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գիտական աստիճանի հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

ԵՐԵՎԱՆ 2024

MINISTRY OF EDUCATION, SCIENCE, CULTURE AND SPORTS OF RA
YEREVAN STATE UNIVERSITY

SHIRVANYAN ANAHIT HAMBARDZUM

THE ACTION MECHANISMS OF STRESS FACTORS ON SOME YEAST GROWTH PARAMETERS AND ANTIOXIDANT ENZYMES ACTIVITIES

SYNOPSIS

of dissertation for conferring of science degree of
Candidate of Biological Sciences
In the specialty of 03.00.04-Biochemistry

YEREVAN 2024

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝ կ.գ.դ., պրոֆ. Կ. Ա. Թռչունյան

Պաշտոնական ընդդիմախոսներ՝ կ.գ.դ., պրոֆ. Կ. Բ. Ենկոյան
կ.գ.թ, դոց. Հ. Լ. Հայրապետյան

Առաջատար կազմակերպություն՝ ՀՀ ԳԱԱ Հ. Բունիաթյանի անվան կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2024թ. հունիսի 14-ին ժամը 14^{00}-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲԿԳԿ-ի Կենսաֆիզիկայի 051 մասնագիտական խորհրդի նիստում (0025, Երևան, Ալեք Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:

Ատենախոսության սեղմագիրն առաքված է 2024թ. մայիսի 7-ին:

051 մասնագիտական խորհրդի գիտական քարտուղար, կ.գ.դ., դոց.՝ Մ.Ա. Փարսադանյան

The theme of dissertation has been approved at Yerevan State University

Academic advisor: D.Sc., Prof. K. A. Trchounian

Official opponents: D.Sc., Prof. K. B. Yenkoyan
Ph.D., Assoc. Prof. H. L. Hayrapetyan

Leading organization: H. Buniatian Institute of Biochemistry of NAS RA

The defense of the dissertation will be held on 14th June 2024, at 14:00, at the session of 051 Scientific Specialized Council on Biophysics of HESC of RA at Yerevan State University (0025, Yerevan, Alex Manoogian str. 1, YSU, Faculty of Biology).

The dissertation is available at the library of Yerevan State University.

The synopsis has been sent on 7th May 2024.

Scientific Secretary of 051 Specialized Council, D.Sc., Assoc. Prof. M. A. Parsadanyan

INDRODUCTION

Topic's significance. Metabolic regulation systems respond to various environmental conditions. However, the dynamic changes in metabolic pathways under stress conditions in yeast cells are not well understood. The cellular response to environmental factors involves both specific and general mechanisms. The biological goal of the stress response is to protect cells from potential harm and prepare them for future detrimental effects of the same or different types of environmental factors. Understanding the molecular basis of adaptation to stress factors during fermentation is important for the successful construction of genetically modified yeast strains. These strains can have multiple stress tolerances, making them suitable for high-yield production of valuable end products like bioethanol. In terms of production, obtaining a large quantity of biomass is crucial. However, yeast viability can be affected by various factors, including temperature, nutrition, oxidative transitions, inhibitory substances, osmotic pressure, sulfide levels, pH levels, and oxygen limitation. Additionally, extreme changes in the environment throughout fermentation stages, such as high sugar levels at the beginning and high alcohol levels later, can also impact yeast cells. These factors can damage yeast cells, leading to inhibition of biomass production and cell death, ultimately reducing the production of desired products. Glycerol synthesis is an important factor in the central metabolism and stress biology of *S. cerevisiae*. Further research is needed to understand the cellular mechanisms involved in responding to and adapting to osmotic stress induced by glycerol. Under osmotic stress conditions, the membrane transitions into a gel state, resulting in reduced fluidity due to the adherence of phospholipid heads. Therefore, studying ion channels under osmotic stress conditions is relevant and can contribute to enhancing yeast adaptation. While the role of antioxidant enzymes in aerobic conditions has been extensively studied, limited information is available regarding their activation or activity in real fermentation conditions. Developing yeast cells that are resistant to various stresses is essential for increasing the yield of fermentable products and biofuels. However, the systemic interactions between cellular maintenance, growth, and stressors are still not well understood, and the precise mechanisms of adaptation remain unclear. The increasing demand for renewable energy sources, such as bioethanol production, is driven by the rising volumes of energy consumption, the accumulation of greenhouse gases, and their impact on climate change. Biofuels are considered environmentally friendly, have a lesser impact on global warming compared to fossil fuels, and are sustainable and renewable. In 2019, the total annual ethanol production reached 29 billion gallons. Therefore, it is crucial to study the growth characteristics of yeast under conditions of high inhibitor content (such as formaldehyde, acetic acid, heavy metals) and high osmotic conditions to assess the utilization of waste in yeast-based production processes. Facilitating sustainable ethanol production from raw materials and waste is particularly important

considering the lack of waste management systems in Armenia and the increasing demand for renewable resources in the international market.

Research goals and tasks. The aim of the research was to investigate the impact of glycerol-induced osmotic stress on the metabolic and bioenergetic parameters of *S. cerevisiae* ATCC 9804 and ATCC 13007 strains and to elucidate the molecular mechanisms of their adaptation depending on the growth conditions.

Constituted tasks of the research were:

- Investigating the influence of glycerol-induced osmotic stress on the growth parameters of *S. cerevisiae* ATCC 9804 and ATCC 13007 strains, including specific growth rate, growth yield, and viability, depending on oxygen availability and pH of the medium.
- Studying changes in the osmolality of the medium during the cultivation of *S. cerevisiae* ATCC 9804 and ATCC 13007 strains under physiological and osmotic stress conditions, depending on the growth conditions.
- Assessing the impact of glycerol-induced osmotic stress on the damage to biomembranes by osmotic stress in *S. cerevisiae* ATCC 9804 and ATCC 13007 strains.
- Investigating the characteristics of potassium, sodium, and proton ion fluxes and their transporters under glycerol-induced osmotic stress conditions in ATCC 9804 and ATCC 13007.
- Identifying the influence of osmotic stress factors on the activity of antioxidant enzymes and the mechanisms of their regulation in strains of *S. cerevisiae* ATCC 9804 and ATCC 13007 under conditions of respiratory and fermentative metabolism.
- Studying the redox balance in *S. cerevisiae* ATCC 9804 and ATCC 13007 strains under physiological and osmotic stress conditions, using the thiol/disulfide ratio as a biomarker.
- Determining the impact of osmotic stress on yeast bioenergetic parameters, particularly on total and DCCD-sensitive ATPase activity.
- Investigating possible metabolic shifts under osmotic stress by studying qualitative and quantitative changes in end products.

Scientific novelty and practical value of the study. Two Crabtree-positive industrial yeast strains, *S. cerevisiae* ATCC 9804 and ATCC 13007, were used in this study due to their intriguing biotechnological potential. The ATCC 9804 strain was isolated from palm wine or makgeolli, a traditional Korean rice wine, while the ATCC 13007 strain was isolated from Irish beer and could synthesize extracellular glucoamylase. This characteristic makes it suitable for bioethanol production through waste processing. However, there is a lack of literature on the functioning and regulation of membrane transporters, metabolic pathways, and adaptation of these strains under stress conditions. Therefore, this study aimed to investigate the molecular mechanisms of stress response and adaptation pathways in these strains

for the first time. Additionally, the researchers examined the metabolic characteristics and differences in adaptation pathways of yeast under osmotic stress conditions depending on growth conditions and oxygen availability. By uncovering the metabolic dynamics of yeast in response to stress factors, this study provides valuable insights into how yeast copes with environmental challenges. These findings lay the foundation for the development of genetically modified stress-resistant yeast strains, which could greatly contribute to the efficient production of ethanol, biomass, and valuable materials derived from waste processing. Furthermore, data on the activity of antioxidant enzymes, redox balance, and metabolic changes in response to osmotic stress can be applied in biomedical research. Understanding the molecular mechanisms underlying yeast adaptation to stress can provide valuable insights into similar processes in higher eukaryotic organisms, leading to the development of new therapeutic strategies for various diseases. Furthermore, studying the mechanisms of yeast adaptation to osmotic stress can also be applied in biotechnologies aimed at environmental protection. Therefore, the knowledge gained from understanding how microorganisms respond to changing environmental conditions can be applied to develop bioremediation strategies or create microbial consortia for environmental restoration purposes.

