Zinc-elicited stress tolerance in fungi

Tolerância ao estresse provocada pelo zinco em fungos

Tolerancia al estrés por zinc en hongos

Received: 01/11/2023 | Revised: 01/21/2023 | Accepted: 01/23/2023 | Published: 01/26/2023

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Abstract
Fungi for industrial and agricultural uses must endure a variety of physical and chemical challenges, collectively referred to as stresses. The fungal response to stress conditions may be due to impaired growth and metabolism. Understanding the physiology of stress responses can help alleviate the detrimental effects when the fungus is applied agricultural and industrial uses. The present study aims to review the physiological and morphological effects of zinc ions on fungi. Zinc is essential for the activity of zinc metalloenzymes, such as alcohol dehydrogenase and aldehyde dehydrogenase. It can activate riboflavin synthesis and increase protein content in fermenting yeast. However, excess zinc is toxic to the cell, and the cellular level must be finely controlled within an adequate range between 0.1 and 0.5 mM. In conclusion, a relationship between zinc and yeasts is observed but has not yet been fully defined in the fermentation process. Thus, studies are still needed to evaluate the physiological and morphological effects of zinc ions in fungi used in agriculture, such as tolerance of conidia produced in culture media with zinc or to conditions of oxidative stress, osmotic stress, ultraviolet radiation, and heat.
Keywords: Zinc sulfate; Fungi; Stress tolerance; Toxicity.

Resumo
Os fungos para uso industrial e agrícola devem suportar uma variedade de desafios físicos e químicos, referidos coletivamente como estresses. A resposta fúngica às condições de estresse pode ser devida ao crescimento e metabolismo prejudicados. Compreender a fisiologia das respostas ao estresse pode ajudar a aliviar os efeitos prejudiciais quando o fungo é aplicado em usos agriculturais e industriais. O presente estudo tem como objetivo revisar os efeitos fisiológicos e morfológicos dos íons zinco sobre fungos. O zinco é essencial para a atividade de metaloenzimas de zinco, como álcool desidrogenase e aldeído desidrogenase. Pode ativar a síntese de riboflavina e aumentar o teor de proteína na levedura de fermentação. No entanto, o excesso de zinco é tóxico para a célula, e o nível celular deve ser rigorosamente controlado dentro de uma faixa adequada entre 0,1 e 0,5 mM. Em conclusão, observa-se uma relação entre zinco e leveduras, mas ainda não totalmente definida no processo de fermentação. Assim, ainda são necessários estudos que avaliem os efeitos fisiológicos e morfológicos dos íons zinco em fungos usados na agricultura, como tolerância de conídios produzidos em meios de cultura com zinco ou a condições de estresse oxidativo, estresse osmótico, radiação ultravioleta e calor.
Palavras-chave: Sulfato de zinco; Fungos; Tolerância ao estresse; Toxicidade.

Resumen
Los hongos para uso industrial y agrícola deben resistir una variedad de desafíos físicos y químicos, denominados colectivamente como estreses. La respuesta fúngica a las condiciones de estrés puede deberse a un crecimiento y metabolismo deficientes. Comprender la fisiología de las respuestas al estrés podría ayudar a aliviar los efectos nocivos cuando el hongo se aplica en usos agrícolas e industriales. El presente estudio tiene como objetivo revisar los efectos fisiológicos y morfológicos de los iones de zinc sobre hongos. El zinc es esencial para la actividad de las metaloenzimas

de zinc, como el alcohol deshidrogenasa y el aldehído deshidrogenasa. Puede activar la síntesis de riboflavina y aumentar el contenido de proteínas en la levadura de fermentación. Sin embargo, el exceso de zinc es tóxico para la célula y el nivel celular debe controlarse estrictamente dentro de un rango adecuado entre 0,1 y 0,5 mM. En conclusión, se observa una relación entre el zinc y la levadura, pero aún no completamente definida en el proceso de fermentación. Por lo tanto, aún se necesitan estudios para evaluar los efectos fisiológicos y morfológicos de los iones de zinc sobre los hongos utilizado en la agricultura, como la tolerancia de las conidias producidas en medios de cultivo con zinc o condiciones de estrés oxidativo, estrés osmótico, radiación ultravioleta y calor.
Palabras clave: Sulfato de zinc; Hongos; Tolerancia al estrés; Toxicidad.

