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RESPONSE TO THERMAL AND ETHANOL STRESS IN INDUSTRIAL STRAINS OF SACCHAROMYCES CEREVISIAE: EFFECTS ON CELL GROWTH AND PROTEIN PRODUCTION

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ABSTRACT

The synergism between thermal and ethanol stress directly affects the functionality and survival of yeasts in industrial environments, compromising their cellular physiology. Therefore, this study aims to compare the proteins associated with thermal and ethanol stress, as well as to investigate the impacts of these factors on cell growth and total protein production. The Fleischmann®, Santa Adélia, and Pedra-2 yeasts were cultivated in 2% YPD medium. The biomass was recovered by centrifugation and inoculated in sugarcane juice at 22ºBrix, pH 5.0, and incubated at 30 and 40ºC, with ethanol concentrations ranging from 0, 5, 12, and 16% (v.v-1). Lysis occurred in Tris-HCl and glass beads, and total protein quantification was performed using the Bradford method, followed by readings in a spectrophotometer at 595 nm. Heat shock proteins (HSPs), especially HSP70 and HSP104, play a crucial role in cellular protection and maintenance under adverse conditions. The Fleischmann® lineage showed lower tolerance, with a significant reduction in cell growth. On the other hand, the Pedra-2 lineage demonstrated greater robustness, standing out for its greater biomass production and stress tolerance. Principal component analysis (PCA) confirmed the correlation between total protein production and stress tolerance, consolidating the Pedra-2 lineage as the most promising for industrial applications. This study provides valuable contributions to understanding the interaction of heat shock proteins with severe stress factors in industrial yeasts, with the aim of improving efficiency and promoting sustainability in fermentation processes.

Keywords: Protein Quantification. Cell Growth. Industrial Yeasts.

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INTRODUCTION

Saccharomyces cerevisiae is widely used in biotechnological processes due to its versatility and adaptability, being one of the main yeasts used in large-scale bioethanol production (AZHAR et al., 2017). This yeast also plays an important role in making second-generation ethanol viable, aligning with the Sustainable Development Goals (SDGs), which encourage the use of renewable energy and industrial innovation. Despite its efficiency, the fermentation environment presents significant challenges, such as high concentrations of ethanol, thermal variations, and osmotic stresses, which directly impact cellular metabolism, plasma membrane integrity, and cell viability (WALKER & BASSO, 2020).

According to Lairón-Peris et al. (2021), stress factors such as temperature variations, high ethanol concentrations, osmotic stress, pH fluctuations, and the presence of contaminating agents exert negative pressure on the biochemical, physiological, and genetic mechanisms in yeast. These factors can cause changes in cellular metabolism, impacting the formation of undesirable metabolites and reducing fermentation efficiency (COERTJENS et al., 2023). Paradoxically, although ethanol is a natural product of yeast metabolism, its high concentration in the fermentation medium becomes toxic to these microorganisms. This toxicity causes changes in metabolic pathways and cellular compartments, compromising the biotechnological potential of yeasts (MUELLER et al., 2020). Bearing in mind that ethanol has the ability to cross the cell membrane, increasing its fluidity and permeability, these changes in prolonged fermentation times and high temperatures can lead to loss of viability and apoptosis (JIN et al., 2022).

In high concentrations, ethanol can generate drastic effects, such as phospholipid accumulation in the plasma membrane. In this environment, it interacts with unsaturated fatty acids and proteins located in the membrane, altering its structural and functional dynamics (CHETTY et al., 2022). These interactions compromise the mobility of fatty acid chains and membrane proteins, increasing the polarity of the region and making it difficult to exchange polar molecules between the extracellular and intracellular environment (BERTRAND et al., 2020). These changes directly affect the positioning of proteins and phospholipids, impairing the yeast's ability to maintain the concentration gradient of essential compounds present in the plasma membrane. This impairment results in the inhibition of glucose metabolism, fundamental for cell growth and survival (FRALLICCIARDI et al., 2022).

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Among the adaptive responses of yeast, stress conditions trigger protein production mechanisms, which play a crucial role in protecting against damage caused by thermal and ethanol stress. These proteins allow cells to recover their metabolism and integrity, maintaining essential physiological processes, even under adverse conditions. According to Rosenzweig et al. (2019), Heat Shock Proteins (HSPs) function as “molecular chaperones,” preventing the aggregation of proteins denatured at high temperatures, among which the proteins HSP40, HSP60, HSP70, and HSP90 stand out. Furthermore, as noted by Auesukaree (2017), HSPs contribute to the recovery of damaged proteins, ensuring the maintenance of cellular homeostasis under stress conditions.

