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# Oxidative stress and antioxidant response in a thermotolerant yeast

Jorge A. Mejía-Barajas[a], Rocío Montoya-Pérez[a], Rafael Salgado-Garciglia[a], Leopoldo Aguilera-Aguirre[b], Christian Cortés-Rojo[a], Ricardo Mejía-Zepeda[c], Melchor Arellano-Plaza[d], Alfredo Saavedra-Molina[a,*]

[a] Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Químico-Biológicas, Morelia, Mich., Mexico
[b] University of Texas Medical Branch at Galveston, Galveston, TX, USA
[c] Unidad de Biomedicina, FES Iztacala, UNAM. Tlalnepantla, Mexico
[d] Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Guadalajara, Mexico

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ABSTRACT

Stress tolerance is a key attribute that must be considered when using yeast cells for industrial applications. High temperature is one factor that can cause stress in yeast. High environmental temperature in particular may exert a natural selection pressure to evolve yeasts into thermotolerant strains. In the present study, three yeasts (*Saccharomyces cerevisiae*, MC4, and *Kluyveromyces marxianus*, OFF1 and SLP1) isolated from hot environments were exposed to increased temperatures and were then compared with a laboratory yeast strain. Their resistance to high temperature, oxidative stress, and antioxidant response were evaluated, along with the fatty acid composition of their cell membranes. The SLP1 strain showed a higher specific growth rate, biomass yield, and biomass volumetric productivity while also showing lower duplication time, reactive oxygen species (ROS) production, and lipid peroxidation. In addition, the SLP1 strain demonstrated more catalase activity after temperature was increased, and this strain also showed membranes enriched in saturated fatty acids. It is concluded that the SLP1 yeast strain is a thermotolerant yeast with less oxidative stress and a greater antioxidant response. Therefore, this strain could be used for fermentation at high temperatures.



## Introduction

Yeasts are eukaryotic unicellular fungi that are widely distributed in natural environments. They are used in many industrial processes, such as the production of alcoholic beverages, biomass, and metabolic products. Currently, the majority of yeast biotechnology applications are with the species *Saccharomyces cerevisiae*. However, the limited stress resistance of *S. cerevisiae* has led to an increased focus on the potential of the

* *Corresponding author at*: Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mich. 58030, Mexico.
E-mail: saavedra@umich.mx (A. Saavedra-Molina).
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non-*Saccharomyces* spp. yeasts, such as the *Pichia* spp., *Debaryomyces* spp., and *Kluyveromyces* spp. The *Kluyveromyces* spp. is usually considered to be a thermotolerant yeast, with important commercial relevance to high temperature fermentation during ethanol production. High temperature is one of the most important factors affecting microbial activity, microbial growth rate and biomass yield.[1] The capacity to tolerate high temperatures is related to oxidative stress and the antioxidant response. High temperature increases oxidative stress and overexpression of antioxidant enzyme genes in *S. cerevisiae*.[2,3] However, this effect has not been studied in thermotolerant yeasts. Even less is known about yeasts isolated from damaged environments, where several types of stress affect communities, such as osmotic, temperature, pH, and oxidative stress. These conditions could apply a natural selection pressure on yeast to evolve into thermotolerant strains. Arellano-Plaza et al.[4] reported that the *Saccharomyces cerevisiae* (MC4) and *Kluyveromyces marxianus* (OFF1 and SLP1) yeast strains were able to resist oxidative stress for a long period of time compared with W303-1A (*S. cerevisiae* reference strain). The MC4, OFF1, and SLP1 yeast strains were isolated from spontaneous mezcal fermentation carried out at handcraft mezcal distilleries in Oaxaca, San Luis Potosí, and Guerrero (all Mexican states). Mezcal production occurs between October and May, when the environmental temperature decreases to −5 °C in winter, and can increase to 45 °C in spring. Little information is currently available regarding oxidative stress induction and the antioxidant response to increased temperatures in thermotolerant yeasts. The aim of this work was to select a thermotolerant yeast (yeasts that were isolated in regions of Mexico with high-temperature environments) and study its oxidative stress and antioxidant response.

