Revista Cubana de Química. Vol.37.2025
https://cubanadequimica.uo.edu.cu/index.php/cq
Research Papers

OENOLOGICAL POTENTIAL OF *Saccharomyces cerevisiae* AND *Kluyveromyces marxianus* STRAINS FOR USE IN MIXED FERMENTATIONS

POTENCIAL ENOLÓGICO DE CEPAS DE *Saccharomyces cerevisiae* Y *Kluyveromyces marxianus* PARA SU USO EN FERMENTACIONES MIXTAS

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Recibido: 18 de noviembre de 2025 Aprobado: 14 de diciembre de 2025

ABSTRACT

Wine fermentation is a complex process involving several yeast species. *Saccharomyces cerevisiae* species is particularly effective due to its fermentative efficiency, while non-*Saccharomyces* yeasts contribute to the aromatic complexity of wines. This study aimed to characterize five strains (four *S. cerevisiae* and one *Kluyveromyces marxianus*), from different isolation sources, in terms of characteristics of oenological interest. The results showed that the CCEBI 2015 and CCEBI 2050 strains from *S. cerevisiae* species, and *K. marxianus* CCEBI 2011 tolerate high concentrations of glucose (30 % w/v), ethanol (10 % v/v) and SO_2 (120 mg/l) and have low or no H_2S production. No antagonism was observed between *K. marxianus* and *S. cerevisiae* strains, suggesting their compatibility for mixed fermentations. These results indicate that these strains are promising candidates for use in mixed starter cultures for winemaking.

Keywords: *Kluyveromyces marxianus*; *Saccharomyces cerevisiae*; wine, mixed culture; oenological characteristics.

RESUMEN

La fermentación del vino es un proceso complejo en el que intervienen diversas especies de levadura. La especie *Saccharomyces cerevisiae* destaca por su eficiencia fermentativa y las levaduras no-*Saccharomyces*, por su contribución a la complejidad aromática de los vinos. Este estudio tuvo como objetivo caracterizar cinco cepas (cuatro *S. cerevisiae* y una *Kluyveromyces marxianus*), procedentes de diferentes fuentes de aislamiento, en cuanto a características de interés enológico. Los resultados mostraron que las cepas CCEBI 2015 y CCEBI 2050 de la especie *S. cerevisiae* y *K. marxianus* CCEBI 2011 toleran altas concentraciones de glucosa (30 % w/v), etanol (10 % v/v) y SO_2 (120 mg/L) y presentan baja o nula producción de H_2S. No se observó antagonismo entre *K. marxianus* y las cepas de *S. cerevisiae*. Estos resultados avalan el empleo de estas cepas como candidatas prometedoras, para su uso en cultivos iniciadores mixtos para vinificación.

Palabras clave: *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, vino, cultivo mixto, características enológicas.

INTRODUCTION

The biochemical transformation of must into wine is a process carried out by two main groups of oenological yeasts: *(i)* those belonging to the genus *Saccharomyces*, in particular those of the species *Saccharomyces cerevisiae*, with a key role in the transformation of must sugars into alcohol and *(ii)* non- *Saccharomyces* yeasts, a heterogeneous group that includes autochthonous strains, therefore related to a specific area, and which have been recently studied for their positive effects on wine fermentation.[1] Controlled fermentation from multiple starter cultures represents a microbial approach to achieve the dual purpose of having a less risky process and a distinctive final product. Indeed, the interactions developed between the members of the microbial consortium strongly modulate the final sensory properties of wine.[2]

For the above reasons, several authors have studied fermentations with mixtures of different yeast species, either applied simultaneously or in sequential cultures. In the first case (coinoculation), selected non- *Saccharomyces* yeasts are inoculated at a high viable cell concentration together with *S. cerevisiae*; in the second (sequential inoculation), selected non-*Saccharomyces* yeasts are inoculated first, at high cell densities, allowing fermentation for a given time, before inoculation of *S. cerevisiae* to complete the fermentation. The use of both practices is feasible; the choice of a more appropriate inoculation strategy is based on the potential interaction between the yeasts.[3]

Yeast cells are subjected to a variety of stress factors during winemaking processes, such as high osmolarity, high sulfite levels, nutrient depletion, acid stress, anaerobiosis, and a constantly increasing ethanol concentration. In the wine industry, one of the main trends is the use of new starter cultures to create high-quality wines for which there are two possibilities: natural selection of new strains and genetic improvement of existing ones. Currently, the strains used as starters they are originally isolated from the natural environment and selected based on advantageous characteristics.[4]