During the fermentation process, *S. cerevisiae* presents specific adaptive responses to face the stress caused by ethanol. This compound affects the fluidity of the plasma membrane, in addition to damaging macromolecules such as proteins and deoxyribonucleic acid. To minimize the adverse effects of ethanol, yeast activates defense mechanisms that involve a set of proteins, such as Hsp122, Ssb1, Ssb24, HSP106, Gpd18, and Hsp318, which assist in several mechanisms. HSPs can act in association, aiming at the preservation and maintenance of cellular integrity in the presence of thermal and ethanolic stress. According to Ajmal (2023), heat and ethanol stress proteins form a family of proteins whose expression is triggered and positively regulated in response to various stress conditions.

The synergism between stress factors plays a critical role in cellular functioning. Among these factors, temperature variation is particularly significant, as it can cause protein denaturation, leading to improper folding and aggregation of these molecules. This process compromises the stability of the cell wall, a fundamental structure for maintaining the vitality and integrity of the cell. Studies on the production profile of proteins generated in response to thermal and ethanolic stress can deepen our understanding of the challenges faced by yeasts under industrial conditions. This knowledge is crucial for identifying more robust strains capable of optimizing efficiency in bioethanol production, contributing to the sustainability of industrial processes and the preservation of natural resources. In this context, study aims to compare the proteins associated with the response to thermal and ethanol stress in industrial strains of *Saccharomyces cerevisiae*, as well as to evaluate cell growth and total protein production under different ethanol concentrations.

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MATERIAL AND METHODS

STUDY DEVELOPMENT LOCATION

The study was carried out in the Laboratório de Biotecnologia, Bioquímica e Biotransformação located at Centro de Estudos de Recursos Naturais – CERNA at Universidade do Estado de Mato Grosso do Sul, Dourados/MS.

PROTEINS ASSOCIATED WITH HEAT AND ETHANOL STRESS

To gather information about heat and ethanol shock proteins and their conformational structures, a survey was conducted on websites, scientific articles, and online protein databases. The data were collected, analyzed, sorted, and tabulated according to the information relevant to the study.

MICROORGANISMS USED

The yeasts used in this study were *S. cerevisiae* Fleischmann® (FLEI®), purchased from local stores, and the selected strains Santa Adélia 1 (SA-1) and Pedra-2 (PE-2), available in the Laboratório de Biotecnologia, Bioquímica e Biotransformação, located at Centro de Estudos de Recursos Naturais – CERNA at Universidade do Estado de Mato Grosso do Sul, Dourados/MS.

GROWING CONDITIONS

To obtain cell biomass, yeasts were cultivated in 2% YPD liquid medium, containing 1.0% ($p.v^{-1}$) yeast extract, 1.0% ($p.v^{-1}$) peptone, and 2.0% ($p.v^{-1}$) glucose. The flasks were autoclaved at 120 ºC for 20 minutes, to which 0.10 grams of lyophilized yeast were added and incubated at 30 °C for 10 hours at 250 rpm. After growth, the cells were collected and centrifuged (800 x g for 20 min), resuspended, and washed three consecutive times in sterile saline solution (0.85%), and the biomass obtained was used in fermentation tests.

ACTION OF SYNERGISM OF STRESS FACTORS

To determine the synergism of ethanolic and thermal stress, the biomass of 10 $mg.mL^{-1}$ was inoculated into 125 mL Erlenmeyer flasks containing 50 mL of sterile sugarcane juice at a concentration of 22 ºBrix and pH 5.0, adjusted with 1N HCl. The ethanol concentrations used were 0, 5, 12, and 16% ($v.v^{-1}$) of ethyl alcohol (PA 99%). The

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flasks were incubated at temperatures of 30 ºC and 40 ºC at 250 rpm for 8 hours of cultivation.

QUANTIFICATION OF TOTAL PROTEINS

To quantify the total proteins, 1000 µL aliquots of the samples were collected, centrifuged at (800 spins x 10 minutes), and washed three consecutive times with sterile saline solution (0.85%). The precipitate was resuspended in 500 µL of Tris-HCl buffer (pH 7.0) and subjected to cell lysis by alternating cycles of vortexing and ultrasound, monitored with a microscope. Protein quantification was performed using the Bradford (1976) method, employing 100 µL of lysed samples, 1000 µL of distilled water, and 2500 µL of Bradford reagent. The concentration was determined by measurements in a spectrophotometer at 595 nm, based on a standard curve (0.10 to 0.70 $\mu g.mL^{-1}$) generated with Bovine Serum Albumin (BSA).

