

Ability of The *Saccharomyces Cerevisiae* Y904 to Tolerate and Adapt to High Concentrations of Selenium

Layna Amorim Mota (✉ layna.amorim@hotmail.com)

Universidade de Sao Paulo Centro de Energia Nuclear na Agricultura <https://orcid.org/0000-0002-7684-3958>

Ana Paula Maria da Silva

ESALQ-USP: Universidade de Sao Paulo Escola Superior de Agricultura Luiz de Queiroz

Eric Alberto da Silva

IPEN: Instituto de Pesquisas Energeticas e Nucleares

Gabriela Maria Ferreira Lima Leite

ESALQ-USP: Universidade de Sao Paulo Escola Superior de Agricultura Luiz de Queiroz

Rubens Perez Calegari

Universidade de Sao Paulo Centro de Energia Nuclear na Agricultura

Antonio Sampaio Baptista

ESALQ-USP: Universidade de Sao Paulo Escola Superior de Agricultura Luiz de Queiroz

Research Article

Keywords: Cultivation in high selenium, Organic selenium, Organominerals, Selenium tolerance

Posted Date: May 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-499880/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The rational use of by-products is essential for the development of a sustainable society. Worldwide, the alcoholic fermentation industry generates a large surplus of yeasts, on the scale of millions of tons. So there is a need for beneficial applications to humanity of this surplus. Yeasts, in turn, have the ability to bioaccumulate minerals and enable their bioavailability after cell autolysis. Among these minerals, we highlight selenium (Se), which participates in the formation of antioxidant enzymes. The objectives of the work were to define the minimum and maximum concentration of Se that yeasts (*Saccharomyces cerevisiae* – Y904) support and the concentrations that they tolerate once adapted. To this end, a test of tolerance to Se was carried out, using treatments with different concentrations of Se. The adaptive process started at the maximum concentration obtained in the tolerance test of $60 \mu\text{g mL}^{-1}$, with an increasing addition of $6 \mu\text{g mL}^{-1}$, reaching up to $246 \mu\text{g mL}^{-1}$ of Se. The macromorphological characteristics and number of colony forming units were evaluated. It was identified that yeasts without adaptation grew on substrate containing up to $60 \mu\text{g mL}^{-1}$ of Se and those adapted, up to $246 \mu\text{g mL}^{-1}$ of Se. In addition to the reduction in yeast growth speed, from the concentration of $84 \mu\text{g mL}^{-1}$ of Se in the medium, morphological changes in colony color were observed. It is concluded that non-adapted yeasts support up to $60 \mu\text{g mL}^{-1}$ of Se and, after the adaptive process, they support $246 \mu\text{g mL}^{-1}$ of Se in the medium.

Introduction

Brazil is currently the second largest producer of fuel ethanol in the world, generating approximately 33 billion liters per year (Nova cana 2020). In the ethanol production process, at the end of each fermentation cycle, there is a large surplus of yeasts of the *Saccharomyces cerevisiae* species, around 20 kg of yeasts per m^3 of ethanol produced, which generates about 660,000 tons (DESMONTS 1966). These single-celled microorganisms have the ability to transform sugars into ethanol, carbon dioxide, energy and other by-products. In the ethanol production process, yeasts are used only as agents of biotransformation of sugar into ethanol, and after this biochemical reaction, they can be reused for other purposes, such as enrichment in nutrients of interest (MUSSATTO 2010). In some situations, while the yeast cell recycling, after yeast treatment, part of them can be removed from the process and enriched with minerals, such as Se (SUHAJDA et al. 2000; BASSO et al. 2008). As they also have the ability to bioaccumulate many chemical elements, yeasts are used as sources of micro and macronutrients for human supplementation. Among these nutrients, Se can be highlighted, which is a micronutrient that participates in several antioxidant metabolic routes in human body (RIAZ et al. 2012).

According to TINGGI (2003), Se participates in the conversion of triiodothyronine hormone (T3) into thyroxine hormone (T4) and in the action against toxic and xenobiotic metals. It also participates in the prevention of chronic and non-communicable diseases, in the ubiquinone biosynthesis, which is an important biological process, and it is an essential nutrient for animals and humans that can be obtained as a source for yeast enrichment.

The yeast *S. cerevisiae* used in alcoholic fermentations has the ability to transform inorganic Se into organic compounds, which facilitates its bioavailability in the body and, depending on its growth conditions, it can accumulate remarkable amounts of Se in the form of selenomethionine and selenocysteine (PEDRERO et al. 2009). The organic forms of Se are part of the active site of important selenoproteins, such as glutathione peroxidase, which act to contribute to cell homeostasis by fighting free radicals.

For these reasons, the objectives of this study were to evaluate the tolerance of yeast *S. cerevisiae* to high Se concentrations; to carry out the evolutionary adaptation of yeasts to this mineral and to investigate the morphological variations of yeasts colonies / cells according to the evolutionary adaptation.

Material And Methods

Testing location:

The tests were carried out at the Sugarcane and Bioenergy Technology Laboratory (LTSBio), Sugar and Alcohol Sector, Department of Agribusiness, Food and Nutrition, of ESALQ / USP.

Tolerance study of *Saccharomyces cerevisiae* to Se:

The tolerance tests of the yeast *S. cerevisiae* Y904 to Se, as sodium selenite (Na_2SeO_3) ACS QM[®] with 99.0% purity, were performed in Petri dishes, containing YEPDA culture medium (0.5% Yeast Extract, 1% Peptone, 2% Dextrose and 2% Agar) with and without the addition of Se. The treatments were: 0 $\mu\text{g mL}^{-1}$ (T1); 30 $\mu\text{g mL}^{-1}$ (T2); 60 $\mu\text{g mL}^{-1}$ (T3); 120 $\mu\text{g mL}^{-1}$ (T4) and 240 $\mu\text{g mL}^{-1}$ of Se (T5). In addition, an intermediate treatment of 70 $\mu\text{g mL}^{-1}$ (T6) of Se was performed, so that the limit concentration tolerated by the yeast cell could be ensured. Cultivation was performed in quadruplicates, with 10 mL of substrate and 100 μL of inoculum, under two serial dilutions of 10^{-5} and 10^{-6} CFU mL^{-1} , incubated under $30^\circ\text{C} \pm 2^\circ\text{C}$, from 24 to 48 hours, depending on the appearance of colonies (ASSUNÇÃO 2011).

As a criterion for analyzing tolerance, yeast growth was considered up to 48 hours of incubation, after inoculation in a Se-rich medium. Thus, yeasts growing under these conditions were considered tolerant and those that did not grow were considered susceptible to Se.

Adaptation study of *Saccharomyces cerevisiae* in a medium enriched with sodium selenite:

From the results obtained in the tolerance study, the maximum dose of the nutrient in which the yeasts managed to grow was selected. Then the adaptation process of the yeast *S. cerevisiae* Y904 was started in a culture medium enriched with sodium selenite (Na_2SeO_3) ACS QM[®], 99.0% purity.

The first adaptive cycle was performed with YEPDA enriched with 60 $\mu\text{g mL}^{-1}$ of Se. Subsequently, gradual increases in Se concentrations of 6 $\mu\text{g mL}^{-1}$ per cycle were performed during 32 consecutive

culture cycles. The adaptation process started with $60 \mu\text{g mL}^{-1}$ (D1) and in cycle 32, the concentration of Se in the culture medium was $246 \mu\text{g mL}^{-1}$ (D32).

Petri dishes contained 10 mL of substrate and received 100 μL of inoculum, with dilutions of 10^{-5} and 10^{-6} CFU mL^{-1} . The incubation was carried out at $30 \pm C \pm 2 \text{ }^\circ\text{C}$, for 24 to 48 hours, according to the colonies growth and to the methodology described by ASSUNÇÃO (2011). As the colonies grew, those that grew within the 48-hour period were considered adapted and those that did not grow were considered susceptible. The analyzed macromorphological characteristics of the colonies / cells were: color, size, odor and roughness.