1. Introduction

Fungi for industrial and agricultural uses are subject to a variety of physical and chemical stresses. The fungal response to stress conditions may be due to growth and metabolism impairments, and understanding the physiology of stress responses to alleviate detrimental influences is important (Rangel, 2011; Rangel et al., 2015a; Rangel et al., 2015b; Rangel, et al., 2018; Walker &Basso, 2019).

Divalent cations, such as Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn2+, and Mn^{2+}, are essential for fungal growth and metabolism and consequently must be obtained from the external environment (Jones & Gadd, 1990; Walker & Basso, 2019; Walker & Birch, 1999). However, above certain concentrations all divalent cations, especially those called heavy metals, are toxic and can damage cell metabolism and ultimately cause cell death (Jones & Gadd, 1990).

Zinc plays a central role in enzyme function in yeast cells, especially alcohol dehydrogenase, and is part of protein structural activity (Walker, 2004). Zinc deficiency can lead to slow or incomplete fermentation (De Nicola & Walker, 2008). The present study aims to understand the physiological and morphological effects of zinc ion on fungi for industrial and agricultural use.

2. Methodology

The study in question is a narrative bibliographical review, where the bibliographical research was adopted based on the analysis of the published literature, including articles published in the last 20 years, in Portuguese and English, in the following databases: Pubmed and Scielo, as well as books and theses available. For the research, descriptors related to the subject under study were used, namely: Zinc Sulfate, Entomopathogenic Fungi, Stress tolerance, Toxicity. For the selection of articles to be used, it was based on previously defined inclusion and exclusion criteria. Only texts from scientific articles, book chapters, dissertations, and theses were selected, texts from: congresses were excluded.

After the search and selection, the texts were read in full, the theoretical construction was defined through content analysis, as well as the research design and the main results of the study, organizing it in the form of topics.

3. What is Zinc Sulfate?

Zinc sulfate ($ZnSO_4$) is the mineral goslarite, known as white vitriol, which is a colorless crystalline chemical compound, and all forms are soluble in water. This multifunctional element is essential for all physiological processes; can act as a catalyst, structural and regulatory element; and play, an important role in gene expression (Zhao & Bai, 2009). Zinc may be present in bound or cell-free form depending on cell type and state. The concentration can vary from femtomolar to millimolar (De Nicola & Walker, 2008). In animals, mineral deficiency is associated with serious pathological conditions mainly due to dietary deficiency, the presence of chelating compounds in food, and disturbances in the gastrointestinal absorption process or high urinary levels. In some situations, the deficiency of this mineral depresses growth of yeast, and the cells tend to swell and form clumps (Obata, et al., 1996).

4. How Important is Zinc to Fungi?

Zinc is an essential micronutrient that plays a role in yeast metabolism. It functions as a co-factor of essential enzymes, such as alcohol and aldehyde dehydrogenases, which are directly involved in ethanol production (Tosun & Ergun, 2007).

Cation bioavailability directly influences sugar metabolism of yeast in brewery and distillery fermentations. The right balance is vital to successful ethanol production. In particular, knowledge of the interactions between K^{+}, Mg^{2+}, Ca^{2+}, and Zn^{2+} as well as their relative bioavailability in industrial yeast growth is important to optimally manipulate their fermentation levels (Chandrasena, et al., 1997).

Zinc plays an important role in fermentative metabolism because it is essential for the activity of zinc metalloenzymes, including alcohol dehydrogenase, aldehyde dehydrogenase. Zinc can also activate riboflavin synthesis and increase the protein content in fermenting yeast. Moreover, it can stimulate the transport of maltose and maltotriose into the cells, thus increasing the rate of fermentation (Walker, 2004).

Zinc is a cofactor of many proteins and is indispensable for their catalytic activity and/or structural stability. It is also a ubiquitous component of enzymes involved in transcription and Zn finger proteins regulate gene expression. In the yeast *Saccharomyces cerevisiae*, zinc is probably required for the function of almost 3% of the proteome (Böhm et al., 1997). In addition to its involvement in the structure and function of proteins, the interaction of zinc with lipids contributes to membrane fluidity, and its interaction with nucleic acids helps to prevent deleterious radical reactions (Vallee & Auld, 1990). Deficiency of this essential trace element can have serious consequences. For example, in beer fermentation, zinc deficiency in the wort leads to a slow fermentation and thus a deterioration in the quality of the beer. While accurate monitoring of zinc concentration in such industrial fermentations is important, the formation of complexes with polyphenols, proteins, and other compounds implies that zinc concentration does not always accurately predict its bioavailability to yeast (Kreder, 1999).