Main points to present at the defense.

1. The response to osmotic stress varies depending on the yeast strain and growth conditions. Yeast growth is suppressed by osmotic stress in microaerophilic and acidic conditions.
2. Na^+/K^+ exchange plays a key role in the stress response during aerobic growth, while K^+/H^+ exchange is important in microaerophilic conditions.
3. Inhibition of the plasma membrane and mitochondrial H^+-ATPase disrupts not only proton fluxes but also potassium and sodium fluxes across the yeast membrane.
4. The activity of antioxidant enzymes activity depends on the selected strain, nutrient media composition, redox balance, and stress conditions. Superoxide dismutase plays a major role in antioxidant defense processes in aerobic conditions in both strains, while catalase enzyme activity is crucial in microaerophilic conditions.
5. Thiol groups play a key role in regulating the activity of antioxidant enzymes in aerobic conditions compared to microaerophilic growth conditions.

Work approbation. Main results of the dissertation were discussed at seminars in Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty of Yerevan State University, and at scientific conferences: Scientific Conference "Biotechnology: Science and Practice, Innovation and Business" for Young Researchers (Yerevan, Armenia, 2021), 46th FEBS Congress (Lisbon, Portugal, 2022) and 47th FEBS Congress (Tours, France, 2023).

Publications. According to experimental data observed in dissertation, 6 papers, including 1 article in peer-reviewed journal, 2 articles in a journal included in the list of HESC of the RA and 3 abstracts were published.

Volume and structure of dissertation. The dissertation contains the following chapters: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and discussion (Chapter 3), conclusions and cited literature (total 151 papers and books). The dissertation consists of 130 pages, 4 tables and 27 figures.

MATERIALS AND METHODS

Yeast, growth media and conditions. Research object: *S. cerevisiae* ATCC 13007 (prototroph, pof+ STA, diploid, European Nucleotide Archive (ENA) - ERR5358805) and ATCC 9804 (GenBank: Z95939.1; ENA-ERR5285363) strains have been purchased from Microbial Depository Center of Scientific and Production Center "Armbiotechnology" NAS RA. Yeast strains were stored at -80°C in liquid YPD (yeast extract: 10 g L^{-1}, peptone: 20 g L^{-1}, D-glucose: 20 g L^{-1}) medium containing 15% glycerol, and/or at 4°C on solid YPDA medium (yeast extract: 10 g L^{-1}, peptone: 20 g L^{-1}, D-glucose: 20 g L^{-1}, agar: 17 g L^{-1}). Yeasts were grown under microaerophilic (flask-to-medium ratio of 1, without shaking) or aerobic (flask-to-medium ratio of 1/2.5, with rotary shaking at 130 rpm) conditions at 30°C. Medium pH (5; 6.5; 7.2) was adjusted by monopotassium phosphate or 0.1 N HCl (Shirvanyan A. et al., 2021).

Determining the influence of osmotic stress on yeast specific growth yield and viability. The overnight culture cells were transferred to fresh YPD medium at a concentration of 1.5% (v/v) (physiological conditions). To create osmotic stress conditions, yeast cells were grown similarly under aerobic or microaerophilic conditions in peptone medium containing glycerol (YPG, yeast extract: 10 g L^{-1}, peptone: 20 g L^{-1}, glycerol: 5 g L^{-1}). The specific growth rate (SGR) of cells was determined using UV-VIS spectrophotometer (Cary 60, Agilent Technologies, Germany) by monitoring changes in culture optical density at 600 nm relative to the medium (Shirvanyan et. al., 2021). To assess the influence of osmotic stress on cell viability, the colony-forming unit (CFU) was determined. The number of viable cells was expressed in CFU mL^{-1}.

Determination of the malondialdehyde (MDA) level. To study the processes of lipid peroxidation in the cell the amount of MDA - one of its end end-products, were determined spectrophotometrically at 532 nm using reaction with thiobarbituric acid. The results were expressed in μM/mg of protein unit.

Determination of ion fluxes across the plasma membrane. H^+, K^+, and Na^+ fluxes through the plasma membrane of intact cells (ΔJ_{H+}, ΔJ_{K+}, ΔJ_{Na+} respectively) were determined potentiometrically upon addition of glucose (aqueous solution, 20 g L^{-1}) or glycerol (aqueous solution, 5 g/L), as described. Proton flux was determined using a pH electrode, potassium flux-using a potassium-selective electrode (HI4114,

Hanna Instruments, Portugal) with the sensitive module HI4114-51, and sodium flux using an Na^+-selective electrode FC300B, connected to an HI 5222 pH-ISE-ORP ionometer (Hanna Instruments, USA) (Shirvanyan et al., 2023). The results were calculated using the calibration curve. To understand the relationship between the changes in ion fluxes related to bioenergetic processes, cells were treated with a specific inhibitor of H^+-ATPase, *N, N′*-dicyclohexylcarbodiimide (DCCD, 0.5 mM), for 15 minutes. Results were expressed in mmol mL^{-1} min per 10^9 cells.

Determination of the activity of antioxidant enzymes. The total catalase activity (CAT, 1.11.1.6) was determined spectrophotometrically by measuring the decrease in hydrogen peroxide absorption at 240 nm. Results were expressed in Units mg^{-1} protein. The total superoxide dismutase (SOD, 1.15.1.1) activity was determined using Beauchamp and Fridovich method and expressed in Units mg^{-1} protein. To understand the influence of redox processes on the activity of CAT and SOD enzymes, the total CAT and SOD activity was also studied in samples treated with a specific blocker of free SH-groups-N-ethylmaleimide (NEM, 0.5 mM).

SH groups determination. SH-groups were determined by the reaction of cell extract with Ellmann's reagent (5, 5'-dithiobis-2-nitrobenzoic acid) using glutathione as a standard. Samples, treated with 0.5 mM NEM, a thiol-group specific modifier, were measured by the same principle. Protein was measured by Lowry assay using BSA as a standard. Accessible SH groups were determined as the difference of total and NEM treated samples. SH groups level was determined according to Beer–Lambert law and expressed in nmol L^{-1} mg^{-1} protein.

ATPase assay. Mitochondria were isolated using differential centrifugation. Protein concentration was determined by the method of Lowry in whole cell extract and mitochondrial fraction using BSA as a standard. The F_OF_1-ATPase activity was determined by the increase of inorganic phosphorus in the incubation medium by Taussky and Shorr method. Enzyme activity was expressed in nmol P_{in} mL^{-1} min^{-1} mg^{-1} mitochondrial protein. In DCCD-treated (0.5 mM) samples ATPase activity was measured in the same manner. In the assays, as a source of potassium, 100 mM KCl was added when indicated (Gevorgyan H. et al., 2023).