Scanning Electron Microscopy (Sem) Analysis:

After the yeast adaptation tests to the minimum, average and maximum concentrations of sodium selenite equivalent at T1 ($60 \mu\text{g mL}^{-1}$), T11 ($120 \mu\text{g mL}^{-1}$) and T31 ($240 \mu\text{g mL}^{-1}$), respectively, SEM analysis was performed in the treatments.

The biomass of each treatment was stored in a 0.5 mL microtube containing the modified Karnovsky reagent, composed of 2.5% glutaraldehyde, 2.5% formaldehyde 0.05M, sodium cacodylate buffer solution at pH 7.2, and CaCl_2 0.001 M. The samples preparation followed the protocol of KITAJIMA (1999), in which a drop of poly-L-lysine was added to the coverslip and this was placed to rest for 15 to 20 minutes, then a sample drop was added in suspension keeping at rest for more 30 minutes in the coverslip. The coverslips with separators between one sample and another were placed in the "cage" to dehydrate inside the "cage" in a beaker in increasing concentrations of acetone: 30%, 50%, 70% and 90% for 30 minutes at each concentration, and 100%, 3 times of 30 minutes each, to then be dried to the critical point, using CO_2 . Finally, the coverslips were fixed in the *stubs* submitted to the metallization process, so that the samples could be observed and analyzed in the SEM with an increase of 10,000 times.

Results

Tolerance of *Saccharomyces cerevisiae* to Se:

In the treatments used to define the dose of tolerance to Se, concentrations of $0 \mu\text{g mL}^{-1}$ (T1), $30 \mu\text{g mL}^{-1}$ (T2), $60 \mu\text{g mL}^{-1}$ (T3), $120 \mu\text{g mL}^{-1}$ (T4) and $240 \mu\text{g mL}^{-1}$ (T5) of sodium selenite were added to the medium. The results obtained showed that the cultivation of yeasts under the conditions of the T1 (control), T2 and T3 treatments obtained colonies growth within 48 hours after inoculation. The composition of the T3 medium was the maximum Se concentration that yeasts managed to grow. On the other hand, no colonies growth was observed within 48 hours after incubation, on substrates subjected to treatments T4 and T5. In the T1 treatment (without the addition of sodium selenite) with 10^{-5} CFU mL^{-1} dilution of the inoculum, the largest number of colonies was obtained per unit volume of substrate, with 3.9×10^2 CFU mL^{-1} . These colonies showed white color, circular shape, not rough and sizes varying

between 0.8 and 5 mm in diameter. Under the conditions of treatments T2 and T3, smaller increases in the number of colonies were observed, with approximately 2.8 and 0.1×10^2 CFU mL⁻¹, respectively. It was also identified that these colonies showed white color, circular shape and not rough. However, ranging from 2 and 5 mm in diameter. In the conditions of treatments T4 and T5, no growth of colonies was observed within 48 hours of incubation, as can be seen in Fig. 1, with the images of the treatments of tolerance.

As no colony growth was identified at a concentration of $120 \mu\text{g mL}^{-1}$ (T4) in YEPDA culture medium, it was necessary to define the tolerance interval between 60 (T3) and 120 (T4) $\mu\text{g mL}^{-1}$. However, at the concentration of $70 \mu\text{g mL}^{-1}$ of sodium selenite (T6), there was no growth of colonies. Therefore, it was defined that $60 \mu\text{g mL}^{-1}$ (T3) was the maximum concentration with cell growth without the need for an adaptive process.

Adaptation of *Saccharomyces cerevisiae* to Se:

The adaptive process carried out for 64 days in 32 consecutive cultivation cycles made expansion of tolerance capacity of yeasts to Se from $60 \mu\text{g mL}^{-1}$ to up to $246 \mu\text{g mL}^{-1}$ possible. As the concentration was increased, changes in the color of the yeast colonies were observed (Figs. 2, 3, 4 and 5).

At the beginning of the adaptation process, the colonies were light beige and shiny, as the doses of Se in the medium were increased. Then the yeast colonies began to show a darker color so that, after 32 cultivation cycles, the colonies had an intense reddish brown color. In addition, there was a reduction in the number of colonies and an increase in the roughness of the colonies on the entire surface, but mainly at the edges. Changes in the odor of the colonies were also observed, which began to show similarity to the smell of hydrogen sulfide.

In Fig. 6a it is possible to clearly see the color change from beige to orange red, when the yeasts were grown at $96 \mu\text{g mL}^{-1}$ medium. In Fig. 6b, colonies with intense orange red coloring and roughness at the edges were found when the yeasts were grown at $150 \mu\text{g mL}^{-1}$.

Although yeasts grow in concentrations from 222 to $246 \mu\text{g mL}^{-1}$, a reduction in the growth speed of colonies that started to develop after 24 or 36 hours of incubation was observed. While at concentrations of 60 , 66 and $72 \mu\text{g mL}^{-1}$, yeast colonies could be observed in the first 12 hours of incubation.

With the presence of high concentrations of Se in the substrate, morphological changes were observed in the yeast cells. At T6 ($90 \mu\text{g mL}^{-1}$ of sodium selenite in the culture medium) was possible to verify cell clusters, increase in size and transverse area of the cells, using an optical microscope (Fig. 7).

The changes promoted in the yeast cell wall were observed by means of scanning electron microscopy, through which it was possible to observe the wrinkling of the surface of the yeast cells and changes in the shape of the cells when they were subjected to concentrations of 60 , 120 and $240 \mu\text{g mL}^{-1}$ of sodium selenite (Fig. 8), once the characteristic of yeast without the presence of Se is smooth.

Discussion

The yeasts submitted to Treatment T2 ($60 \mu\text{g mL}^{-1}$ of sodium selenite) grew within the estimated time of 24 to 48 hours, without the need for cell adaptation of the yeasts to the Se; therefore, the maximum concentration of yeast (*S. cerevisiae*), strain Y904, supports without the adaptive process. Similar results were found in the works of Assunção (2011) and Rajashree et al. (2013), using the same species of yeast, but from another strain.

According to Assunção (2011), the objective was to evaluate the inhibitory effect of 0.0 concentrations; 5.6; 34.8; 49.7 and $94.0 \mu\text{g mL}^{-1}$ of sodium selenite in YEPDA in the growth of the yeast *S. cerevisiae* EVN 166, in 24 h. As a result, the colony-forming unit (CFU mL^{-1}) was the same for all Se concentrations after 24 h of growth; however, a slightly lower value of CFU mL^{-1} was observed for the $94.0 \mu\text{g mL}^{-1}$ of sodium selenite.

The study by Rajashree et al. (2013), aimed to analyze the toxicity of Se (0, 10, 20, 30, 40, 50, 75, 100, 125, $150 \mu\text{g mL}^{-1}$ of sodium selenite) in yeast cells *S. cerevisiae* NCYC 1026 and the effects on biomass production, in sterile Sabouraud Dextrose, under aseptic conditions and incubation for 72 hours at 30°C . As a result, it obtained the highest concentration of $75 \mu\text{g mL}^{-1}$ with no change in biomass production, and $50 \mu\text{g mL}^{-1}$ taking into account the bioaccumulation of Se by the cell.

According to the studies by Nagodawithana et al., Kaur et al. (2006), Stabnikova et al. (2008) and Marinescu et al. (2011), the decrease in the number of yeast cells is directly related to the increase in the concentration of sodium selenite in the culture medium, because the higher amounts of sodium selenite in the culture medium has a strong inhibitory effect on growth of yeast. Explained by Kieliszek et al. (2019a), the slowdown in yeast growth may be a result of the occurrence of oxidative stress caused by the presence of high concentrations of Se in the culture medium, which can lead to another phenomenon called the level of lipid peroxidation.