Kluyveromyces marxianus is frequently isolated from dairy products, such as cheese and kefir, but is also isolated from habitats such as fruits, decaying plant tissues, insects and agro-industrial waste.[5] Its long history of use allows it to be a generally recognized as safe (GRAS) microbial species. *K. marxianus* is considered a yeast with prebiotic and probiotic potential, capable of utilizing a broad spectrum of carbon sources, including carbohydrates and organic acids; it also has other beneficial characteristics, such as its high growth rate and thermotolerance, make it a particularly attractive host for applications in a variety of food and biotechnology industries.[5-9] Its potential use in wine production has recently been suggested due to several potentially interesting aspects such as its enzymatic activity and aroma production.[9] The objective of this research was to characterize five yeast strains from different isolation sources, in as for oenological characteristics of interest in order to use them in mixed *Saccharomyces*/non- *Saccharomyces* starter cultures in wine production.

MATERIALS AND METHODS

Microorganisms

For the evaluation of the oenological characteristics, four strains of *Saccharomyces cerevisiae* and one non-*Saccharomyces* strain belonging to the species *Kluyveromyces marxianus* were used; in addition a strain of *Candida intermedia* was used as a positive control in the hydrogen sulfide production test. All strains were part of the Microbial Culture Collection of the Centro de Estudios de Biotecnología Industrial (CCEBI, Universidad de Oriente, Santiago de Cuba). The general data of these yeast strains are presented in Table 1. Throughout this work, the strains was stored on YPD agar slants (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose and 15 g/L agar) at 4 °C. For inoculums preparation, 100-ml conical flasks containing 10 mL of YPD medium were inoculated with a single loopful of the yeasts from the YPD agar slants. The flasks were then incubated at 30°C in a rotary shaker at 200 min^{-1} for 12-16 h. All media components were supply for BIOCEN (Bejucal, Mayabeque, Cuba).

Table 1- Yeast strains used in the research. Source: CCEBI

Strain	Source of origin
Kluyveromyces marxianus CCEBI 2011	Coffee waste
Saccharomyces cerevisiae CCEBI 2015	Wine
Saccharomyces cerevisiae CCEBI 2017	Wine
Saccharomyces cerevisiae CCEBI 2021	Grapes
Saccharomyces cerevisiae CCEBI 2050	*Chicha*[*]
Candida intermedia CCEBI 2034[**]	Coffee waste

[*]Fermented corn drink, traditional in South America.
[**]Used as positive control in the H_2S production test.

Evaluation of high osmolarity, ethanol and sulfur dioxide tolerances

The growth of the strains was evaluated at increasing concentrations of glucose (5, 10, 15, 20, 25, 28 and 30 % w/v) (osmotolerance test), ethanol (5, 8, 10, 12, 14 and 15 % v/v) and sulfur dioxide (20,40,80 and 120 mg-SO_2/L, added as sodium metabisulfite) according to procedures describes for Ruiz *et al*.,[10] Barone *et al*.[5] and Escribano-Viana *et al*.,[11] with some modifications. In brief, 20 µl of a pure yeast culture, grown for 24 h in YEPD medium, were added to tubes containing 5 mL of YEPD, to which were added either glucose, ethanol or sodium metabisulfite to reach the concentrations required for each test. The cultures were incubated under shaking conditions at 30 ºC and 120 min^{-1}. Tolerance was determined by comparing the growth of the studied strains under stress conditions with their growth in YEPD medium after 24 hours of incubation. Growth measurements were made by reading the optical density (OD) at 600 nm. The assays were performed in duplicate for each test concentration.

Production of hydrogen sulfide (H_2S)

One drop (20 µl) of yeast culture grown 24 h in YPD medium was deposited onto plates on modified BiGGY agar, and incubated for 96 hours at 28-30 ^oC. Modified BiGGY agar was composed of (per liter): 5 g ammonium and bismuth citrate, 10,0 g glycine, 3 g sodium sulfate, 1 g yeast extract, 10 g dextrose, 16 g agar; pH 6.8. As a qualitative measure of H_2S production, the color of the colonies was taken into account: white, no production of hydrogen sulfide; light brown, low production and dark brown or black, high production.[12] The test was performed in triplicate.