STATISTICAL ANALYSIS

Data analysis was performed using Excel 2019 software and consisted of evaluating the mean and standard deviation. RStudio 4.4.2 software was used to perform Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

During the fermentation process, yeasts encounter various stress conditions, particularly thermal and ethanolic stress, which induce significant physiological and biochemical changes, negatively affecting ethanol production. Heat shock proteins (HSPs) play a critical role in protecting and maintaining the integrity of *S. cerevisiae* under adverse conditions. These proteins help maintain cellular functionality by dissolving protein aggregates, aiding in the folding and stabilization of proteins, and participating in the synthesis of reserve carbohydrates. As chaperones, they also play a vital role in safeguarding plasma membrane proteins from the effects of heat stress. Under ethanol stress, HSPs respond to cell membrane damage, participate in ribosome biogenesis mechanisms, act as co-chaperones, and assist in osmotic regulation and reducing cellular toxicity. Collectively, these proteins ensure the vitality and functionality of *S. cerevisiae* in hostile environments, supporting its survival and adaptability, as detailed in Table 1.

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Among the heat shock proteins (HSPs) in *S. cerevisiae*, HSP40 stands out, working in conjunction with HSP70 to facilitate proper protein folding. This interaction is essential for the reactivation and restructuring of denatured or aggregated proteins, a process regulated by the activation of the ATPase associated with HSP40, which plays a fundamental role in the reconformation of polypeptides (Geng et al., 2024). Similarly, HSP60 specifically prevents misfolding and protein aggregation after exposure to heat stress conditions, ensuring the structural and functional integrity of these molecules (Bandyopadhyay; Bose; Chattopadhyay, 2019).

Table 1. Heat shock proteins (HSPs) activated in the presence of heat and ethanol stress and their respective functions in *S. cerevisiae*.

Heat stress protein	Ethanol stress protein	Reference
HSP40, HSP60[1], acts as molecular chaperones, assisting in the correct folding of proteins and preventing cellular integrity.	GPD1[2], Glycerol-3-phosphate dehydrogenase, involved in osmotic regulation HSP31, Molecular chaperone involved in detoxification.	[1]Hu et al. (2022) [2]Hubmann; Guillouet and Nevoigt. (2011)
HSP70[1] Protein folding and prevention of aggregation.	Ssb1, Ssb2[2], Chaperones involved in ribosome biogenesis.	[1]Usman et al. (2017) [2] Rodrigues et al. (2023)
HSP90 (HSP82)[1], Protein folding and stabilization under stress.	HSP110[2], Family co-chaperone involved in the stress response.	[1]Rios; Hunsberger and Johnson (2024) [2]Masser et al. (2019)
Hsp104[1], Dissolution of denatured protein aggregates.	HSP12[2], Protection of the plasma membrane against damage.	[1]Knier et al. (2022) [2]Horianopoulos and Kronstad (2021)
TPS1 e TPS2[1], responsible for the synthesis of trehalose, a disaccharide that acts as a reserve and protector.	AHP1, is a peroxiredoxin involved in protection against oxidative damage, protecting the cell against toxic byproducts generated in the fermentation process.	[1]Hu et al. (2022) [2]Picazo and Molin (2021)

Source: Prepared by the authors.

Another essential chaperone is HSP90, whose presence increases significantly in situations of cellular stress, rising from 1-2% to up to 4-6% of the total cytoplasmic proteins. This protein is responsible for correct folding, degradation of damaged proteins, and prevention of aggregates, in addition to acting in the transport of proteins to specific organelles. These functions make HSP90 a key player in cellular adaptation to hostile environments (MCnutt et al., 2024).

HSP104, in turn, is a hexameric ATPase located in the cytoplasm that forms an asymmetric ring structure, allowing the solubilization of aggregated proteins. Working in

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synergy with other chaperones, such as HSP40, HSP70, and HSP90, HSP104 reactivates protein aggregates essential for cellular recovery after thermal and ethanolic stresses. The absence of these proteins results in a significant drop in cell viability under extreme conditions, highlighting their importance in yeast survival (Segal-Kischinevzky et al., 2022).

In addition to these proteins, others play complementary roles. HSP12, for example, is associated with the plasma membrane, and its expression is amplified in response to ethanol, contributing to membrane integrity and helping to maintain cellular homeostasis. The chaperones Ssb1 and Ssb2 play critical roles in ribosome biogenesis, ensuring the efficiency of protein synthesis in stress situations (Jay-Garcia et al., 2023; Rodrigues et al., 2023). The co-chaperone Sse1 enhances the activity of HSP70, helping in protein folding and maintaining cellular functionality in adverse environments (Bhattacharya; Picard, 2021).

