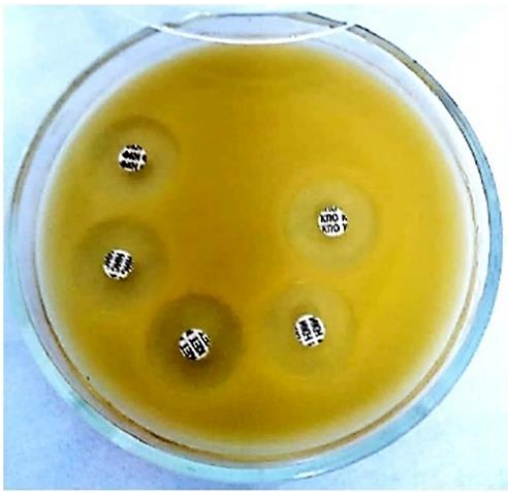

Figure 3. Colonies of the isolated marine yeast *Saccharomyces cerevisiae* Dag-1 during the study of susceptibility to antifungal drugs Ketoconazolum, Clotrimazolum, Fluconazolum, Nystatinum, and Itraconazolum.

terrestrial yeasts, it is clear that marine cultures are phenotypically different from the typical yeast *S. cerevisiae* (Kurtzman et al., 2011). The studied strain Dag-1 is phenotypically unique compared to other terrestrial representatives of this species: it demonstrated resistance to inhibitors; capable of fermenting catalase, amylase, β-glucosidase; assimilation of sugars D-arabinose, D-cellobiose, D-xylose; alcohols D-sorbitol, D-inositol, D-mannitol, usually present in lignocellulosic hydrolysates; resistant to antibiotics ketoconazole, nystatin; clotrimazole, fluconazole, and itraconazole; exhibited antagonistic properties to pathogenic bacteria *E. coli*, *St. aurens*, and *Kl. Oxitoka*. All distinctive features indicate the possibility of using Dag-1 in biotechnology utilizing lignocellulosic raw materials. Information about the *S. cerevisiae* Dag-1 strain, isolated from the coastal zone of the Caspian Sea, has expanded our understanding of the distribution of the species.

Acknowledgment

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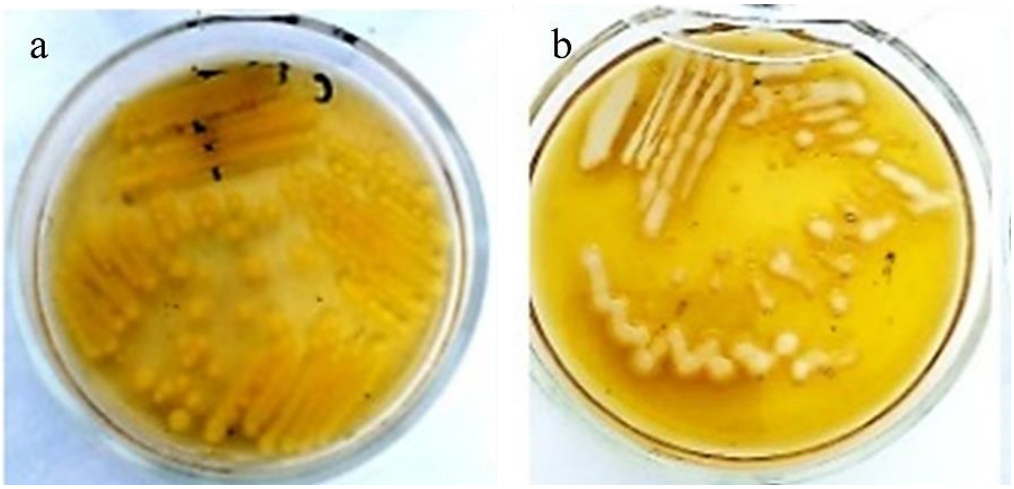

Figure 4. Growth of the marine strain *Saccharomyces cerevisiae* Dag-1 on Sabouraud medium. (a) on Sabouraud medium with potassium tellurite and (b) duration: 3 days.

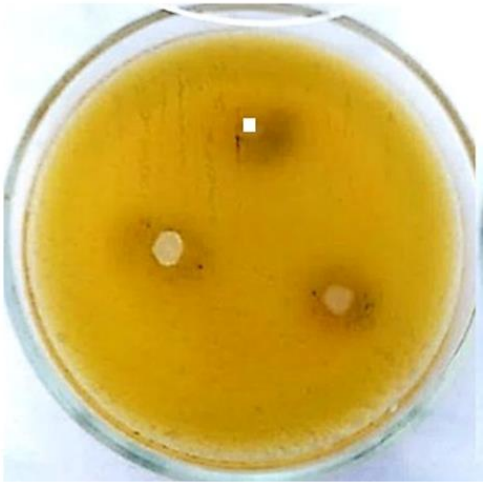

Figure 5. Pathogenic resistance of the marine culture *Saccharomyces cerevisiae* Dag-1 against *Escherichia coli*, *Staphylococcus aurens*, and *Klebsiella oxitoka* bacteria.

(Makhachkala, Republic of Dagestan, Russian Federation).

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