Antagonism test using the cross-streak method

YPD agar plates were inoculated with the *K. marxianus* CCEBI 2011 strain using a single inoculum streak in the center of the plate. After two days of incubation at 28°C, the plates were inoculated with *S. cerevisiae* strains using a single streak at a 90° angle to the *Kluyveromyces marxianus* strain. Microbial interactions were analyzed by observing the size of the inhibition zone. A negative control was performed using plates with YPD medium growing only *S. cerevisiae* strains.[13] The assay was performed in triplicate.

Statistical analysis

Normality and homogeneity of variance tests were performed. Once these conditions were met, analysis of variance (ANOVA) was performed, followed by Tukey means comparison test. Differences at $p < 0,05$ were considered to be significant. All statistical analyses were performed using the Statgraphics 19.0 (Statistical Graphics, Rockville, MD) software.

RESULTS AND DISCUSSION

Osmotolerance

One of the methods currently being studied to enhance wine aromas and flavors is the use of non-conventional yeasts, which produce organoleptic diversity when used in the early stages of fermentation and, when co -cultivated with *S. cerevisiae*, allow for the production of wines with high alcohol levels. However, in order to utilize these yeasts, as in the case of native *S. cerevisiae* and non-*Saccharomyces* strains, it is necessary to determine the relevant oenological characteristics that support their use.

Figure 1 shows the growth of different yeast strains at increasing glucose concentrations. Strains CCEBI 2015 and CCEBI 2050, belonging to the species *S. cerevisiae*, and strain CCEBI 2011, corresponding to the species *K. marxianus*, did not show significant changes in their growth up to a concentration of 30 % (w/v) glucose in the medium, the maximum concentration evaluated in our research. On the other hand, strains CCEBI 2017 and CCEBI 2021 (both *S. cerevisiae*) showed a significant decrease in growth as the glucose concentration increased; however, CCEBI 2021 maintained a notable growth up to 15 % (w/v), unlike CCEBI 2017, whose growth declined dramatically as glucose concentration increased and was virtually nonexistent for concentrations of 15 % (w/v) and higher. These results indicate that it is unlikely that a high-alcohol wine will be obtained with this strain, which would limit its use as a starter culture for industrial purposes. Different letters indicate significant differences according to Tukey's test for $p < 0,05$; error bars correspond to the standard deviation for each treatment (Figure 1).

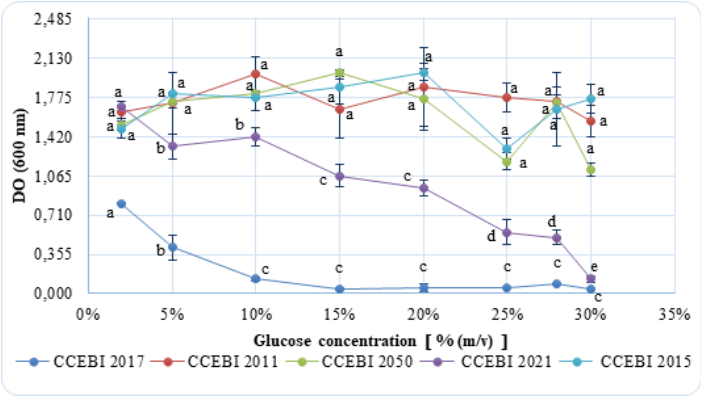

Fig. 1- Growth of the yeast strains at the different glucose concentrations (YPD medium plus glucose; 24 hours of incubation)

It is a widely documented fact which is accepted by the scientific community that the species *S. cerevisiae* it is resistant to high concentrations of sugars; however, sugar concentrations in the range of 200 to 300 g/L have been reported to significantly decrease its growth rate.[14] In the case of *K. marxianus*, a recent characterization carried out by Lappe -Oliveras *et al*.[15] on seven isolates of this species, from the pulque and mezcal production processes, showed that at a temperature of 30 ºC and 42 ºC this species is able to tolerate sugar concentrations of up to 30 % (w/v). In the context of sequential inoculations, osmotolerance is essential for strains used in musts with high sugar levels, such as those used in the production of sweet or high-alcohol wines. Strains CCEBI 2015, CCEBI 2050, and CCEBI 2011, which showed stable growth up to high glucose concentrations, seem to be ideal for this type of fermentation.