Finally, the GPD1 protein plays a crucial role in osmotic regulation and ethanol tolerance, being responsible for promoting glycerol synthesis. This compound acts as an osmoprotector, helping to minimize the damage caused by ethanol to the plasma membrane and cellular metabolism, ensuring greater cell resistance in adverse conditions. Moreover, the HSP31 protein, a multifunctional chaperone, contributes significantly to cellular protection by neutralizing the effects of toxic byproducts generated during the fermentation process, such as ethanol itself (Nava-Ramírez; Gutiérrez-Terrazas; Hansberg, 2023). Its action is essential for cellular detoxification and for maintaining cellular viability, reinforcing the adaptive capacity of cells in stressful environments (Shen et al., 2022).

The interaction between HSP70 and HSP104 proteins is essential for cellular protection and maintenance under thermal and ethanolic stress conditions (Figure 1). HSP70, with a molecular weight of 70 kDa and 642 amino acids, acts in the unfolding and stabilization of proteins, while HSP104, with a molecular weight of 104 kDa and 908 amino acids, solubilizes protein aggregates, reactivating them to functional states. Together, these chaperones work synergistically to prevent protein denaturation and aggregation while promoting plasma membrane fluidity, ensuring cellular adaptation and resilience to thermal, ethanolic, and other stresses. They play a fundamental role in fermentation processes by maintaining cellular integrity and supporting yeast survival, making them key targets for developing strains with enhanced performance in biotechnological applications.

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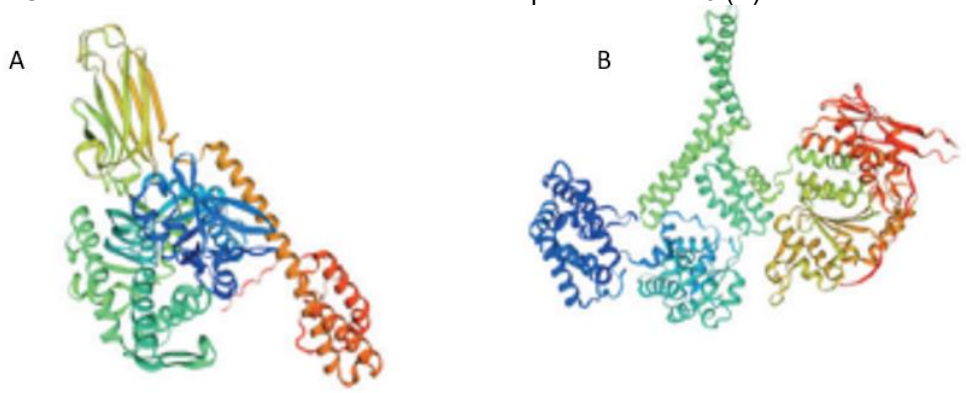
Figure 1. Conformational structure of the heat shock proteins HSP70 (A) and ethanolic HSP104 (B).



Source: Adapted from the National Library of Medicine NIH online database (2024). Model created using SWISS-MODELL.

The response of *S. cerevisiae* to ethanol stress involves a complex network of proteins, which help to protect and repair damaged proteins, as well as proteins involved in maintaining membrane integrity and osmotic balance. This response allows the yeast to survive and continue to grow even in high concentrations of ethanol, as occurs during alcoholic fermentation (Jhariya et al., 2021; Liszkowska; Berlowska, 2021; Da Silva Fernandes et al., 2022).

In evaluating cell growth under thermal and ethanolic stress, the results indicate that the analyzed yeasts experienced stress synergism, displaying distinct growth behaviors likely due to their varying capacities to withstand the imposed stress conditions. The Fleischmann® strain (Figure 2A) exhibited the highest sensitivity among the tested yeasts, showing a significant reduction in cell growth at both temperatures, particularly under high ethanol concentrations. At 30°C, the progressive increase in ethanol concentration negatively impacted cell performance, whereas at 40°C, the combined effects of thermal and ethanolic stress further limited growth. This response suggests that Fleischmann® has lower tolerance to the applied conditions.

In contrast, the Santa Adélia strain (Figure 2B) displayed an intermediate response to stress. At 30°C, it maintained gradual growth despite increasing ethanol concentrations; however, at 40°C, the higher temperature intensified sensitivity, leading to a more pronounced decline in cell growth at 12% and 16% ethanol. Although it demonstrated some stress tolerance, Santa Adélia exhibited efficient growth and relatively higher resilience. The Pedra-2 strain (Figure 2C) emerged as the most tolerant strain, showing superior growth under the tested stress conditions. At 30°C, cell growth remained stable even at higher ethanol concentrations. At 40°C, while a gradual reduction in growth was observed, Pedra-2