With the increase in the concentration of sodium selenite, the yeasts showed an intense reddish brown color. Changes in colony odor were also observed. It was similar to the smell of hydrogen sulfide. According to Suhajda et al. (2000), this may occur due to the substitution of Sulfur by Se in the enzymes. The Se compounds follow the same metabolic pathways and the metabolites are analogous to that of S. These changes in cell staining can be explained by the biotransformation that happens inside the cell, when the selenite (transparent coloring) is reduced to Se amorphous (reddish coloring) (KONETZKA 1977).

In the work of Bierla et al. (2013), the substitution and degree of substitution of sulfur for Se in methionine and cysteine, in Se-rich yeasts, using plasma mass spectrometry inductively coupled by capillary HPLC (ICP-MS), used in parallel to capHPLC-ESI-MS was investigated. As a result, substitution of cysteine sulfur is three times less frequent than that of methionine sulfur. Taking into account the

amounts of methionine and cysteine available in yeast cells, they concluded that the estimate of selenocysteine concentration in Se-rich yeasts was 15 to 30% of selenomethionine.

Birringer et al. (2002) demonstrated that the small amounts of organic Se compounds in plants, yeasts, bacteria or animals are isologs of sulfur compounds and the enzymes involved in the metabolism and trans-sulfurization pathway do not normally discriminate between sulfur and Se compounds.

Also according to Birringer et al. (2002), Se causes morphological changes in yeast, possibly altering the structure of the cell wall and membrane complex. In the work by Kieliszek et al. (2019b), Se supplementation increased the participation of unsaturated acids, such as linoleic acid and linolenic acid, in the biomass of *Candida utilis* ATCC 9950 and *S. cerevisiae* MYA-2200. As the biosynthesis of these acids may be associated with increased desaturase activity and lipid peroxidation, these processes may be directly linked to changes in yeast cell morphology. Some changes already mentioned in the literature are: increase in the size of cells, shrinkage of yeasts, thickening of the cytoplasm or changes in the structure of the vacuole (Kieliszek 2016).

The morphological change in yeasts according to the increase in the concentration of sodium selenite was also found in the work of Rajashree et al. (2013), where the control group without the addition of sodium selenite had yeast cells with a smooth edge surface, while the treatments with sodium selenite acquired roughness on the surface. Damage to the cell wall was observed in yeast cells, which was caused by high concentrations of sodium selenite, resulting in a reduction in the number of yeast cells. According to Kieliszek et al. (2016), the addition of sodium selenite (salt) to the medium causes osmotic stress in the yeast cells, and as a response, the formation of grooves in the cell wall and consequently the wrinkling occurs, thus being the identified roughness.

The results of the optical microscopy images (Fig. 7) corroborate with those observed in the study conducted by Kieliszek et al. (2016), where the analysis of microscopic images of yeasts of the species *C. utilis* TCC 9950, demonstrated that the concentrations of 20, 30 and 40 $\mu\text{g mL}^{-1}$ of sodium selenite in the substrate caused a significant increase in size and cross-sectional area of cells due to agglomerations and vacuoles among them, when compared to cells without the addition of Se. Comparing with other similar results, Rajashree et al. (2013) identified the change in the surface of the yeasts by means of SEM, observing that the smooth surface of *S. cerevisiae* without the addition of sodium selenite, contrasted yeast cells with rough surfaces when grown in a medium enriched with 50 $\mu\text{g mL}^{-1}$ of sodium selenite, while those grown in 100 $\mu\text{g mL}^{-1}$ were partially damaged (with small cracks).

Despite being of the same species, *S. cerevisiae*, and having the ability to bioaccumulate several elements and, as a result, tolerate higher concentrations in the medium, the different strains show different behaviors to stress and specific variations. There are studies with yeasts of the same species that tolerate different concentrations of Se (WHITE 1987). As in the results obtained by Wang et al. (2010), the addition of 90 $\mu\text{g mL}^{-1}$ in the late exponential development phase of yeast of lineage GS2, was the highest tolerated concentration taking into account the decrease in biomass production.

In the present work, the adaptive process allowed the yeast to tolerate up to $246 \mu\text{g mL}^{-1}$ of sodium selenite in the culture medium. Due to the gradual increase of Se in the medium and the metabolic interactions caused by Se, the yeasts presented different characteristics, such as, the reduction of the cell multiplication speed, roughness, intense hydrogen sulfide odor and increase in the intensity of the reddish brown color. Such results were similar to those cited in the literature. Suhajda et al. (2000), enriched *S. cerevisiae* also using sodium selenite, obtained a reddish color in the yeast cells.

The adaptive ability of yeast can be acquired when exposed to the appropriate selection pressure (WHITE 1987). According to Bronzetti et al. (2001), the addition of high concentrations of sodium selenite to the culture medium demonstrated to have a mutagenic effect in the yeast cells of the species *S. cerevisiae* D7, generating a 70% decrease in cell survival when compared to the control group.

The change in all these characteristics (change in color, roughness, odor, size and number of cells) with the increase in the concentration of sodium selenite, demonstrates how the yeasts have undergone modifications and / or adaptations in order to survive the stress caused by the enrichment of the culture medium with Se, which may induce to believe that adaptive evolution of yeast cells has occurred.

Declarations

Acknowledgements

This study was supported and financed by the foundation Coordination for the Improvement of Higher Education Personnel (CAPES) of public interest.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001.

Conflicts of interest/Competing interests

I declare that there is no conflict of interest between the authors of the article entitled: “Ability of the *Saccharomyces cerevisiae* Y904 to tolerate and adapt to high concentrations of selenium” submitted for consideration at the scientific seriodical World Journal of Microbiology and Biotechnology.

Availability of data and material

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Code availability

Not applicable

Author contributions

Conceptualization: Layna Amorim Mota and Antonio Sampaio Baptista; Methodology: Layna Amorim Mota and Ana Paula Maria da Silva; Formal analysis: Layna Amorim Mota, Gabriela Maria Ferreira Lima Leite and Eric Alberto da Silva; Investigation: Layna Amorim Mota, Ana Paula Maria da Silva, Rubens Perez Calegari, Eric Alberto da Silva and Gabriela Maria Ferreira Lima Leite; Resources: Antonio Sampaio Baptista; Data curation: Layna Amorim Mota, Ana Paula Maria da Silva, Rubens Perez Calegari and Eric Alberto da Silva; Writing-original draft preparation: Layna Amorim Mota; Writing-review and editing: Rubens Perez Calegari, Antonio Sampaio Baptista, Ana Paula and Layna Amorim Mota; Visualization: Antonio Sampaio Baptista; Supervision: Antonio Sampaio Baptista; Funding acquisition: Antonio Sampaio Baptista. All authors have read and agreed to the published version of the manuscript.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. Amanullah M, Zaman GS, Rahman J, Rahman SS (2012) Lipid peroxidation the levels of antioxidant enzymes in hypertension. *Free Radicals Antioxidants* 2(2):12–18. <https://doi.org/10.5530/ax.2012.2.2.3>
2. Arthur JR, McKenzie RC, Beckett GJ (2003) Selenium in the immune system. *J Nutr*, 133(5), 1457S–1459S. <https://doi.org/10.1093/jn/133.5.1457S>
3. Assunção MADS (2011) Avaliação da tolerância ao selênio de diferentes espécies de leveduras em ensaios de fermentação. Dissertation, Universidade Técnica de Lisboa
4. Basso LC, De Amorim HV, De Oliveira AJ, Lopes ML (2008) Yeast selection for fuel ethanol production in Brazil. *FEMS yeast research* 8(7):1155–1163. <https://doi.org/10.1111/j.1567-1364.2008.00428.x>
5. Bierla K, Bianga J, Ouerdane L, Szpunar J, Yiannikouris A, Lobinski R (2013) A comparative study of the Se/S substitution in methionine and cysteine in Se-enriched yeast using an inductively coupled plasma mass spectrometry (ICP MS)-assisted proteomics approach. *Journal of proteomics* 87:26–39. <https://doi.org/10.1016/j.jprot.2013.05.010>
6. Birringer M, Pilawa S, Flohé L (2002) Trends in selenium biochemistry. *Natural product reports* 19(6):693–718