Musts containing high levels of sugar expose yeasts to hypertonic conditions, resulting in an outflow of water from the cell, which in turn reduces turgor pressure and water availability leading to cytoplasmic contraction.[16,17] Different cellular mechanisms have been proposed to counteract hyperosmotic stress, including the synthesis and accumulation of specific osmotically active compounds (glycerol or trehalose), temporary arrest of the cell cycle, modifications of the transcription and translation patterns,[17] in addition to the modification of the cell wall and the cytoskeleton.[16]

Resistance to ethanol

A range of ethanol concentrations between 0 and 15 % (v/v) added externally to the YPD medium was evaluated. This range of concentrations corresponds to those usually present in wine fermentations. The CCEBI 2015 strain showed the highest degree of tolerance among all the strains studied, with stable growth up to a concentration of 10 % (v/v) of added ethanol; at higher concentrations, there was a progressive decrease in the OD of the culture, indicative of growth inhibition (Figure 2). *K. marxianus* CCEBI 2011 and *S. cerevisiae* CCEBI 2050 only showed a slight decrease in growth up to an ethanol concentration of 8 % (v/v); then a progressive decrease in cell concentration in the culture medium occurred, which was much more drastic in the *K. marxianus* strain (Figure 2). Strains CCEBI 2021 and CCEBI 2017 of the species *S. cerevisiae* showed low tolerance to ethanol, with significant growth inhibition observed at 2 % (v/v) of ethanol added; in both strains, growth was practically zero when the ethanol concentration reached 8 % (v/v). Different letters indicate significant differences according to Tukey's test for $p < 0,05$; error bars correspond to the standard deviation for each treatment (Figure 2).

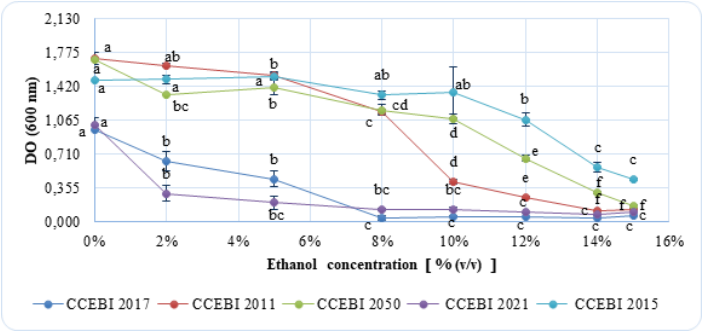

Fig. 2- Growth of the yeast strains at different ethanol concentrations (YPD medium plus ethanol; 24 hours of incubation)

The results described above suggest that CCEBI 2017 and CCEBI 2021 strains are not suitable for producing high-alcohol beverages, such as wines. In sequential mixed cultures, alcohol tolerance is especially important for strains used in the final stages of fermentation, where ethanol concentrations are highest. Strains that display high alcohol tolerance, such as CCEBI 2015 and CCEBI 2050, can complete fermentation even under high-alcohol conditions, ensuring a final product with the desired properties.

Ethanol, being composed of only two carbon atoms, is soluble in both water and lipids, and can therefore cross the plasma membrane and be harmful to yeast cells. Severe ethanol stress impairs transporter activity and endocytosis , induces oxidative stress and changes in cell membranes, and strongly represses poly(A)$^+$ mRNA nuclear export and protein synthesis in yeast cells under laboratory conditions. Similar to severe

heat shock, acute ethanol stress (10 % v/v) also damages proteins, causing the accumulation of denatured insoluble proteins in cells, leading to cytotoxicity.[18]

Yeasts have been used for brewing beer and other beverages alcohol-related fermentations throughout the history of human civilization.[19] Among them, the species *S. cerevisiae* is characterized by the existence of domesticated strains, used in various industrial processes such as the production of biofuels, wine, sake, brewing and baking. Due to its enormous genetic and phenotypic variability,[20] varying degrees of tolerance to ethanol stress have been reported, which would explain the difference in growth between the different strains of this species used in our work, which also come from different environments such as wines, grapes and chicha.

As an example of the above, Lairón-Peris *et al.*,[21] in their study on ethanol tolerance carried out on 61 strains of *S. cerevisiae* of different origins and isolation sources, found that 22 of them had high tolerance to ethanol and 39 showed growth at intermediate and low values. In another study, conducted by Feng *et al.*,[22] the diversity of response of the *S. cerevisiae* species was confirmed at different ethanol concentrations; of a total of 52 isolates from five wine-producing regions in northwest China, all yeast strains could grow and ferment with 14 % (v/v) ethanol, of which 77 % (40 strains) could grow and ferment in the presence of 16 % (v/v) ethanol. These results reflect that, although *S. cerevisiae* it is a microorganism with a reported capacity to grow at high concentrations of alcohol, but it is still susceptible to its toxic effects.