Organic acids, alcohols, and sugar determination. Organic acids and alcohols (ethanol, glycerol, acetate, and succinate) and sugars were determined by HPLC (Agilent 1260 Infinity II LC Bioinert with a refractive index detector (Agilent RID, G1362A, set on positive polarity and optical unit temperature of 55°C) using Macherey-Nagel EC 250/4.6 NUCLEOSIL 120–5 C18 column (250 × 4.6 mm, MN720041.46, Düren, Germany)). Substrate utilization and metabolites production rate was calculated as the difference in concentration (mmol) for the given growth period. Carbon conversion efficiency (CCE) was determined by following equation: $CCE=\Delta C_2/\Delta C_1*100\%$, where ΔC_1 is the difference in substrate carbon concentrations (mmol) during a given growth period and ΔC_2 is the difference in metabolism products carbon concentrations (mmol) during the same growth period. Fermentation balance was calculated by the difference in carbon concentration at the stationery and lag growth phase expressed in %.

Chemicals and data processing. All chemicals of analytical grade were used. Each data point represented was averaged from independent triplicate cultures: the standard deviation was not more than 3%. Statistical analysis was performed by using two-way ANOVA Tukey's statistical test using GraphPad Prism 8.0.2 software (San Diego, CA, USA) (Shirvanyan A. et al., 2023).

RESULTS AND DISCUSSION

The study of changes in SGR and viability of yeast depending on growth conditions.

Enhancing the stress resistance of *S. cerevisiae* is a challenging task. Understanding of the mechanisms of cellular regulation of cell protection in *S. cerevisiae* under various stress conditions is also important. The results indicate that the oxygen availability and pH during growth significantly affect SGR of yeast, with the pH having a more pronounced effect in microaerophilic compared to aerobic growth conditions. In microaerophilic growth conditions, an increase in pH leads to a decrease in SGR of yeast (see Figure 1).

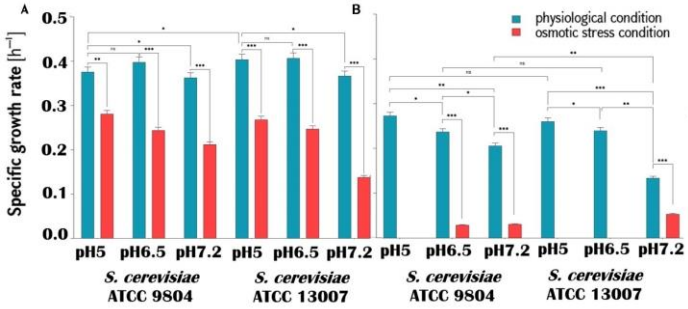

Figure 1. The effect of osmotic stress on the SGR of *S. cerevisiae* ATCC 9804 and ATCC 13007 strains under aerobic (A) or microaerophilic growth depending on pH. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns – non significant

Under physiological conditions, yeast optimally grows at pH 6.5 under aerobic conditions and at pH 5 for microaerophilic conditions. SGR is approximately 1.4-fold (ATCC 9804) and 1.5-fold (ATCC 13007) higher in aerobic conditions, compared to the same parameter under microaerophilic conditions. In osmotic stress conditions, pH 5 is unfavorable for microaerophilic growth of *S. cerevisiae* ATCC 9804, while pH 5 and pH 6.5 are unfavorable for *S. cerevisiae* ATCC 13007 strains (Shirvanyan et al., 2021). Thus, basic conditions (pH 7.2-7.5) are necessary for the growth of yeast under microaerophilic osmotic stress conditions, where the SGR of ATCC 9804 and ATCC 13007 is suppressed by 85% and 60% respectively compared to physiological conditions (Shirvanyan A. et al., 2021). Obtaining large volumes of biomass is crucial for efficient production. The maximum number of viable cells is present during aerobic growth of ATCC 9804 strain under physiological conditions, which is twice as high as the number of viable cells of ATCC 13007 strain (~$6x10^{10}$ cells mL^{-1}). The viability of yeast decreased by 2.6 and 1.7-fold in ATCC 9804 and ATCC 13007 strains, respectively, under osmotic stress during aerobic growth (Shirvanyan et al., 2023)

During microaerophilic growth under osmotic stress in acidic environments, the number of viable cells sharply decreases in both strains, unlike physiological conditions, where at pH 5, the viability of yeast in microaerophilic conditions is twice as high as to aerobic conditions (Shirvanyan A. et al., 2023).

The assessment of osmotic stress on membrane lipids peroxidation processes.

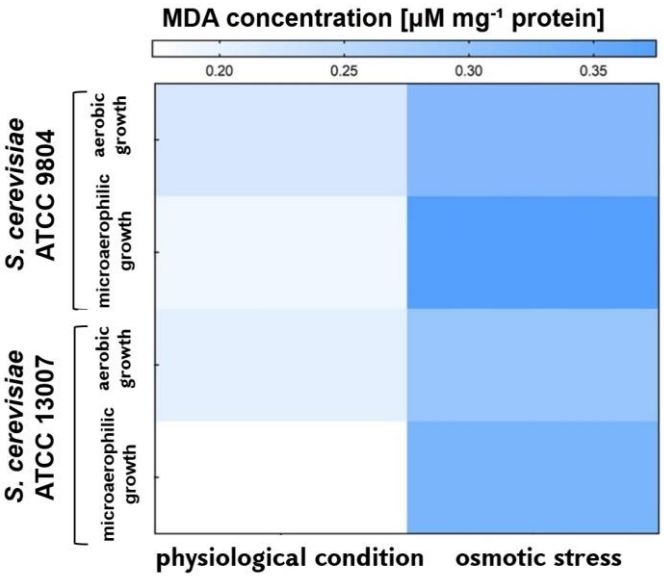

Figure 2: Heat map showing the change in MDA levels (μM per mg of protein) during the growth of *S. cerevisiae* ATCC 9804 and ATCC 13007 under osmotic stress condition.

To assess the impact of stress on biomembranes, it is important to study the processes of lipid peroxidation. Results show a significant increase in the amount of malondialdehyde, doubling its quantity compared to physiological conditions for both strains. This emphasizes the activation of oxidative processes under osmotic stress conditions. The amount of malondialdehyde increases due to oxygen limitation and osmotic stress (see Figure 2). These findings are also consistent with the results of another study, where it was demonstrated that adaptation to high levels of ethanol in yeast occurs through changes in the activity of plasma membrane H^+-ATPase and lipid composition.

Investigation of H^+, K^+ and Na^+ fluxes during growth of different strains of *S. cerevisiae* under physiological and osmotic stress conditions.

Understanding the role of potassium, sodium, and proton transporters in stress conditions is important for exploring yeast adaptation pathways to osmotic stress and the functioning of metabolic regulatory systems. To investigate the role of ion channels and transporters in the adaptation processes to stress conditions, potassium, sodium, and proton fluxes in whole yeast cells were studied depending on the presence of oxygen during growth. In all samples during glucose assimilation under physiological conditions, potassium influx was observed, unlike osmotic stress

conditions, where potassium fluxes were primarily directed towards the extracellular environment (see Figure 3). The most intense potassium flux was confirmed during microaerophilic growth under physiological conditions, where it exceeded the rate of potassium flux under aerobic conditions by approximately 2.5 times for the ATCC 9804 strain and by ~3.4 times for the ATCC 13007 strain. The addition of glucose stimulates accelerated potassium influx, which is important during the adaptation phase to stressful conditions. Addition of the proton ATPase inhibitor DCCD disrupts this process. In *S. cerevisiae* ATCC 9804, the ratio of DCCD-sensitive proton-potassium fluxes remain stable under both physiological and osmotic stress conditions during both aerobic and microaerophilic growth. Unlike the ATCC 13007 strain, potassium flux in physiological conditions during microaerophilic growth of the ATCC 9804 strain is entirely DCCD-dependent. This suggests that the potassium transport systems in the ATCC 9804 stain are related to or metabolically intersect with proton ATPases such as Pma1/2 or FoF_1-ATPase, and these interactions may play a key role in regulating yeast adaptation processes under osmotic stress conditions. This demonstrates tightly regulated crosstalk between potassium and proton transporters of the plasma membrane (Shirvanyan et al., 2023).