7. Bronzetti G, Cini M, Andreoli E, Caltavuturo L, Panunzio M, Della Croce C (2001) Protective effects of vitamins and selenium compounds in yeast. *Mutation Research/Genetic Toxicology Environmental Mutagenesis* 496(1–2):105–115. <https://doi.org/10.1039/B205802M>
8. Canhos VP, Manfio GP (2001) Recursos Microbiológicos para biotecnologia. URL: [http://www.mct.gov.br/Temas/biotec/Tendencias% 20_Vanderlei% 20Fina_. pdf](http://www.mct.gov.br/Temas/biotec/Tendencias%20_Vanderlei%20Fina_.pdf)
9. Caseiro C (2011) A produção e o consumo de biocombustíveis no mundo atual: questões-chave para analisar a sua sustentabilidade. *Campo Território: Revista de geografia agrária* 6(12):6–31
10. Desmonts R (1966) Tecnologia da produção dos fermentos secos de destilaria. *Boletim Inform da Ass Paul Med* 8:1–7
11. Faleiro FG, Andrade SD (2011) Biotecnologia: uma visão geral. Estado da arte e aplicações na agropecuária, Planaltina, p 13–29
12. Goes T, Marra R (2008) Biocombustíveis: uma alternativa para o mundo, uma oportunidade para o Brasil. Área de Informação da Sede-Artigo em periódico indexado (ALICE)
13. ICIDCA. Instituto Cubano de Pesquisa dos Derivados da Cana-de-açúcar. Manual dos derivados da cana-de-açúcar: diversificação, matérias-primas, derivados do 122 bagaço, derivados do melaço, outros derivados, resíduos, energia. Brasília: ABIPTI (1999) p. 49–55, 267–271, 297–301
14. Jobard M, Rasconi S, Sime-Ngando T (2010) Diversity and functions of microscopic fungi: a missing component in pelagic food webs. *Aquat Sci* 72(3):255–268. DOI 10.1007/s00027-010-0133-z
15. Kieliszek M, Błażej S, Bzducha-Wróbel A, Kurcz A (2016) Effects of selenium on morphological changes in *Candida utilis* ATCC 9950 yeast cells. *Biol Trace Elem Res* 169(2):387–393. DOI 10.1007/s12011-015-0415-3
16. Kieliszek M, Błażej S, Bzducha-Wróbel A, Kot AM (A) (2019) Effect of selenium on growth and antioxidative system of yeast cells. *Molecular biology reports*, 46(2), 1797–1808. <https://doi.org/10.1007/s11033-019-04630-z>
17. Kieliszek M, Błażej S, Bzducha-Wróbel A, Kot AM (B) (2019) Effect of selenium on lipid and amino acid metabolism in yeast cells. *Biol Trace Elem Res*, 187(1), 316–327. <https://doi.org/10.1007/s12011-018-1342-x>
18. Kitajima EW, Leite B (1997) Curso introdutório de microscopia eletrônica de varredura. ESALQ, Piracicaba, p 37
19. Konetzka WA (1977) Microbiology of metal transformations. *Microorganisms and minerals*. Marcel Dekker New York, p. 318–342, pp 318–342
20. Leite RDC, Leal MRL (2007) O biocombustível no Brasil. *Novos estudos CEBRAP*, (78), 15–21. <http://dx.doi.org/10.1590/S0101-33002007000200003>
21. Marinescu G, Stoicescu AG, Teodorof L (2011) Industrial nutrient medium use for yeast selenium preparation. *Annals of the University Dunarea de Jos of Galati Fascicle VI–Food Technology*, 35(1)
22. Martiniano SE (2007) Produção de leveduras enriquecidas com selênio a partir de resíduos vegetais. Thesis, Universidade de São Paulo

23. Mehdi Y, Hornick JL, Istasse L, Dufrasne I (2013) Selenium in the environment, metabolism and involvement in body functions. *Molecules* 18(3):3292–3311.
<https://doi.org/10.3390/molecules18033292>
24. Mussatto SI, Dragone G, Guimarães PM, Silva JPA, Carneiro LM, Roberto IC et al (2010) Technological trends, global market, and challenges of bio-ethanol production. *Biotechnology advances* 28(6):817–830. <https://doi.org/10.1016/j.biotechadv.2010.07.001>
25. Nelson DL, Cox MM (2018) *Princípios de Bioquímica de Lehninger-7*. Artmed Editora, São Paulo
26. Pedrero Z, Madrid Y (2009) Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. *Analytica chimica acta* 634(2):135–152.
<https://doi.org/10.1016/j.aca.2008.12.026>
27. Rajashree K, Muthukumar T (2013) Preparation of selenium tolerant yeast *Saccharomyces cerevisiae*. *Journal of Microbiology Biotechnology Research* 3(3):46–53
28. Riaz M, Mehmood KT (2012) Selenium in human health and disease: a review. *Journal of Postgraduate Medical Institute (Peshawar-Pakistan)*, 26(2)
29. Roepcke CBS, Vandenberghe LPS, Soccol CR (2011) Optimized production of *Pichia guilliermondii* biomass with zinc accumulation by fermentation. *Animal feed science technology* 163(1):33–42.
<https://doi.org/10.1016/j.anifeedsci.2010.09.018>
30. Santos C, Fonseca J (2013) Selénio: fisiopatologia, clínica e nutrição. *Associação Portuguesa de Nutrição Entérica e Parentérica*, v. 8, p. 1–9
31. Stabnikova O, Ivanov V, Larionova I, Stabnikov V, Bryszewska MA, Lewis J (2008) Ukrainian dietary bakery product with selenium-enriched yeast. *LWT-food Science Technology* 41(5):890–895.
<https://doi.org/10.1016/j.lwt.2007.05.021>
32. Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. *Annu Rev Microbiol* 60:107–130. <https://doi.org/10.1146/annurev.micro.60.080805.142053>
33. Suhajda A, Hegoczki J, Janzso B, Pais I, Vereczkey G (2000) Preparation of selenium yeasts I. Preparation of selenium-enriched *Saccharomyces cerevisiae*. *J Trace Elem Med Biol* 14(1):43–47.
[https://doi.org/10.1016/S0946-672X\(00\)80022-X](https://doi.org/10.1016/S0946-672X(00)80022-X)
34. Tinggi U (2003) Essentiality and toxicity of selenium and its status in Australia: a review. *Toxicology letters* 137(1–2):103–110. [https://doi.org/10.1016/S0378-4274\(02\)00384-3](https://doi.org/10.1016/S0378-4274(02)00384-3)
35. Wang Z, Zhang L, Tan T (2010) High cell density fermentation of *Saccharomyces cerevisiae* GS2 for selenium-enriched yeast production. *Korean J Chem Eng* 27(6):1836–1840.
<https://doi.org/10.1007/s11814-010-0300-x>
36. White C, GADD GM (1987) The uptake and cellular distribution of zinc in *Saccharomyces cerevisiae*. *Microbiology* 133(3):727–737. <https://doi.org/10.1099/00221287-133-3-727>
37. Zeyl C (2000) Budding yeast as a model organism for population genetics. *Yeast*, 16(8), 773–784.
[https://doi.org/10.1002/1097-0061\(20000615\)16:8<773::AID-YEA599>3.0.CO;2-1](https://doi.org/10.1002/1097-0061(20000615)16:8<773::AID-YEA599>3.0.CO;2-1)