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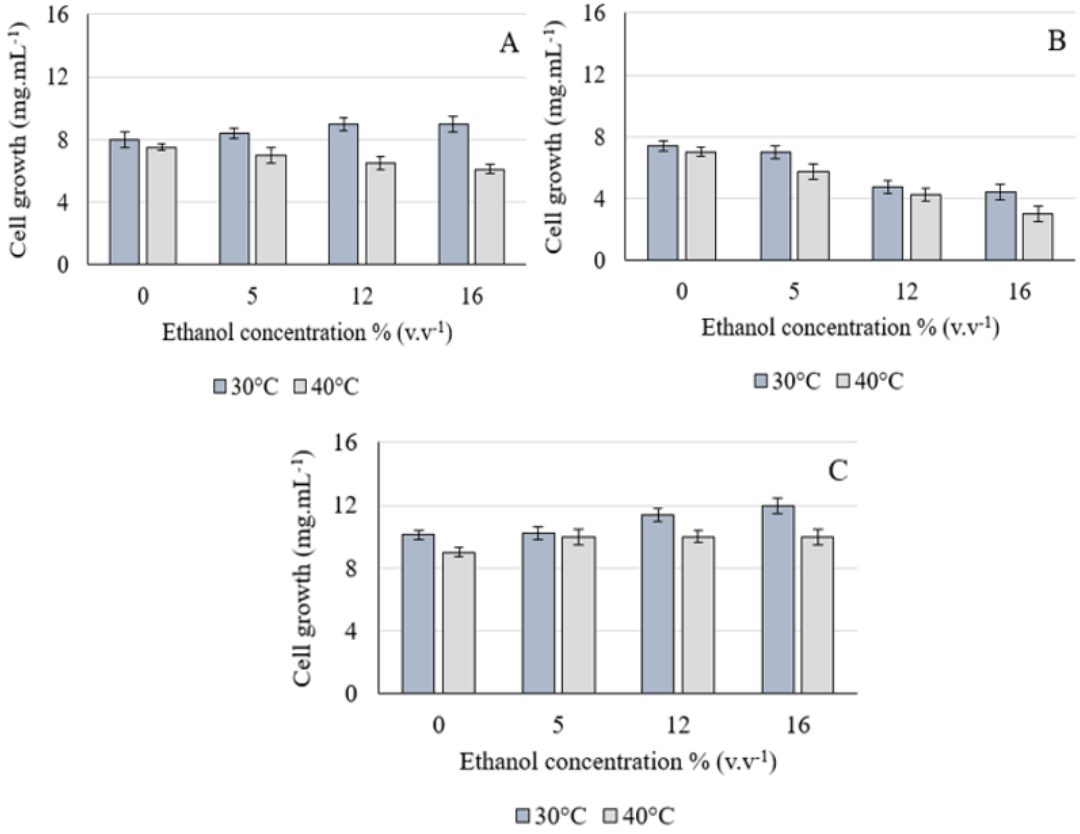
still maintained the highest biomass levels among the three strains. This suggests that Pedra-2 exhibits a more effective interaction of heat shock proteins related to thermal and ethanolic stress, making it an optimal strain for fermentation processes operating under severe conditions.

According to Tobias, Mascarenhas and Batistote (2024), when evaluating the synergism of thermal and ethanol stress in industrial strains of *S. cerevisiae*, including Pedra-2, it highlights the importance of selecting yeasts with high fermentation capacity to improve the efficiency of industrial fermentation, ensuring the sustainability of ethanol production. Iwuozor et al. (2014) reinforce that the use of strains tolerant to extreme conditions in the fermentation process can reduce operating costs by minimizing the need for strict control to maintain process parameter.

Figure 2. Evaluation of cell growth of the yeasts Fleischmann® (A), Santa Adélia (B), and Pedra-2 (C), cultivated in sugarcane juice at a concentration of 22 °Brix, under the action of thermal and ethanol stresses during 8 hours of fermentation.



Source: Research data

Principal Component Analysis (PCA) indicated the formation of clusters that represent the distribution of yeasts according to their protein production profiles, associated