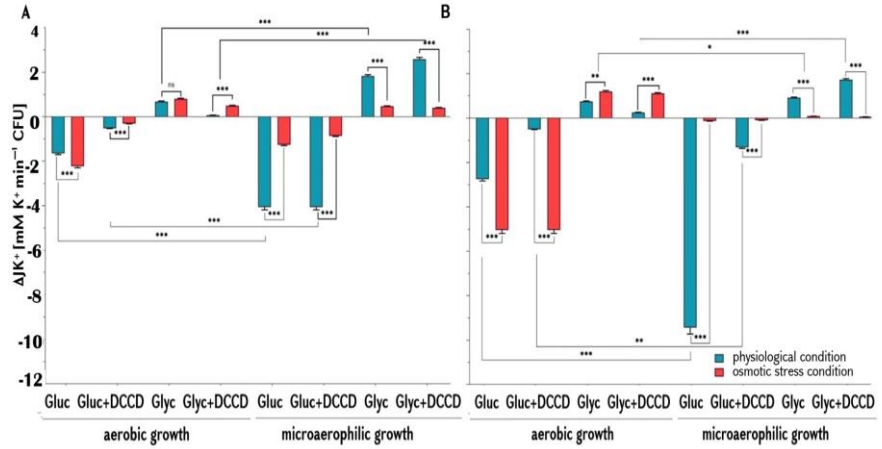

Figure 3. The effect of osmotic stress on the potassium flux rate through the whole-cell membrane of *S. cerevisiae* ATCC 9804 (A) and ATCC 13007 (B) yeast strains depending on the environmental conditions. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns - non significant.

Unlike K^+, proton flux in *S. cerevisiae* cells is directed towards the extracellular environment (see Figure 4). This is consistent with previous studies showing that under fermentation conditions, protons are pumped out of the cell, creating a proton motive force. In both aerobic and microaerophilic growth conditions, cells subjected to osmotic stress alone and treated with DCCD, adding glycerol to the experimental medium stimulates proton influx in both strains, correlating with potassium efflux. Irrespective of the growth conditions (physiological or osmotic stress), adding glucose to the experimental medium stimulates proton efflux in *S. cerevisiae* ATCC 9804 cells, with the rate of proton flow decreasing by 1.3-fold in cells treated with

DCCD. In all other samples, inhibiting the activity of the ATPase by DCCD disrupts proton flux, reducing its rate and even, in some cases, altering the flux direction. These are in good conformity with previous studies, demonstrating that glucose transport in anaerobic *S. cerevisiae* occurs via a diffusion-mediated mechanism, and maximal ethanol production is observed when transport is proton-mediated, with lower ATP output during sugar dissimilation.

Suppression of proton flux can occur due to ethanol accumulation in the surrounding environment and its increased impact on cellular membranes. The results indicate (see Figure 5) that sodium flux is stimulated in both strains during aerobic and microaerophilic growth under osmotic stress conditions. However, the stimulation of sodium flux is lower (by 1.5 and 1.1 times) due to oxygen limitation during growth in ATCC 9804 and ATCC 13007 strains, respectively, compared to aerobic stress conditions (by 2.6-fold and 4.1-fold in ATCC 9804 and ATCC 13007 strains, respectively). Comparing these data with the results of potassium flux, which show the highest potassium flux in microaerophilic growth conditions, it can be concluded that the exchange of Na^+/K^+ plays an important role in yeast adaptation to stress factors in aerobic, and the exchange of K^+/H^+ in microaerophilic conditions. This fact corresponds to and is explained by the evolutionary development of living organisms and the formation of adaptation

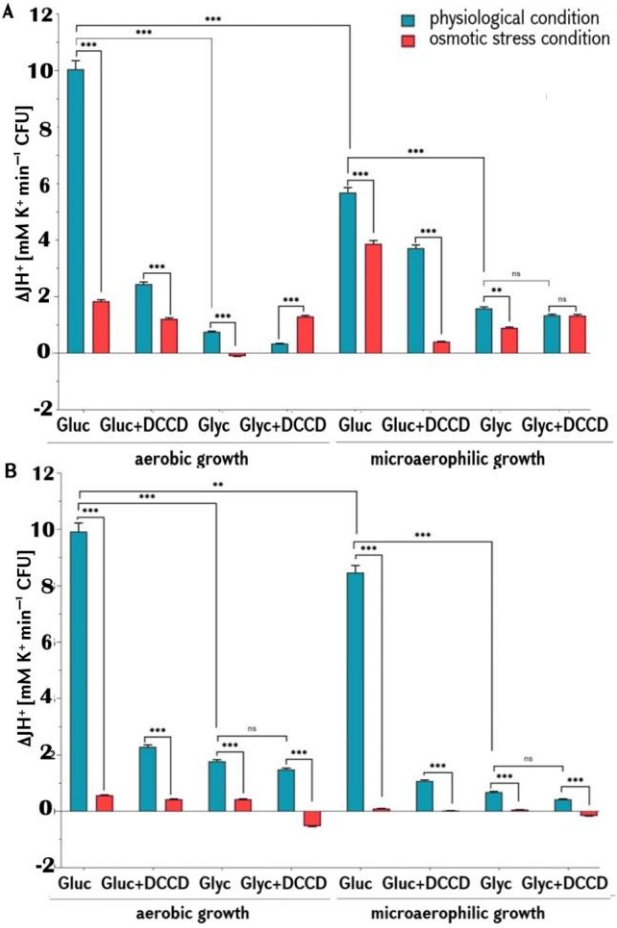

Figure 4. The effect of osmotic stress on the proton flux rate through the whole-cell membrane of *S. cerevisiae* ATCC 9804 (A) and ATCC 13007 (B) yeast strains depending on the environmental conditions. * p < 0.05; ** p < 0.01; *** p < 0.001, ns - non significant.

mechanisms. Thus, it is corroborated that life originated in hydrothermal vents of the World Ocean. These hydrothermal vents were rich in potassium, and the concentration of small inorganic molecules and ions in protocells and their surroundings was the same because the first cells did not have membrane pumps or semi-permeable membranes. Having developed it, they obtained potassium-selective transporters, which, in combination with protons, maintain membrane potential and ATP synthesis. Later, the gradual transition of living organisms from deep-sea hydrothermal vents to higher layers increased the need to adapt to high levels of sodium, enabling transporters to transport sodium. Furthermore, it was shown that yeast K^+ or Na^+ ATPases evolved from an earlier potassium ATPase through the duplication of its gene to better adapt to sodium. They demonstrated that *Schizosaccharomyces pombe* is the only known fungus lacking duplication of this gene and lacking sodium ATPase activity. This duplication was constant for yeast until they were constantly exposed to sodium-rich seawater or similar liquids, and the former proton ATPase evolved into the current Na^+/K^+ ATPase. Emerging from the seas and adapting to aerobic conditions may have led to the predominance of the role of Na^+/K^+ exchange systems in stress factor adjustment.