5. Do Fungi Need Zinc to Grow?

Metal ions including zinc definitely play a role in the regulation of yeast metabolism, where cells require these metals to maintain the structural integrity of the cell and organelles. They act in cell-cell interactions, flocculation phenomenon, gene expression, cell division and growth, mechanisms of nutrient uptake, enzymatic action of metabolism, osmoregulation, and energy maintenance and cell survival. Additionally, yeast cells need metals to act as stress protectors in the face of environmental stressors (Walker, 2004).

6. What Role does Zinc Play in Fungal Physiology?

Zinc limitation causes a strong negative regulation of gene transcription involved in reserve carbohydrate accumulation. The physiological relevance of this response was verified by an analysis of the intracellular glycogen and trehalose contents, which were strongly reduced in Zn-limited cultures. Comparative studies with nitrogen-limited cultures showed that the marked accumulation of storage carbohydrates was specific for Zn limitation and not just a consequence of excess glucose conditions (De Nicola, et al., 2007).

7. How Toxic is Zinc to Fungi?

Excess zinc is toxic, because it can compete with other metal ions for the active sites of enzymes or intracellular transport proteins. For this reason, organisms have evolved mechanisms that tightly control intracellular zinc levels. Zinc homeostasis in yeast can be mediated through the control of zinc uptake, storage of zinc in vacuoles, intracellular binding of zinc by metallothioneins, and efflux of zinc from cells. In *S. cerevisiae*, several proteins involved in zinc absorption and storage have been identified (Zhao & Eide, 1996).

While divalent cations, including Zn^{2+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, and Mn^{2+}, are essential for growth and metabolism, above certain concentrations, all divalent cations, particularly those of the so-called heavy metals, are toxic. They can impair cellular metabolism, including ionic nutrition and ultimately cell death. Their toxicity is related to the strength of coordination abilities and chemical coordination of the particular ion. Toxic effects include blocking functional groups of important biomolecules, e.g., enzymes, inhibiting the transport system for essential ions and nutrients, displacing and replacing essential metal ions from cell locations and biomolecules, denaturing enzymes, and disrupting membrane structures (Jones & Gadd, 1990). Zhao & Bai (2012) mention that excess zinc is toxic to the cell and that the cellular level must be finely controlled within an adequate range, which is normally between 0.1 and 0.5 mM.

8. What Concentrations of Zinc are Used in Culture Medium?

The optimal concentration of zinc in the growth medium is between 4 and 8 µM (Walker, 2004). However, the concentration of this element may vary in different industrial environments, such as wine must, cane molasses, or beer must. In the wine industry, for example, zinc concentration in grape must is between 0.04 and 7.8 ppm, and the average is 0.90 ppm. Normally, in wine production, such zinc levels are satisfactory for the fermentation progress. The average concentration of zinc in beet molasses is 40 ppm, while in sugar cane molasses it is 13 ppm, with an ideal concentration for ethanol production between 1 and 5 ppm (De Nicola & Walker, 2008).

9. Zinc Sulfate Helps Fungi Under Which Types of Stress?

Zinc can be a protector at the time of toxicity, in addition increasing tolerance to heat stress. Furthermore it can increase the content in flocculating yeasts grown in the presence of zinc, significantly impacting growth rate, ethanol yield, and yeast membrane fluidity (Zhao & Bai, 2009).

This element induces stress responses in an effort to ensure survival when exposed to environmental insults, which include: 1) Increased synthesis of trehalose and glycerol, 2) Induction of heat/cold shock protein biosynthesis, 3) Enzymatic induction of stress, structural changes in cell membrane, glutathione production, and ionic homeostasis modulation (Walker, 2004).

10. Conclusion

The relationship of zinc with yeasts in the fermentation process is already well defined. However, studies should evaluate the physiological and morphological effects of zinc ions on fungi of agricultural interest, such as the tolerance of conidia of entomopathogenic fungi to conditions of oxidative and osmotic stress, ultraviolet radiation, and heat produced in zinc supplemented culture media.

Acknowledgments

This research was supported by grants from the National Council for Scientific and Technological Development (CNPq) of Brazil PQ1D 302100/2018-0 and PQ1D 302282/2022-0 and the São Paulo Research Foundation (FAPESP) 2010/06374-1 and 2013/50518-6 for D.E.N.R.

References

Böhm, S., Frishman, D., & Mewes, H. W. (1997). Variations of the C2H2 zinc finger motif in the yeast genome and classification of yeast zinc finger proteins. *Nucleic Acids Research* 25, 2464-2469. 10.1093/nar/25.12.2464.