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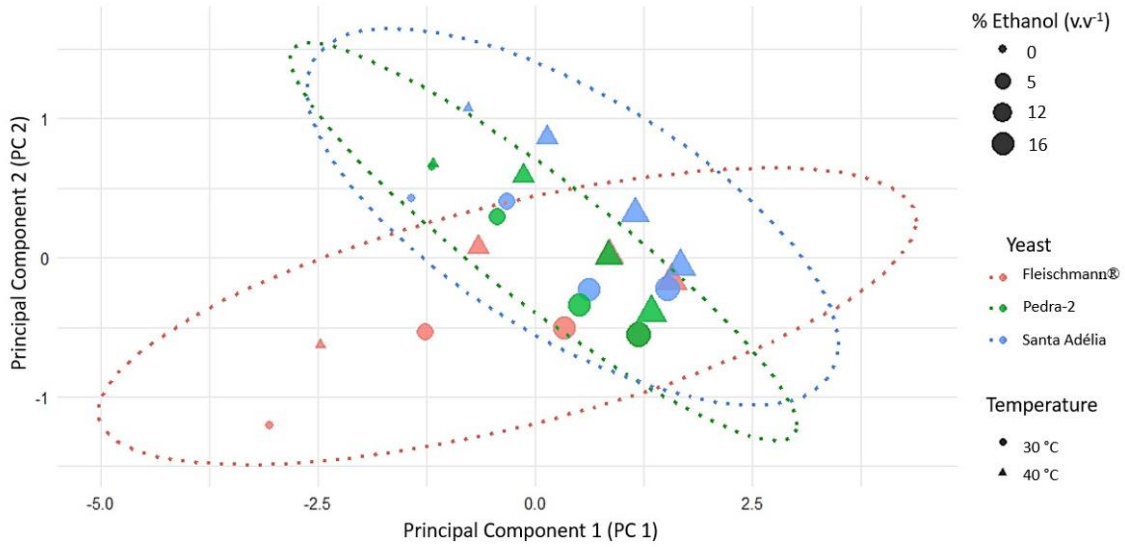
with tolerance to thermal and ethanolic stress (Figure 3). The Fleischmann® lineage presented the lowest protein production profile under the conditions evaluated, characterized by greater dispersion in the graph, which highlights its sensitivity to associated stress. However, the Santa Adélia lineage presented an intermediate protein production. The Pedra-2 lineage stood out for forming a well-defined cluster, reflecting a high protein production profile in relation to the other lineages, which makes it more suitable for extreme industrial applications, where thermal and ethanolic stress are present.

Studies focused on the action of heat shock proteins (HSPs) involved in thermal and ethanol stress are fundamental for obtaining knowledge and understanding of this complex network of proteins that maintain cellular integrity and ensure ethanol production. The ability to maintain yeast adaptation and survival under severe stress conditions involves numerous functions of heat shock proteins. During the fermentation process, in the presence of high temperatures and high ethanol concentrations, there is a significant possibility of creating a hostile environment, which triggers numerous HSPs to combat these stresses, playing a crucial role (Kumar et al., 2024). These mechanisms ensure that yeasts remain active and productive throughout the fermentation process.

Figure 3. Principal Component Analysis (PCA) of the total protein production profile in *S. cerevisiae* strains, cultivated in sugarcane juice at a concentration of 22 °Brix, under the action of thermal and ethanolic stress in 8 hours of fermentation at a temperature of 30 °C (A) and 40 °C (B).



Source: Research data.

Additionally, HSPs enhance the resistance of cells to heat and ethanol stress by stabilizing essential proteins and repairing those that have been damaged (Sahana et al.,

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2024). This ability enables yeast to maintain fermentation capacity even at high ethanol concentrations, reducing cell mortality, preventing metabolic losses, promoting cell integrity, and preserving the efficiency of ethanol production (Chen et al., 2024; Elhalis, 2024). These studies are also essential for understanding the response mechanisms of heat and ethanol shock proteins involved in the protein production profile, which may exhibit greater tolerance and fermentation robustness against associated stress, as well as improve ethanol production in challenging industrial environments.

CONCLUSION

Saccharomyces cerevisiae demonstrates a remarkable ability to adapt to adverse conditions during the fermentation process, mainly due to the action of heat shock proteins (HSPs) that play crucial roles in protecting and repairing cellular functionality against thermal and ethanolic stresses.

The yeast's response to stress conditions triggers a complex network of proteins, which perform essential functions such as folding, oxidation, toxicity reduction, synthesis of new macromolecules, and maintenance of membranes and organelles. However, the proteins that act most prominently are HSP70 for high temperatures and HSP104 for high concentrations of ethanol. The interaction between these proteins allows yeast to maintain homeostasis, integrity, and cell survival.

In the evaluation of cell growth under the action of thermal and ethanolic stress, the yeasts showed different growth patterns, reflecting variations in tolerance capacity and responses to the imposed conditions. The Fleischmann® lineage showed lower tolerance to the stress conditions imposed, while the Santa Adélia yeast showed moderate tolerance, and the Pedra-2 strain exhibited greater tolerance in severe conditions, with promising potential in the industrial production of bioethanol.

Principal component analysis (PCA) revealed a differentiated profile for protein production in the yeasts analyzed. The Fleischmann® lineage showed lower tolerance to the action of thermal and ethanolic stress, with low protein production. However, the Pedra-2 yeast demonstrated greater tolerance to the severe conditions imposed, presenting better protein production and standing out as a promising strain to be utilized in industrial processes that involve extreme stress conditions.