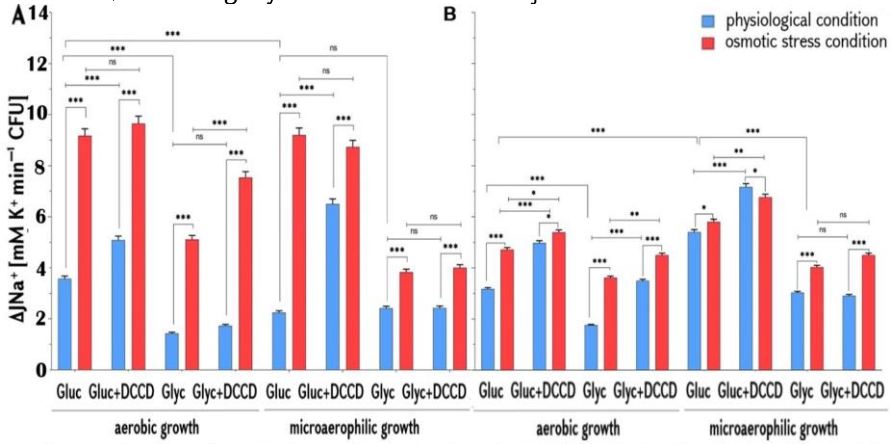

Figure 5. The effect of osmotic stress on the sodium flux rate through the whole-cell membrane of *S. cerevisiae* ATCC 9804 and ATCC 13007 yeast strains during aerobic (A) or microaerophilic (B) growth. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns - non significant.

The increase in sodium flux confirms the previously identified mechanism whereby osmotic stress induced by HOG stimulation of cation transporters in plasma and vacuolar membranes is essential for restoring normal cytosolic ion strength and osmolarity. The latter ensures the generation of an effective transcriptional response to stress factors. Upon the addition of glucose, sodium flux is entirely dependent on DCCD during physiological aerobic growth in both strains. Such dependence is not observed in the case of microaerophilic conditions. This indicates that sodium flux in the processes of adaptation and/or response to stress in aerobic conditions is associated with proton ATPases, and once again underscores the role of Na^+/K^+ exchange systems, such as Na^+, K^+-ATPase, in adapting to stress factors in aerobic

conditions. A completely opposite phenomenon is observed when glucose or glycerol is added to ATCC 9804 cells subjected to osmotic stress; in this case, the increase in osmolarity of the medium stimulates DCCD-dependent sodium flux, indicating the role of K^+/H^+ exchange systems in the stress-adaptation processes of this strain when grown under oxygen limitation. In contrast to ATCC 9804 strain, sodium flux in ATCC 13007 cells subjected to osmotic stress, regardless of oxygen availability during growth, is DCCD-independent (Shirvanyan A. et al., 2023).

The impact of osmotic stress on the activity of antioxidant enzymes in *S. cerevisiae*.

One of the targets of osmotic stress is the oxidative-reductive processes within cells, and antioxidant enzymes play a significant role in cellular response and adaptation to it, regulating the disrupted redox balance. During aerobic growth of the ATCC 9804 strain, no significant changes in CAT activity were observed under the influence of osmotic stress factors, unlike the ATCC 13007 strain, where the enzyme activity increased by ~1.5 times. Removal of available sulfhydryl groups using a unique inhibitor, NEM, stimulated enzyme activity under physiological conditions in both aerobic and microaerophilic growth of the ATCC 9804 strain. Conversely, binding of available sulfhydryl groups in the ATCC 13007 strain did not alter enzyme activity (see Figure 6).

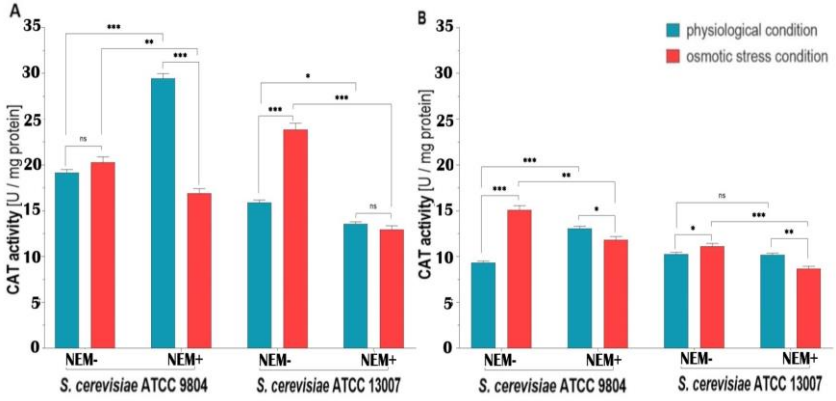

Figure 6. The effect of osmotic stress on the catalase activity in *S. cerevisiae* yeast strains ATCC 9804 and ATCC 13007, cultivated under aerobic (A) or microaerophilic (B) conditions. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns - not significant

This indicates that the CAT enzyme activity in the ATCC 13007 strain is not dependent on the quantity of available sulfhydryl groups under physiological conditions. The role of sulfhydryl groups in regulation of CAT enzyme activity increases under osmotic stress conditions, where binding of available SH groups in both aerobic and microaerophilic growth conditions lead to a 24% decrease in enzyme activity in ATCC 9804 and a 28% decrease in ATCC 13007. During growth of the ATCC 9804 and 13007 strains under microaerophilic conditions, CAT activity decreases by 2 and 1.5 times, respectively, compared to physiological aerobic growth conditions. Under osmotic stress in microaerophilic conditions, the enzyme activity of

the ATCC 9804 strain increases by 63%. In both strains, SOD is twice as active under aerobic physiological conditions compared to microaerophilic conditions (see Figure 7). Under osmotic stress, enzyme activity increases, especially in aerobic conditions, where the ATCC 13007 strain exhibits 1.5 times higher SOD activity. Binding of available SH groups during growth in both aerobic and microaerophilic physiological conditions stimulate enzyme activity by 53% (aerobic) and 22% (microaerophilic) in the ATCC 9804 strain and by 90% (aerobic) and 59% (microaerophilic) in the ATCC 13007 strain. Remarkably, binding of available sulfhydryl groups during aerobic growth of both strains under osmotic stress increases SOD activity, unlike microaerophilic conditions. This indicates that sulfhydryl groups play a distinct role in regulating enzyme activity under aerobic conditions, which sharply differs from the regulation of enzyme activity under microaerophilic conditions. The role of antioxidant enzymes under oxygen limitation remains unclear, and increasing research is aimed at identifying it.

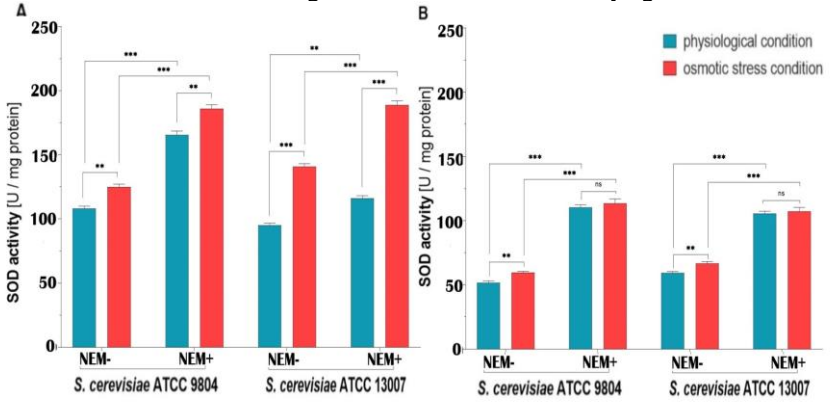

Figure 7. The effect of osmotic stress on the SOD activity in *S. cerevisiae* yeast strains ATCC 9804 and ATCC 13007, cultivated under aerobic (A) or microaerophilic (B) conditions. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns - not significant

Our study demonstrates that not only glutathione peroxidase and thioredoxin reductase depend on thiol groups but also play a significant role in the direct or indirect regulation of CAT and SOD activity. The study reveals the role of antioxidant enzymes and regulatory mechanisms of their activity under osmotic stress conditions. The results can be used for effective manipulation of these enzymes in genetic engineering to develop resilient strains (Shirvanyan A. et al., 2023).

The study of sulfhydryl groups level changes in yeast under physiological and osmotic stress conditions.