Figures

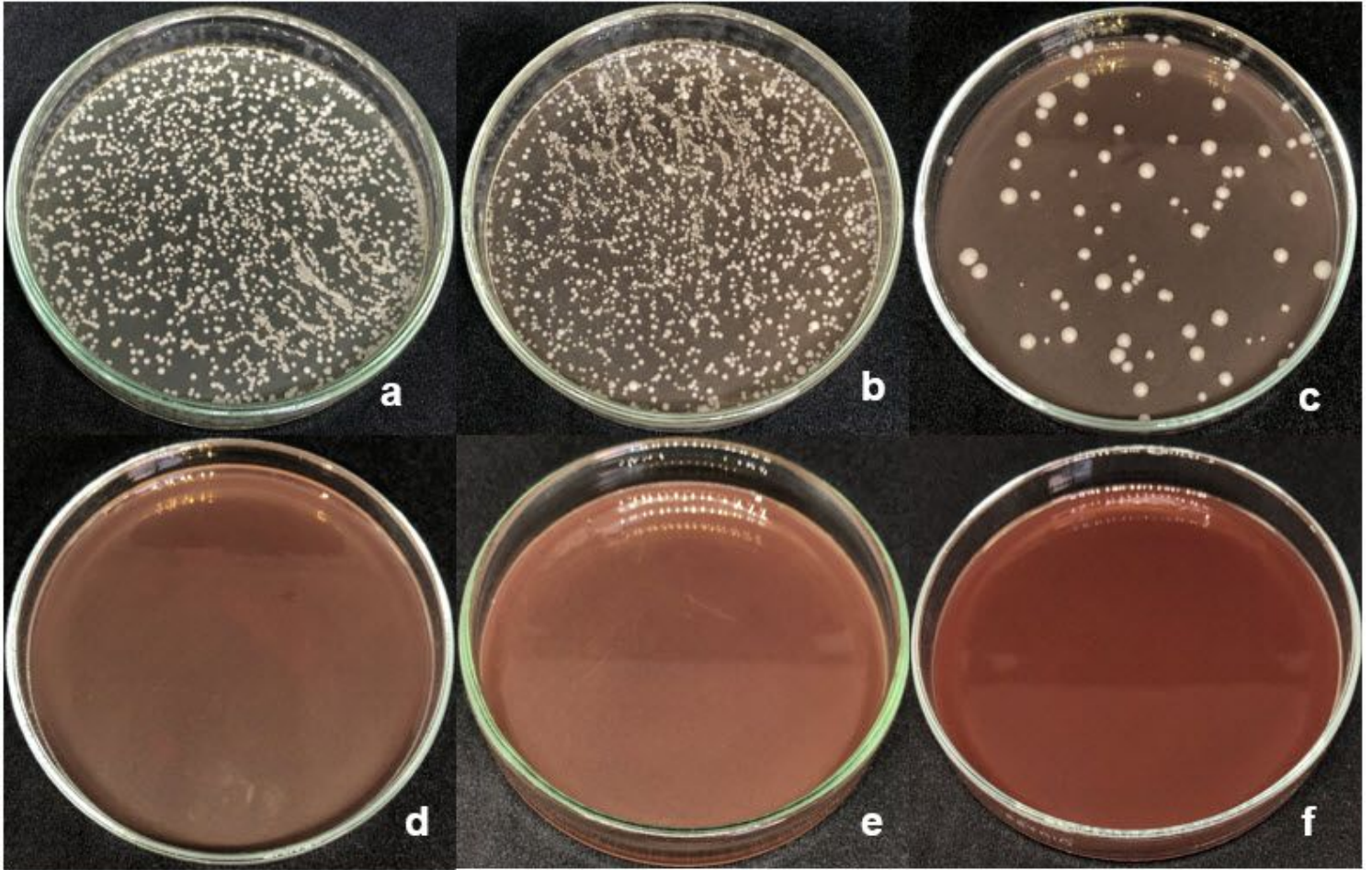


Figure 1

Yeast growth in YEPD medium, dilution 10^{-5} under 30°C for 48 hours. (a) Treatment T1, (b) Treatment T2; (c) Treatment T3; (d) Treatment T6; (e) Treatment T4; (f) Treatment T5

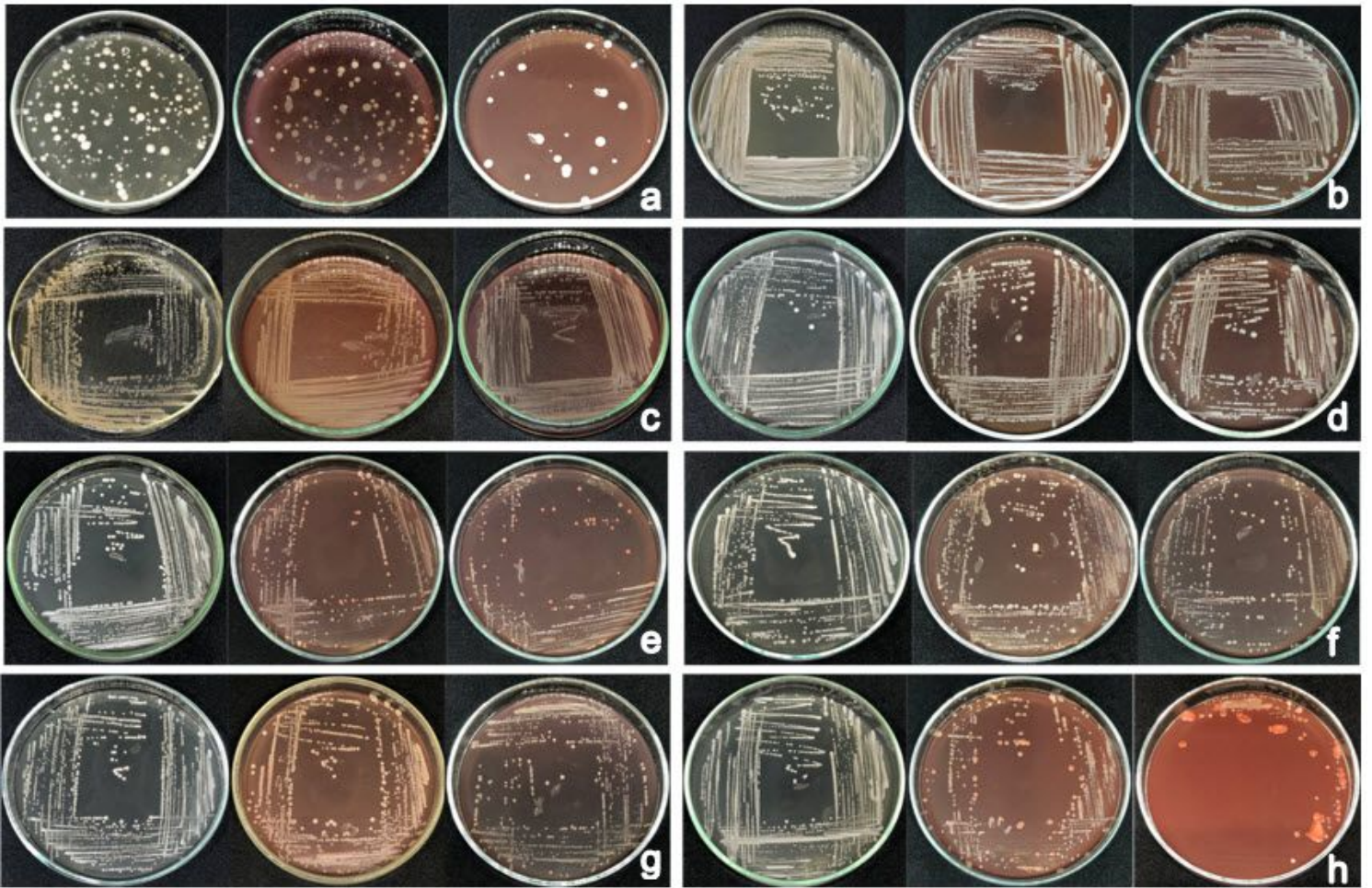


Figure 2

Yeast growth in YEPD medium at 30°C for 48 hours in the control group, dilution 10⁻⁵ and 10⁻⁶ respectively, according to the image for each letter. (a) Treatment T1; (b) Treatment T2; (c) Treatment T3; (d) Treatment T4; (e) Treatment T5; (f) Treatment T6; (g) Treatment T7; (h) Treatment T8