## Materials and methods

### *Yeast strains*

Yeast strains were obtained from the culture collection of the CIATEJ (*Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, México*)[5] and from the ATCC (American Type Culture Collection, Rockville, MD, USA). Two *K. marxianus* yeast strains, SLP1 and OFF1, were isolated at handcraft mezcal distilleries in the Mexican State of San Luis Potosi and Guerrero, respectively, and one *S. cerevisiae*, MC4, was isolated at an Oaxaca state distillery. The ATCC yeast, W303-1A, was also used for comparison.

### *Growth conditions*

Yeast growth was studied using YPD media containing 1% yeast extract, 2% peptone, and 2% glucose as the carbon source. Cells were grown at 30 °C and shaken at 180 rpm for 24 h.

### *Cell viability*

Yeast strains were grown as mentioned above. The yeasts were then collected and inoculated $1 \times 10^7$ cell/mL in fresh YPD medium. The culture was incubated for 24 h under the same conditions. The cells were quantified, and yeast suspensions were cultured on YPD agar plates and incubated for 24 h at temperatures from 30 to 45 °C. After 24 h, the colony forming units (CFU) were determined.[6] The CFU at room temperature were taken as 100%.

Effect of temperature on specific growth rate, biomass yield, volumetric productivity, and duplication time.

The specific growth rate ($\mu$) was calculated by cell growth, measured by optical density (OD) of the cell suspensions, and estimated using the Lineweaver–Burk equation. For biomass determination yeast cells in broth were harvested, washed with distilled water, and dried in an oven at 80 °C until reaching a constant weight. The biomass (Dw) was reported in dry cell mass (g/L). The volumetric productivity of biomass (Qp) was calculated by dividing the biomass yield by the corresponding culture time. The duplication time (Td) was calculated with the equation ln(2)/K, where *K* is the rate constant.

### *Temperature increase*

An increase in temperature was applied as described by Kim et al., [7] with some modifications. Yeast strains were grown in a 10 mL YPD (2%) medium for 24 h at 30 °C and 180 rpm. A concentration of pre-cultured cells of $A_{600} = 0.03$ was transferred to fresh YPD (2%) media, incubated at 30 °C and 180 rpm until the stationary phase. Then, the cultured cells were incubated for 2 h at 40 °C.

### *Intracellular reactive oxygen species*

Intracellular reactive oxygen species in yeast cultures were determined using fluorescence assays with 2′,7′-dichlorodihydrofluorescein diacetate.[8] The cultured cells obtained from a pre-culture were incubated for 2 h at 40 °C, cells were counted and diluted in YPD medium to a final concentration of $0.5 \times 10^7$ cells. A 5-mM stock solution of dichlorofluorescein diacetate was added to each sample and incubated in the dark for 15 min at 30 °C. Afterwards, cells were harvested by centrifugation, washed, and re-suspended in 1930 μL 50 mM Tris/HCl buffer (pH 7.5). The cells were permeabilized by adding chloroform and SDS and vortexing at high speed for 20 s. The tubes were left to settle for 10 min. Cells were pelleted by centrifugation, and the supernatant fluorescence was measured using a Shimadzu RF-5301 fluorometer (excitation, 502; emission, 521 λ).

### *Lipid peroxidation*

The extent of lipid peroxidation was determined through the thiobarbituric acid (TBA) assay.[9] Temperature increase was generated as previously described. Cells were re-suspended in Tris–HCl buffer, pH 7.4, containing 10% trichloroacetic acid, and glass beads were added. The samples were broken by agitation on a vortex. After centrifugation at 300 g, supernatants were mixed with EDTA 0.1 M and 1% (w/v) thiobarbituric acid in NaOH 0.05 M. The reaction mixture was heated for 15 min in a boiling water bath, and then centrifuged. The absorbance at 532 nm was measured in a Perkin–Elmer spectrophotometer.



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