To study redox changes under osmotic stress conditions, the ratio of reduced and oxidized thiol groups in yeast cells grown under aerobic and microaerophilic conditions and subjected to osmotic stress was investigated. The results (see Figure 8) show that the total number of oxidized thiol groups in the stationary growth phase remains constant regardless of growth conditions, oxygen availability, or strain type,

presumably due to disulfide bonds of constitutive proteins. In contrast, upon transition from aerobic growth to microaerophilic growth, the number of reduced thiol groups in physiological conditions increases in both strains, likely due to the assimilation of a large amount of amino acids containing thiol groups from the surrounding environment and the activation of glutathione biosynthesis. Under osmotic stress during aerobic growth, the number of reduced thiol groups increases by 45% and 34% in ATCC 9804 and ATCC 13007 strains, respectively. This increase may be associated with the activation of non-enzymatic antioxidant glutathione biosynthesis. A similar pattern was observed in other yeast strains such as *Candida utilis*, where glutathione biosynthesis increased by 70% under osmotic stress induced by sodium chloride. Under osmotic stress conditions during microaerophilic growth, the number of thiol groups decreases by approximately 50% in both strains. This suggests that peroxide enzymes, whose activity is regulated by these free thiol groups, play a central role in yeast adaptation to aerobic rather than microaerophilic growth conditions.

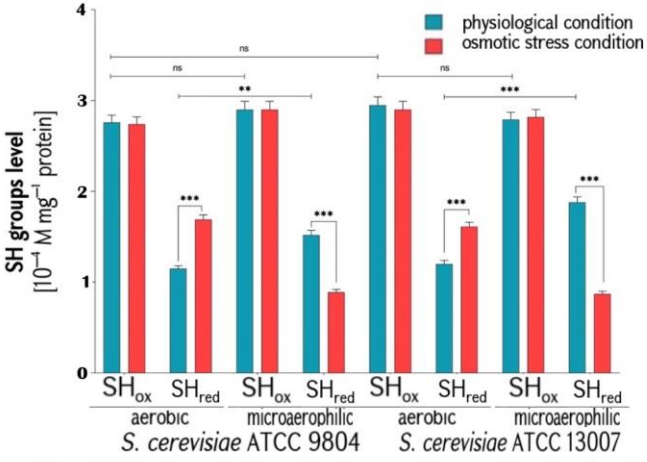

Figure 8. The impact of osmotic stress on the quantity of reduced and oxidized thiol groups in *S. cerevisiae* ATCC 9804 and ATCC 13007. * p < 0.05; ** p < 0.01; *** p < 0.001, ns - not significant.

This can be explained by the absence of a preferred carbon source, glucose, and extremely slow assimilation of glycerol, as mentioned earlier. As a result, cells cannot meet their energy demands, so they utilize amino acids from the surrounding environment as an energy source. Thus, it has been shown that yeast growth on glycerol medium is highly dependent on the medium composition and the presence of additives such as amino acids or nitrogenous bases, but the mechanism by which these compounds affect growth under these conditions remains unknown. It is also known that upon oxidation of 1 molecule of reduced glutathione (2GSH + NADP = GSSG + NADPH), the Gibbs free energy is 58 kJ, which would be sufficient for the synthesis of 2 moles of ATP. Therefore, under microaerophilic stress conditions, the reduction in the number of reduced sulfhydryl groups may be an important mechanism for regulating the cell's bioenergetic charge and, consequently, yeast survival under these conditions.

Changes in the activity of mitochondrial ATPase under osmotic stress.

Results show that mitochondrial proton-ATPase (see Figure 9) reveals that it is more active under aerobic conditions, approximately threefold higher than that observed under microaerophilic physiological conditions in both strains. It should be noted that during fermentative metabolism, ATPase acts in the direction of ATP hydrolysis, whereas under aerobic respiration, it operates in the opposite direction, toward ATP synthesis.

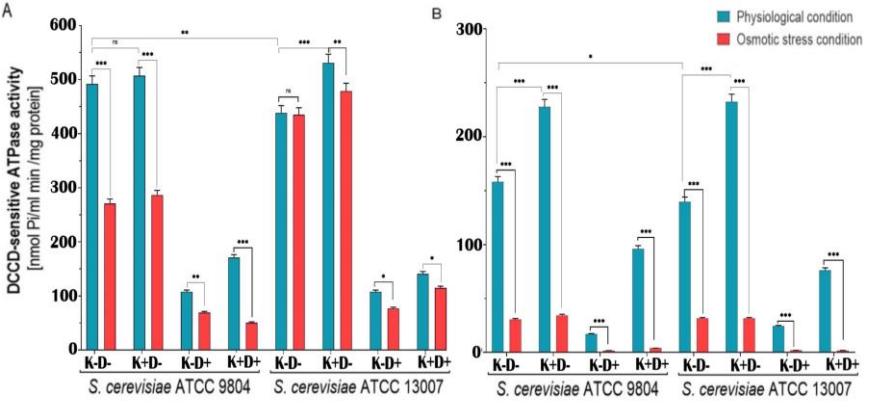

Figure 9. DCCD-sensitive mitochondrial F_0F_1-ATPase activity of *S. cerevisiae* ATCC 9804 and ATCC 13007 cells at physiological or osmotic stress conditions during aerobic (A) or microaerophilic (B) growth. The DCCD (0.5 mM) and potassium ions (100 mM) were added into the assay medium when indicated "D+" or "K+". *p<0.05; **p<0.01; ***p<0.001, ns-non significant.

During the growth of cells subjected to osmotic stress under oxygen-limiting conditions, the overall activity of ATPase is significantly low and not dependent on DCCD, indicating that other ATPases, such as V-type (vacuolar) ATPases or Na^+/K^+-ATPases, contribute more to these conditions. In all investigated conditions, the addition of potassium to the experimental medium stimulated ATPase activity. The latter increased by 21% during aerobic growth and approximately 1.7-fold during microaerophilic growth of ATCC 13007 strain under physiological conditions, correlating with potassium influx and proton and sodium efflux under these conditions. Unlike ATCC 13007, the influence of potassium ions on ATPase activity in aerobic conditions was negligible in the other studied strain. ATPase activity is suppressed approximately fivefold under microaerophilic growth conditions of yeast subjected to osmotic stress. This may be due to a decrease in mitochondrial biomass, especially its protein mass, caused by oxygen limitation. Potassium also affects the sensitivity of mitochondrial ATPase to DCCD: its effect increases DCCD sensitivity by 3-4 times during microaerophilic growth of different strains (Shirvanyan A. et al., 2023).

Analysis of intermediate compounds of different yeast strains under physiological and osmotic stress conditions.