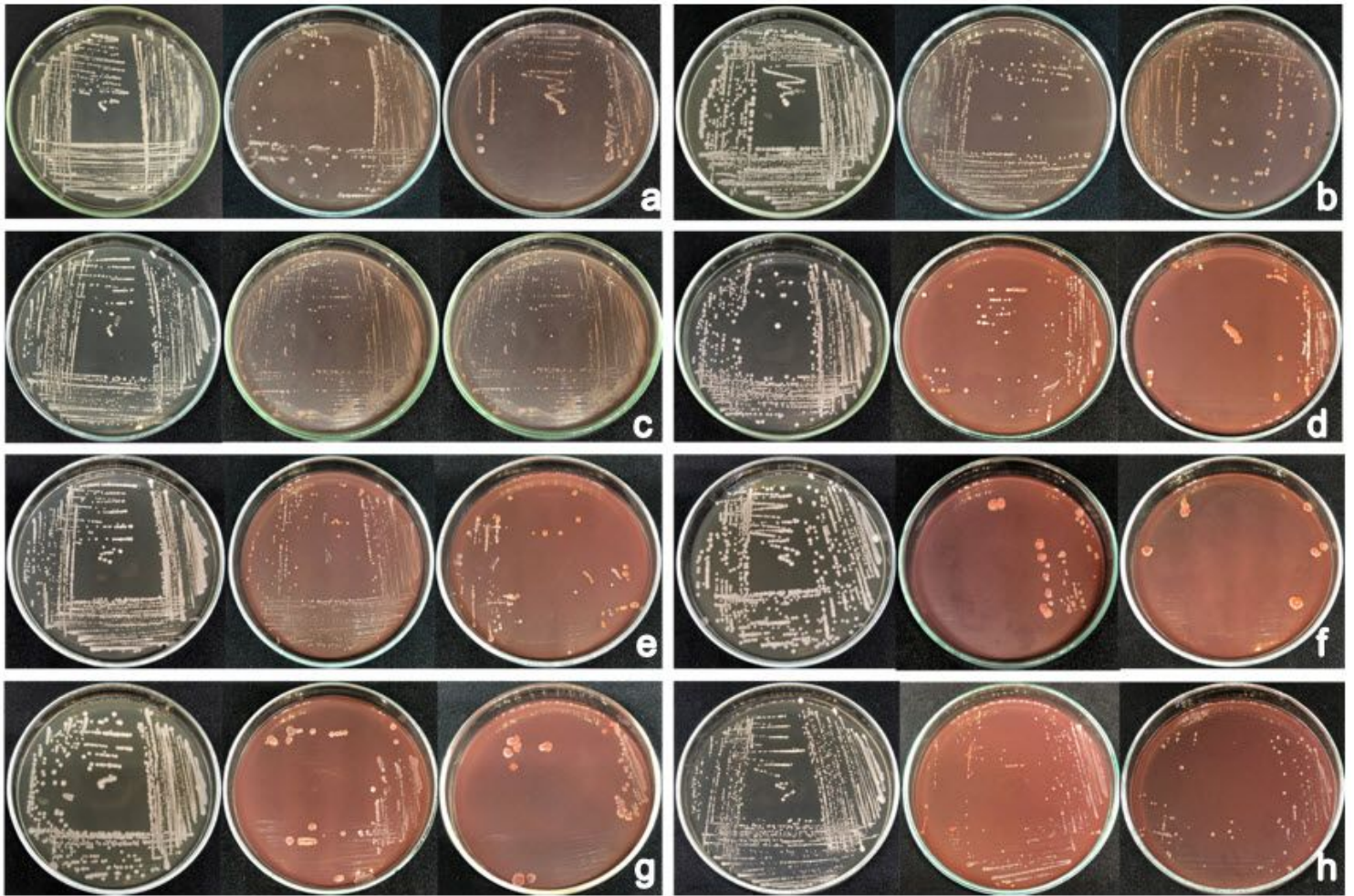


Figure 3

Yeast growth in YEPD medium at 30°C for 48 hours in the control group, dilution 10⁻⁵ and 10⁻⁶ respectively, according to the image for each letter. (a) Treatment T9; (b) Treatment T10; (c) Treatment T11; (d) Treatment T12; (e) Treatment T13; (f) Treatment T14; (g) Treatment T15; (h) Treatment T16)

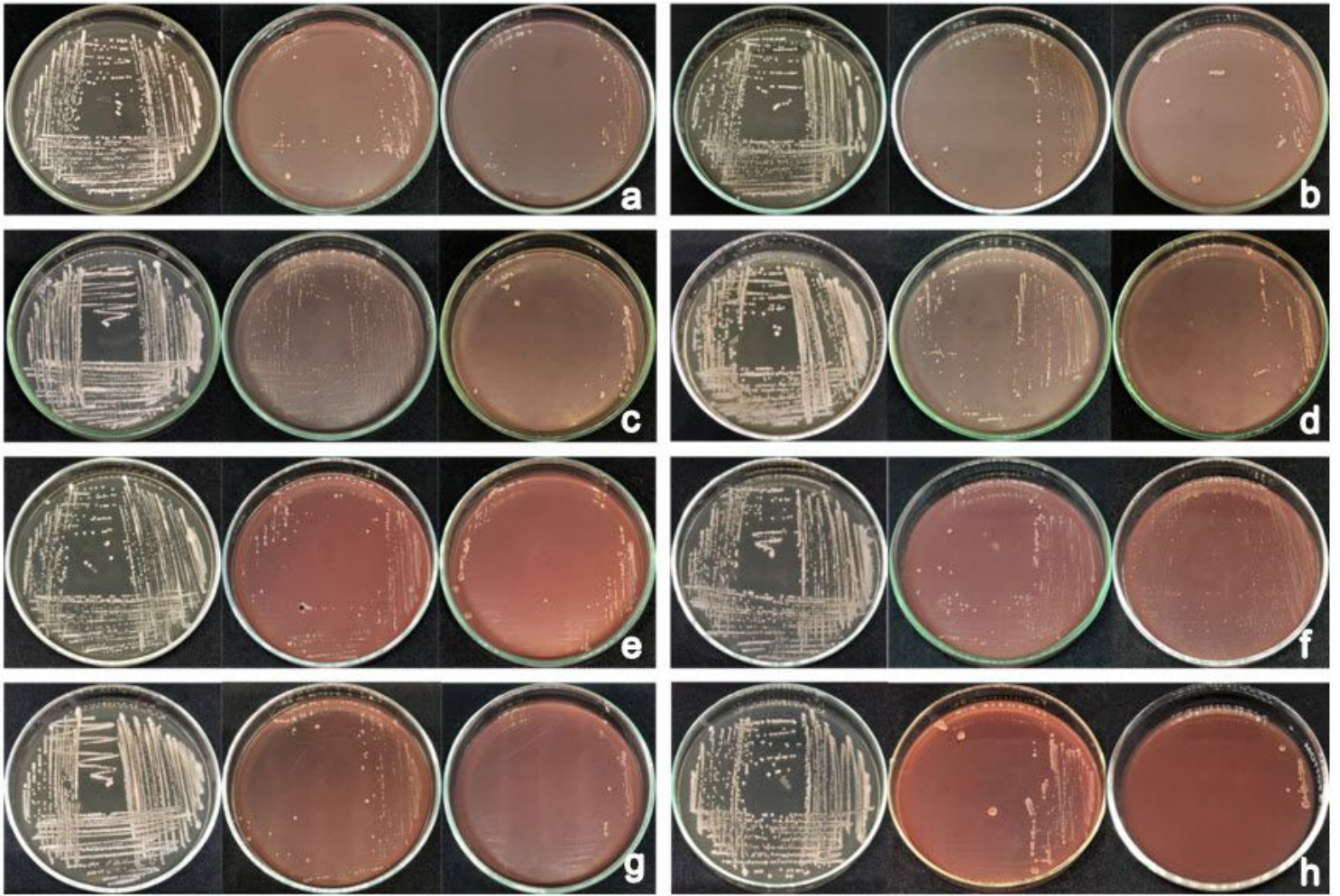


Figure 4

Yeast growth in YEPD medium at 30°C for 48 hours in the control group, dilution 10⁻⁵ and 10⁻⁶ respectively, according to the image for each letter. (a) Treatment T17; (b) Treatment T18; (c) Treatment T19; (d) Treatment T20; (e) Treatment T21; (f) Treatment T22; (g) Treatment T23; (h) Treatment T24