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REFERENCES

1. AJMAL, Mohammad Rehan. Protein misfolding and aggregation in proteinopathies: causes, mechanism and cellular response. **Diseases**, v. 11, n. 1, p. 30, 2023.

2. AUESUKAREE, Choowong. Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. **Journal of bioscience and bioengineering**, v. 124, n. 2, p. 133-142, 2017.

3. AZHAR, Siti Hajar Mohd et al. Yeasts in sustainable bioethanol production: A review. **Biochemistry and biophysics reports**, v. 10, p. 52-61, 2017.

4. BANDYOPADHYAY, Arnab; BOSE, Indrani; CHATTOPADHYAY, Krishnananda. Osmolytes ameliorate the effects of stress in the absence of the heat shock protein Hsp104 in *Saccharomyces cerevisiae*. **PLoS One**, v. 14, n. 9, p. e0222723, 2019.

5. BERTRAND, Brandt et al. Biophysical characterization of the insertion of two potent antimicrobial peptides-Pin2 and its variant Pin2 [GVG] in biological model membranes. **Biochimica et Biophysica Acta (BBA)-Biomembranes**, v. 1862, p. 183105, 2020.

6. BHATTACHARYA, Kaushik; PICARD, Didier. The Hsp70–Hsp90 go-between Hop/Stip1/Sti1 is a proteostatic switch and may be a drug target in cancer and neurodegeneration. **Cellular and Molecular Life Sciences**, p. 1-17, 2021.

7. BRADFORD, Marion M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical biochemistry**, v. 72, n. 1-2, p. 248-254, 1976.

8. CHEN, Anqi et al. Osmotic Tolerance in *Saccharomyces cerevisiae*: Implications for Food and Bioethanol Industries. **Food Bioscience**, p. 104451, 2024.

9. CHETTY, Bronwyn Jean et al. Improvement of cell-tethered cellulase activity in recombinant strains of *Saccharomyces cerevisiae*. **Applied Microbiology and Biotechnology**, v. 106, n. 18, p. 6347-6361, 2022.

10. COERTJENS, Nicole Carneiro et al. Evaluation of stress factors in the metabolism of Pedra-2 yeast. **Scientific Electronic Archives**, v. 16, n. 3, 2023.

11. DA SILVA FERNANDES, Flávia et al. Current ethanol production requirements for the yeast *Saccharomyces cerevisiae*. **International journal of microbiology**, v. 2022, n. 1, p. 7878830, 2022.

12. determining factors in the permeability of membranes to small solutes. Nature Communications, v. 13, p. 1605, 2022.

13. ELHALIS, Hosam. Expanding the Horizons of Saccharomyces cerevisiae: Nutrition, Oenology, and Bioethanol Production. **Sustainability**, v. 16, n. 24, p. 11151, 2024.

Revista ARACÊ
ISSN: 2358-2472

14. FRALLICCIARDI, Jacopo et al. Membrane thickness, lipid phase and sterol type are determining factors in the permeability of membranes to small solutes. **Nature communications**, v. 13, n. 1, p. 1605, 2022.

15. GENG, Yuanwei et al. Genome-Wide Identification and Interaction Analysis of Turbot Heat Shock Protein 40 and 70 Families Suggest the Mechanism of Chaperone Proteins Involved in Immune Response after Bacterial Infection. **International Journal of Molecular Sciences**, v. 25, n. 14, p. 7963, 2024.

16. HORIANOPOULOS, Linda C.; KRONSTAD, James W. Chaperone networks in fungal pathogens of humans. **Journal of Fungi**, v. 7, n. 3, p. 209, 2021.

17. HU, Chen et al. Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. **MedComm**, v. 3, n. 3, p. e161, 2022

18. HUBMANN, Georg; GUILLOUET, Stephane; NEVOIGT, Elke. Gpd1 and Gpd2 fine-tuning for sustainable reduction of glycerol formation in *Saccharomyces cerevisiae*. **Applied and environmental microbiology**, v. 77, n. 17, p. 5857-5867, 2011.

19. IWUOZOR, Kingsley O. et al. Advancements in high gravity fermentation strategies for optimizing ethanol production from sugarcane-based substrates. **Sugar Tech**, v. 26, n. 4, p. 1016-1032, 2024.

20. JAY-GARCIA, Lina M. et al. Yeast Chaperone Hsp70-Ssb Modulates a Variety of Protein-Based Heritable Elements. **International Journal of Molecular Sciences**, v. 24, n. 10, p. 8660, 2023.