To elucidate various metabolic pathways and the mechanisms regulating them in yeast under osmotic stress conditions depending on the growth conditions, a high-performance liquid chromatographic analysis of intermediates was conducted. The results show that glucose assimilation in *S. cerevisiae* cells begins at the third hour of growth, and by the 24th hour, it is completely assimilated by both strains under both aerobic and microaerophilic growth conditions. For instance, the rate of glucose assimilation during aerobic growth in physiological conditions is 4.6 mmol/h in all samples, whereas under microaerophilic conditions, the rate of glucose assimilation decreases by almost half in both strains. The maximum ethanol production (82.1±2.5 mmol (ATCC 9804); 117.25±3.5 mmol (ATCC 13007)) is observed at 24-32 hours of growth under physiological conditions. In physiological conditions, the maximum ethanol yield (mmol) per glucose (mmol) is 0.28 and 0.74 for ATCC 9804 strain and 0.37 and 1.06 for ATCC 13007 strain under aerobic and microaerophilic growth, respectively. During aerobic growth in physiological conditions, the glycerol production rate was 4 μmol/h and 1.3 μmol/h in *S. cerevisiae* ATCC 9804 strains and ATCC 13007, respectively, significantly lower than the glycerol production rate under microaerophilic conditions (98 μmol/h and 175 μmol/h for the same strains). Under oxygen-limiting conditions in physiological conditions, the ethanol/ (glycerol + acetic acid) ratio, calculated per mole of glucose or glycerol, remains constant for both yeasts. Under microaerophilic growth conditions, yeast subjected to osmotic stress did not exhibit fermentation end products. However, a small formation of formic acid, absent during glucose fermentation, was detected. The absorption of formic acid by yeast was also observed by other groups. To assess the metabolic activity of yeast, the ratio of each mole of glucose or glycerol (succinic+malic acid) /pyruvic acid and ethanol/(glycerol + acetic acid) was determined under microaerophilic conditions in the stationary growth phase. Under aerobic conditions, the ratio (succinic+malic acid)/pyruvic acid, calculated per mole of glucose or glycerol, did not differ significantly between ATCC 9804 and ATCC 13007 strains under both physiological and osmotic stress conditions. However, under stress, the ratio decreased by 1.4 times compared to physiological conditions. The fermentation equilibrium, indicating the rate of carbon transformation, was 75% in *S. cerevisiae* ATCC 13007 and 52% in *S. cerevisiae* ATCC 9804 at 24-32 hours of growth under physiological conditions. In the case of *S. cerevisiae* ATCC 13007, the low carbon transformation values may be due to the high biomass production yield as well as the production of volatile compounds such as ethanol, acetic acid, or carbon dioxide. In the case of *S. cerevisiae* ATCC 9804 strains, the fermentation equilibrium remains open, likely due to the production of other secondary intermediates such as organic acids, which require further investigation for identification (Shirvanyan A. et al., 2023).

CONCLUSIONS

The following conclusions were made based on experimentally obtained results:

1. The SGR of yeast cells subjected to osmotic stress decreases under oxygen limitation and acidic conditions. Compared to physiological conditions, the SGR of reduced by 85% and 60% in the *S. cerevisiae* ATCC 9804 and ATCC 13007 strains, respectively.

2. In the stationary phase of microaerophilic growth of *S. cerevisiae* ATCC 9804 under physiological conditions, the medium osmolality exceeds that of aerobic growth by 1.3 times. The change in medium osmolality depends on the nature of the respiratory or fermentative metabolism of the yeast, the strain, and the availability of oxygen during growth.

3. Unlike *S. cerevisiae* ATCC 13007, the potassium influx in ATCC 9804 strain depends entirely on DCCD during growth under physiological microaerophilic conditions. The affinity of potassium transporters to K^+ decreased when transitioning from aerobic to microaerophilic growth, as well as under osmotic stress conditions, and was particularly low in samples inhibited by DCCD. The highest affinity to potassium was recorded under aerobic growth conditions for strain ATCC 9804 (Km=0.075 mmol), and the lowest was in microaerophilic growth conditions for ATCC 13007 strain inhibited by DCCD (Km=1.12 mmol).

4. Sodium efflux increases under osmotic stress conditions. It is DCCD-dependent in both strains during aerobic growth under physiological, but not stress conditions. The Na^+/K^+ exchange plays a decisive role in the adaptation of aerobic yeast to osmotic stress conditions, while the K^+/H^+ exchange plays a decisive role in overcoming stress under oxygen limitation.

5. Both strains exhibit CAT and SOD activity twice as high under aerobic physiological conditions compared to microaerophilic growth conditions. Under osmotic stress conditions, enzyme activity increases, especially in aerobic conditions, where ATCC 13007 strain exhibits 1.5 times higher SOD activity.

6. Binding of available SH-groups during growth in both aerobic and microaerophilic physiological conditions stimulate enzyme activity by 53% (aerobic) and 22% (microaerophilic) in ATCC 9804 strain, and by 90% (aerobic) and 59% (microaerophilic) in ATCC 13007. Under aerobic growth, binding of available sulfhydryl groups under osmotic stress increases enzyme activity, unlike microaerophilic conditions.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. Shirvanyan A., Mirzoyan S., Trchounian K. 2021. Fruit wastes as a substrate for yeast biomass production. Biotechnology: Science and practice, innovation, and business". International Scientific and Practical Conference, abstracts, 2021, part 1, 74-75 pp. ISBN: 978-9939-1-1354-8.

2. Shirvanyan A. H., Mirzoyan S. N., Trchounian K.A. 2021. Peculiarities of growth parameters of *Saccharomyces cerevisiae* under different conditions.

Proceedings of the YSU B: Chemical and Biological Sciences, Vol.55, No. 3 (256), 2021, 254-265 pp. https://doi.org/10.46991/PYSU:B/2021.55.3.255.

3. Shirvanyan A., Mirzoyan S., Trchounian K. Potassium and proton ions transport during glucose fermentation in *Saccharomyces cerevisiae* under glycerol-induced osmotic stress at different pHs." In FEBS OPEN BIO, 2022, vol. 12, pp. 179-180. https://doi.org/10.1002/2211-%205463.13440.

4. Shirvanyan, A., Mirzoyan, S., & Trchounian, K. (2023). Relationship between proton/potassium fluxes and central carbon catabolic pathways in different *Saccharomyces cerevisiae* strains under osmotic stress conditions. Process Biochemistry, 133, pp. 309-318. https://doi.org/10.1016/j.procbio.2023.09.015.

5. Shirvanyan A. H. 2023. Evaluation of ethanol and biomass production rate by different *Saccharomyces cerevisiae* strains depending on external ph and temperature. proceedings of the YSU B: Chemical and Biological Sciences, 57(2 (261), 141-153. https://doi.org/10.46991/PYSU:B/2023.57.2.141.

6. Shirvanyan A., Mirzoyan S., Trchounian K. Regulation of catalase and superoxide dismutase activities by sodium and potassium ions in *Saccharomyces cerevisiae*. The 47th FEBS Congress: Together in Biosience for a better future. FEBS OPEN BIO, vol. 13 (2), 2023, 107-108pp. https://doi.org/10.1002/2211-5463.13646.

ՇԻՐՎԱՆՅԱՆ ԱՆԱՀԻՏ ՀԱՄԲԱՐՁՈՒՄԻ

ԽՄՈՐԱՍՆԿԵՐԻ ԱՃԻ ԵՎ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ՖԵՐՄԵՆՏՆԵՐԻ ԱԿՏԻՎՈՒԹՅԱՆ ՎՐԱ ՏԱՐԲԵՐ ՍԹՐԵՍԱՅԻՆ ԳՈՐԾՈՆՆԵՐԻ ԱԶԴԵՑՈՒԹՅԱՆ ՄԵԽԱՆԻԶՄՆԵՐԸ

Ամփոփագիր

Բանալի բառեր՝ *S. cerevisiae, օսմոսային սթրես, աճի տեսակարար արագություն, կենսունակություն, կալիումի, նատրիումի և պրոտոնի հոսքեր, Աէֆազային ակտիվություն, կատալազ, սուպերօքսիդ դիսմուտազ*

Սթրեսային պայմաններում խմորասնկերի նյութափոխանակային փոփոխությունների ուսումնասիրումը առանցքային է սթրեսակայուն շտամների զարգացման և արտադրական գործընթացների բարելավման համար: Սույն աշխատանքում ուսումնասիրվում է գլիցերոլով խթանված օսմոսային սթրեսի ազդեցությունը *S. cerevisiae* ATCC 9804 և ATCC 13007 շտամների, մասնավորապես աճի պարամետրերի, իոնների հոսքերի, կենսաթաղանթների վնասման, հակաօքսիդանտային ֆերմենտների ակտիվության, վերօքս հավասարակշռության և կենսաէներգետիկական ցուցանիշների վրա կախված աճման ընթացքում թթվածնի հասանելիությունից և pH-ից (5, 6.5 և 7.2):