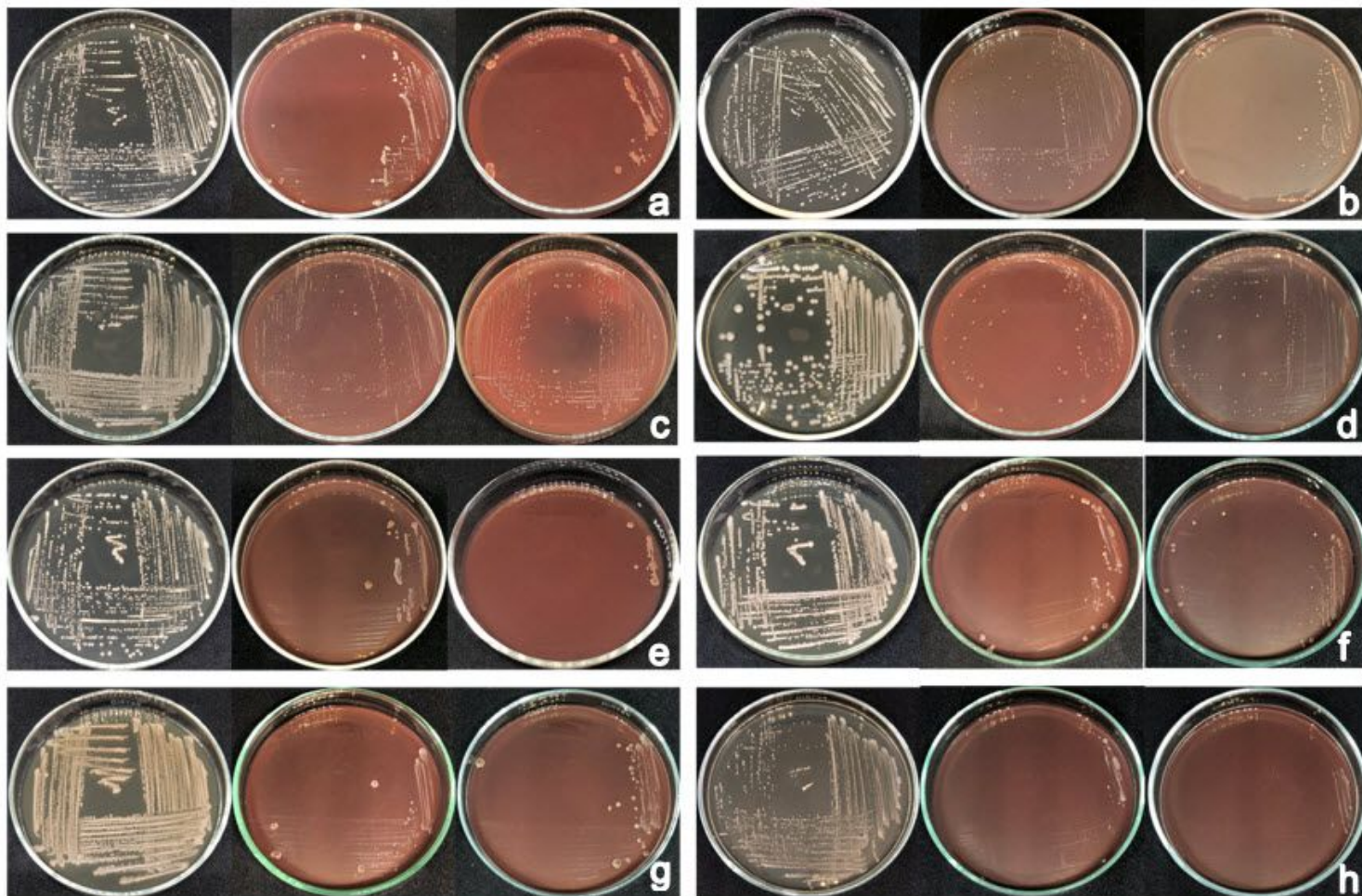


Figure 5

Yeast growth in YEPD medium at 30°C for 48 hours in the control group, dilution 10⁻⁵ and 10⁻⁶ respectively, according to the image for each letter. (a) Treatment T25; (b) Treatment T26; (c) Treatment T27; (d) Treatment T28; (e) Treatment T29; (f) Treatment T30; (g) Treatment T31; (h) Treatment T32

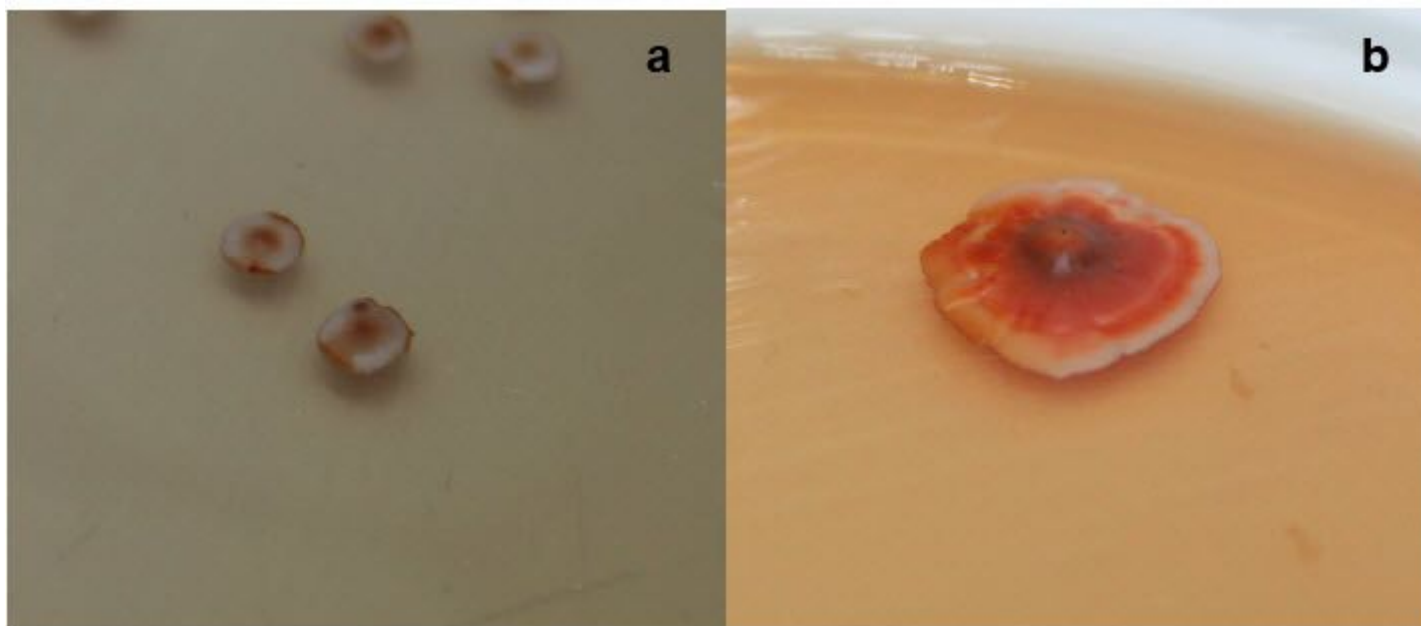


Figure 6

Adaptation of yeast cells *Saccharomyce cerevisiae*. (a) Treatment T7 e (b) Treatment T16