Chandrasena, G., Walker, G. M., & Staines, H. J. (1997). Use of response surfaces to investigate metal ion interactions in yeast fermentations. *Journal of the American Society of Brewing Chemists* 55, 24-29. 10.1094/asbcj-55-0024.

De Nicola, R., Hazelwood, L. A., De Hulster, E. A. F., Walsh, M. C., Knijnenburg, T. A., Reinders, M. J. T., Walker, G. M., Pronk, J. T., Daran, J. -M., & Daran-Lapujade, P. (2007). Physiological and transcriptional responses of *Saccharomyces cerevisiae* to zinc limitation in chemostat cultures. *Appl Environ Microb* 73, 7680-7692. doi:10.1128/AEM.01445-07.

De Nicola, R., & Walker, G. M. (2008). Interaction between yeasts and zinc, in: Satyanarayana, T., Kuntze, G. (Eds.), Yeast Biotechnology: Diversity and Applications. *Springer Science & Business Media BV*, Dordecht, pp. 237-257.

Jones, R. P., & Gadd, G. M. (1990). Ionic nutrition of yeast—physiological mechanisms involved and implications for biotechnology. *Enzyme Microb Tech* 12, 402-418. https://doi.org/10.1016/0141-0229(90)90051-Q.

Kreder, G. C. (1999). Yeast assimilation of Trub-Bound Zinc. *Journal of the American Society of Brewing Chemists* 57, 129-132. 10.1094/asbcj-57-0129.

Obata, H., Hayashi, A., Toda, T., & Umebayashi, M. (1996). Effects of zinc deficiency on the growth, proteins, and other constituents of yeast, *Saccharomyces cerevisiae*, cells. *Soil Science and Plant Nutrition* 42, 147-154. 10.1080/00380768.1996.10414698.

Rangel, D. E .N. (2011). Stress induced cross-protection against environmental challenges on prokaryotic and eukaryotic microbes. *World J. Microbiol. Biotechnol.* 27, 1281-1296. 10.1007/s11274-010-0584-3.

Rangel, D. E .N., Alder-Rangel, A., Dadachova, E., Finlay, R. D., Kupiec, M., Dijksterhuis, J., Braga, G. U. L., Corrochano, L. M., & Hallsworth, J. E. (2015a). Fungal stress biology: a preface to the Fungal Stress Responses special edition. *Curr. Gen.* 61, 231-238. 10.1007/s00294-015-0500-3.

Rangel, D. E. N., Braga, G. U .L., Fernandes, É. K. K., Keyser, C. A., Hallsworth, J .E., & Roberts, D. W. (2015b). Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. *Curr. Gen.* 61, 383-404. 10.1007/s00294-015-0477-y.

Rangel, D. E. N., Finlay, R.D ., Hallsworth, J. E., Dadachova, E., & Gadd, G. M. (2018). Fungal strategies for dealing with environmental and agricultural stress. *Fungal Biol*. 122, 602-612. 10.1016/j.funbio.2018.02.002.

Tosun, A., & Ergun, M. (2007). Use of experimental design method to investigate metal ion effects in yeast fermentations. *Journal of Chemical Technology & Biotechnology* 82, 11-15. https://doi.org/10.1002/jctb.1616.

Vallee, B. L., & Auld, D. S. (1990). Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* 29, 5647-5659. 10.1021/bi00476a001.

Walker, G. M. (2004). Metals in Yeast Fermentation Processes, Advances in Applied Microbiology. *Academic Press*, pp. 197-229.

Walker, G. M., & Basso, T. O. (2019). Mitigating stress in industrial yeasts. *Fungal Biol.* https://doi.org/10.1016/j.funbio.2019.10.010.

Walker, G. M., & Birch, R. M. (1999). Environmental stress responses in industrial yeasts, 5th Aviemore Conference on Malting, Brewing & Distilling. *Campbell Institute of Brewing*, London, pp. 195-197.

Zhao, H., & Eide, D. (1996). The yeast ZRT1 gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. *Proceedings of the National Academy of Sciences* 93, 2454-2458. doi:10.1073/pnas.93.6.2454.

Zhao, X. Q., & Bai, F. W., (2012). Zinc and yeast stress tolerance: Micronutrient plays a big role. *J Biotechnol* 158, 176-183. https://doi.org/10.1016/j.jbiotec.2011.06.038.

Zhao, X. Q., & Bai, F. W. (2009). Mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. *J Biotechnol* 144, 23-30. S0168-1656(09)00205-3 [pii]10.1016/j.jbiotec.2009.05.001.