Ստացված արդյունքները ցույց են տալիս, որ օսմոսային սթրեսի ենթարկված խմորասնկերի աճի տեսակարար արագությունը նվազում է

թթվածնի սահմանափակման և թթվային պայմաններում: Ֆիզիոլոգիական պայմանների համեմատ *S. cerevisiae* ATCC 9804 շտամի աճի տեսակարար արագությունն ընկճվում է 85%-ով, իսկ *S. cerevisiae* ATCC 13007 շտամի դեպքում՝ 60%-ով:

Ի տարբերություն ATCC 13007 շտամի, կալիումի հոսքը լիովին ԴՑԿԴ-կախյալ է ATCC 9804 շտամի ֆիզիոլոգիական միկրոաերոֆիլ պայմաններում աճի ժամանակ: Նատրիումի արտահոսքը մեծանում է օսմոսային սթրեսային պայմաններում: Այն ԴՑԿԴ-զգայուն է երկու շտամերում էլ՝ ֆիզիոլոգիական, բայց ոչ սթրեսային պայմաններում աերոբ աճի ընթացքում: Na^+/K^+ փոխանակությունը վճռորոշ դեր է կատարում աերոբ պայմաններում *S. cerevisiae* ATCC 9804 և ATCC 13007 խմորասնկերի օսմոսային սթրեսային պայմաններին հարմարվելու մեջ, մինչդեռ K^+/H^+ փոխանակությունը՝ թթվածնի սահմանափակման պայմաններում սթրեսի հաղթարահման մեջ: H^+-ԱԵՖազի արգելակումը խաթարում է պրոտոնների, կալիումի և նատրիումի հոսքը թաղանթների միջով:

S. cerevisiae ATCC 9804 և ATCC 13007 շտամերում ՍՕԴ-ի և ԿԱՏ-ի ակտիվությունը կրկնակի բարձր է աերոբ ֆիզիոլոգիական պայմաններում՝ միկրոաերոֆիլ աճման պայմանների համեմատությամբ: Օսմոսային սթրեսի ազդեցությամբ ֆերմենտի ակտիվությունը մեծանում է հատկապես աերոբ պայմաններում, որտեղ ATCC 13007 շտամը ցուցաբերում է 1.5 անգամ ավելի բարձր ՍՕԴ ակտիվություն: Թե՛ աերոբ, թե՛ միկրոաերոֆիլ ֆիզիոլոգիական պայմաններում աճման ընթացքում հասանելի SH խմբերի կապումը խթանում է ֆերմենտի ակտիվությունը 53%-ով (աերոբ) և 22%-ով (միկրոաերոֆիլ) ATCC 9804 շտամում և 90%-ով (աերոբ) և 59%-ով (միկրոաերոֆիլ) ATCC 13007 շտամում: Օսմոսային սթրեսի ազդեցությամբ երկու շտամերի աերոբ աճման ժամանակ հասանելի սուլֆհիդրիլային խմբերի կապումը մեծացնում է ֆերմենտի ակտիվությունը, ի տարբերություն միկրոաերոֆիլ պայմանների:

Այսպիսով, շրջապատող միջավայրի փոփոխվող պայմաններում խմորասնկերի բջջային պատասխանի և հարմարվելու մոլեկուլային մեխանիզմների ուսումնասիրությունը հնարավորություն կտա օպտիմալիզացնել արտադրական գործընթացները: Ստացված արդյունքները մեծ ներդրում կարող են ունենալ տարբեր նյութերի, օրինակ՝ կենսաէթանոլի, սպիտակուցների, վիտամինների արտադրության ելքի բարձրացման համար: Ավելին, սթրեսային պայմաններին խմորասնկային բջիջների արձագանքման ուղիների պարզաբանումը կարևոր է կենսաբժշկական կիրառությունների համար և կարող է նպաստել շրջակա միջավայրի պահպանման և թերապևտիկ ռազմավարությունների մշակման հարցերում:

ШИРВАНЯН АНАИТ АМБАРЦУМОВНА

ВЛИЯНИЕ РАЗЛИЧНЫХ СТРЕССОВЫХ ФАКТОРОВ НА ПАРАМЕТРЫ РОСТА ДРОЖЖЕЙ И АКТИВНОСТЬ АНТИОКСИДАНТНЫХ ФЕРМЕНТОВ

РЕЗЮМЕ

Ключевые слова: *S. cerevisiae, осмотический стресс, удельная скорость роста, жизнеспособность, потоки калия, натрия и протонов, АТФазная активность, каталаза, супероксиддисмутаза*

Изучение метаболических изменений дрожжей в условиях стресса является ключом к созданию стрессоустойчивых штаммов и совершенствованию производственных процессов. В данной работе изучено влияние осмотического стресса стимулированного глицерином на штаммы *S. cerevisiae* ATCC 9804 и ATCC 13007, в частности на параметры роста, потоки ионов, повреждение биомембраны, активность антиоксидантных ферментов, окислительно-восстановительный баланс и биоэнергетические показатели в зависимости от доступности кислорода и pH во время роста (5; 6.5 и 7.2).

Полученные результаты показывают, что удельная скорость роста дрожжей, подвергнутых осмотическому стрессу, снижается в условиях ограничения кислорода и кислотной среды. По сравнению с физиологическими условиями удельная скорость роста штамма *S. cerevisiae* ATCC 9804 снижается на 85 %, а в случае штамма *S. cerevisiae* ATCC 13007 — на 60 %.

В отличие от штамма ATCC 13007 поток калия у штамма ATCC 9804 полностью зависит от DCCD при росте в физиологических микроаэрофильных условиях. Отток натрия увеличивается в условиях осмотического стресса. Он чувствителен к DCCD у обоих штаммов во время аэробного роста в физиологических, но не стрессовых условиях. Обмен Na^+/K^+ играет решающую роль в адаптации к условиям осмотического стресса *S. cerevisiae* ATCC 9804 и ATCC 13007 в аэробных условиях, тогда как обмен K^+/H^+ играет решающую роль в преодолении стресса в условиях ограниченного кислорода. Ингибирование фазы H^+-АТФазы нарушает поток протонов, калия и натрия через мембраны.

У штаммов *S. cerevisiae* ATCC 9804 и ATCC 13007 активность СОД и КАТ в аэробных физиологических условиях вдвое выше, чем в микроаэрофильных условиях роста. Под влиянием осмотического стресса активность фермента возрастает, особенно в аэробных условиях, где штамм ATCC 13007 проявляет в 1.5 раза большую активность СОД. Связывание доступных SH-групп в процессе роста как в аэробных, так и в микроаэрофильных физиологических условиях стимулировало активность ферментов на 53% (аэробные) и 22% (микроаэрофильные) у штамма ATCC 9804 и на 90% (аэробные) и 59% (микроаэрофильные) у штамма ATCC 13007. Связывание доступных сульфгидрильных групп при аэробном росте обоих штаммов под влиянием

осмотического стресса повышает активность фермента в отличие от микроаэрофильных условий.

Таким образом, изучение молекулярных механизмов реакции дрожжевых клеток и адаптации к изменяющимся условиям окружающей среды позволит оптимизировать производственные процессы. Полученные результаты могут внести большой вклад в увеличение выхода различных веществ, например, биоэтанола, белков, витаминов в промышленности. Кроме того, выяснение путей реакции дрожжевых клеток на стрессовые условия важно для биомедицинских применений и может способствовать разработке стратегий сохранения окружающей среды и терапии.