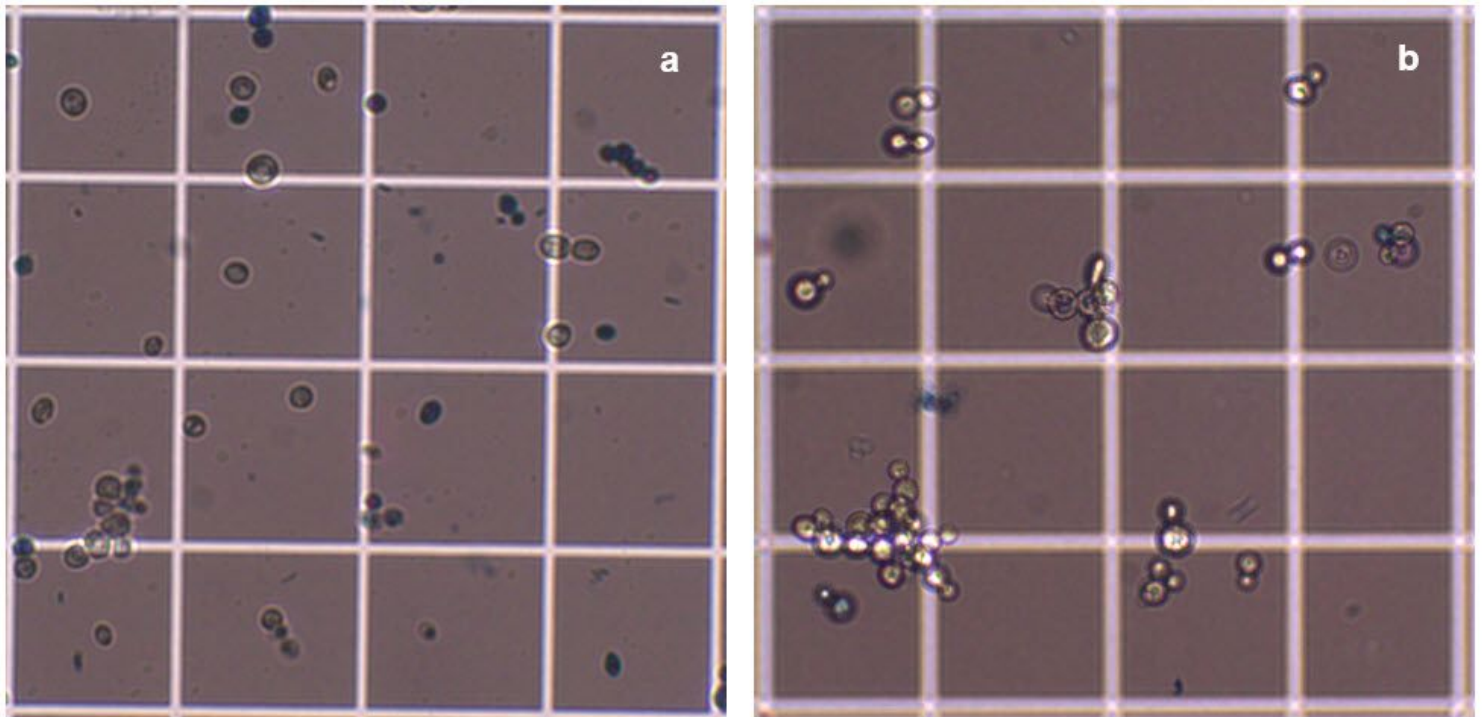


Figure 7

Optical microscopy of yeasts grown in YEPD medium at 30°C after 48 hours, with a 400x magnification. (a) Treatment T1- Enrichment of the medium with 60 $\mu\text{g.mL}^{-1}$ of sodium selenite; (b) Treatment T16 – Enrichment of the medium with 156 $\mu\text{g.mL}^{-1}$ of sodium selenite

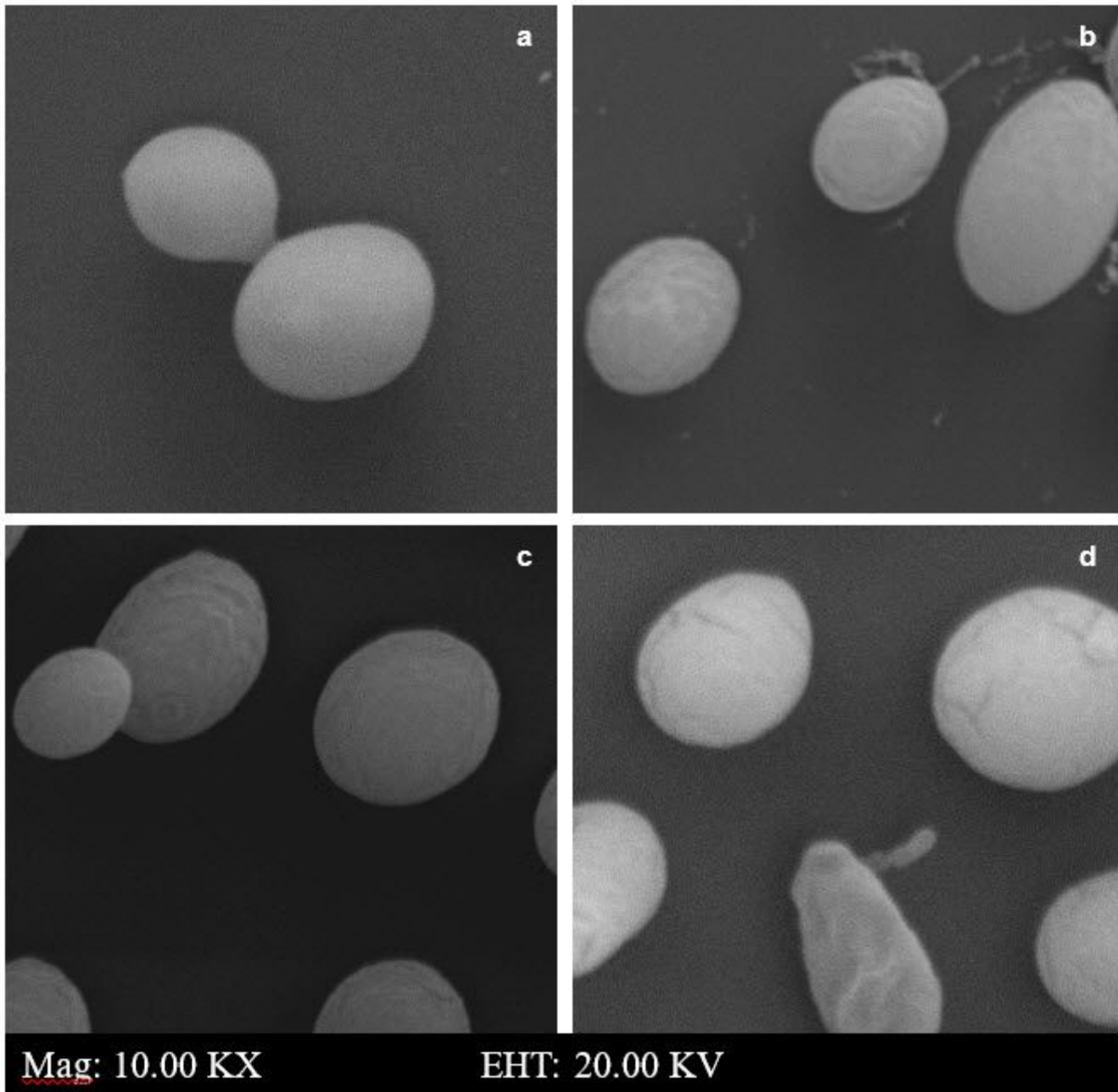


Figure 8

Scanning electron microscopy of *Saccharomyces cerevisiae* yeasts grown in YEPD medium at 30°C after 48 hours, (a) Control Treatment - No enrichment 0 µg.mL⁻¹ of sodium selenite; (b) Treatment T1 – Enrichment of the medium with 60 µg.mL⁻¹ of sodium selenite, (c) Treatment T10 – Enrichment of the medium with 120 µg.mL⁻¹ of sodium selenite e (d) Treatment T31 - Enrichment of the medium with 240 µg.mL⁻¹ of sodium selenite