21. JHARIYA, Upasana et al. Understanding ethanol tolerance mechanism in *Saccharomyces cerevisiae* to enhance the bioethanol production: Current and future prospects. **BioEnergy Research**, v. 14, p. 670-688, 2021.

22. JIN, Xiaofan et al. Wheat gluten peptides enhance ethanol stress tolerance by regulating the membrane lipid composition in yeast. **Journal of Agricultural and Food Chemistry**, v. 70, n. 16, p. 5057-5065, 2022.

23. KNIER, Adam S. et al. The yeast molecular chaperone, Hsp104, influences transthyretin aggregate formation. **Frontiers in Molecular Neuroscience**, v. 15, p. 1050472, 2022.

24. KUMAR, Prabhat et al. Understanding heat-shock proteins' abundance and pivotal function under multiple abiotic stresses. **Journal of Plant Biochemistry and Biotechnology**, p. 1-22, 2024.

25. LAIRÓN-PERIS, María et al. Lipid composition analysis reveals mechanisms of ethanol tolerance in the model yeast *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, v. 87, p. e00440-21, 2021.

Revista ARACÊ
ISSN: 2358-2472

26. LISZKOWSKA, Wiktoria; BERLOWSKA, Joanna. Yeast fermentation at low temperatures: Adaptation to changing environmental conditions and formation of volatile compounds. **Molecules**, v. 26, n. 4, p. 1035, 2021.

27. MASSER, Anna E. et al. Cytoplasmic protein misfolding titrates Hsp70 to activate nuclear Hsf1. **Elife**, v. 8, p. e47791, 2019.

28. MCNUTT, Seth W. et al. Phosphorylation-Driven Epichaperome Assembly: A Critical Regulator of Cellular Adaptability and Proliferation. **Research Square**, 2024.

29. MUELLER, Larissa Pires et al. The effects of thermal and ethanolic stress in industrial strains of *Saccharomyces cerevisiae*. **Research, Society and Development**, v. 9, p. e6819109091-e6819109091, 2020.

30. NAVA-RAMÍREZ, Teresa; GUTIÉRREZ-TERRAZAS, Sammy; HANSBERG, Wilhelm. The Molecular chaperone mechanism of the C-terminal domain of large-size subunit catalases. **Antioxidants**, v. 12, n. 4, p. 839, 2023.

31. PICAZO, Cecilia; MOLIN, Mikael. Impact of hydrogen peroxide on protein synthesis in yeast. **Antioxidants**, v. 10, n. 6, p. 952, 2021.

32. RIOS, Erick I.; HUNSBERGER, Isabel L.; JOHNSON, Jill L. Insights into Hsp90 mechanism and in vivo functions learned from studies in the yeast, Saccharomyces cerevisiae. **Frontiers in Molecular Biosciences**, v. 11, p. 1325590, 2024.

33. RODRIGUES, Joana I. et al. Yeast chaperones and ubiquitin ligases contribute to proteostasis during arsenite stress by preventing or clearing protein aggregates. **FEBS letters**, v. 597, n. 13, p. 1733-1747, 2023.

34. ROSENZWEIG, Rina et al. The Hsp70 chaperone network. **Nature reviews molecular cell biology**, v. 20, n. 11, p. 665-680, 2019.

35. SAHANA, Gandasi Ravikumar et al. A review on ethanol tolerance mechanisms in yeast: Current knowledge in biotechnological applications and future directions. **Process Biochemistry**, v. 138, p. 1-13, 2024.

36. SEGAL-KISCHINEVZKY, Claudia et al. Yeasts inhabiting extreme environments and their biotechnological applications. **Microorganisms**, v. 10, n. 4, p. 794, 2022.

37. SHEN, Dongxu et al. A review of yeast: High cell-density culture, molecular mechanisms of stress response and tolerance during fermentation. **FEMS Yeast Research**, v. 22, n. 1, p. foac050, 2022.

38. SWISS-MODEL Repository. Disponível em: https://swissmodel.expasy.org/. Acesso em 26 de janeiro de 2025.

39. TOBIAS, V. C. M.; MASCARENHAS, M. do S.; BATISTOTE, M. Acción del sinergismo del Estrés térmico y etanólico en cepas industriales de Saccharomyces

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cerevisiae. **Contribuciones A Las Ciencias Sociales**, *[S. l.]*, v. 17, n. 12, p. e13163, 2024.

40. USMAN, Magaji G. et al. Molecular analysis of Hsp70 mechanisms in plants and their function in response to stress. **Biotechnology and Genetic Engineering Reviews**, v. 33, n. 1, p. 26-39, 2017.

41. WALKER, Graeme M.; BASSO, Thiago O. Mitigating stress in industrial yeasts. **Fungal Biology**, v. 124, p. 387-397, 2020.