Regarding the *K. marxianus* strain CCEBI 2011, Camacho *et al.*[23] reported an ethanol tolerance of 8-11 % (v/v), expressing this tolerance as the maximum concentration at which fermentative activity was observed, under microaerophilic conditions. Studies carried out with other strains of the species indicate that they cannot withstand high concentrations of ethanol, since their growth is strongly inhibited when concentrations are higher than 6 % (v/v). It has been postulated that this is due to the low stability of the membrane and the negative regulation of some genes encoding enzymes of the ergosterol biosynthesis pathway, under conditions of ethanol stress.[24] Barone *et al.*[8] found in *K. marxianus* Km L2009 ethanol resistance values similar to those reported in our work; this strain showed growth up to 6 % (v/v). Similar results are also described by Lappe -Oliveras *et al.*[15] when testing the ethanol resistance in seven strains of *K. marxianus* at different temperatures; in this investigation, growth was observed between 2,5 - 7,5 % (v/v) at 42 ºC and 7,5 - 10 % (v/v) at 30 ºC, which demonstrates the effect of temperature on resistance to this metabolite.

Since in this work the ethanol resistance tests were developed under conditions in which only the strain and the alcohol concentration varied, it could be stated that the level of resistance expressed by each member of the species is related to its individual genetic pool. In this sense, several hundred genes associated with ethanol tolerance have been identified, which involve a wide range of functional categories that include protein biosynthesis, amino acid and nucleotide metabolism, transport, cell cycle and growth, as well as lipid, fatty acid and ergosterol metabolism, membrane and cell wall organization, proline and tryptophan biosynthesis, among others.[19]

Resistance to sulfur dioxide

Sulfur dioxide, added in the form of sulfite salts (usually metabisulfites), is one of the most common, economical and effective chemical additives in food and beverages due to its antimicrobial action. In the wine industry, the addition of sulfite suppresses the growth of many non- *Saccharomyces* yeasts, lactic acid bacteria and acetic acid bacteria. Furthermore, it can counteract both enzymatic and chemical oxidations in wine, thus stabilizing its sensory properties during storage and aging.[25] Several mechanisms of its action as a growth inhibitor have been described; For example, it can be oxidized by reactive oxygen species to generate sulfur trioxide free radicals that can damage DNA and destroy tryptophan, in addition to other negative effects, such as ATP depletion by inhibiting glyceraldehyde-3-phosphate dehydrogenase and alcohol dehydrogenase, damage to the plasma membrane, proteins, vitamins and coenzymes.[26]

In the study, all strains evaluated showed stable growth up to 120 mg/L of sulfite (expressed as SO_2), except for CCEBI 2017, which had a decrease in growth from 40 mg/L, without reaching a complete inhibition (Figure 3). The resistance of yeasts to this substance is also related to genetic and metabolic factors. Wine yeasts have four main pathways to survive in the presence of SO_2: (1) entering a viable but non-culturable state; (2) expelling SO_2 from the cell by means of specialized sulfite efflux pumps; (3) reducing SO_2 by incorporating it into the sulfur amino acid biosynthesis pathway; and (4) producing acetaldehyde.[27]

It is known that the sulfite efflux pump Ssu1 to play a key role in the defense of *S. cerevisiae* against this compound,[26] which is encoded by the SSU1 gene. This gene shows a high level of polymorphism and harmful mutations in its coding sequence cause susceptibility to SO_2. The promoter sequence of SSU1 is known to be involved in three different chromosomal rearrangements, one of which generates a sulfite pump allele, SSU1R, with higher expression levels than SSU1 and conferring enhanced sulfite resistance. However, *S. cerevisiae* winemaking strains exhibit different ploidy degrees and heterozygosity levels, so the number of SSU1 and SSU1R could potentially explain the diverse range of resistance observed between strains.[25] In the context of sequential cultivation, SO_2 resistance is crucial, especially for strains used in the initial stages of fermentation where SO_2 concentrations are highest. Strains displaying high resistance, such as CCEBI 2011, CCEBI 2015 and CCEBI 2050, are able to overcome this stress and ensure efficient fermentation from the start. Different letters indicate significant differences according to Tukey's test for $p < 0,05$; error bars correspond to the standard deviation for each treatment.

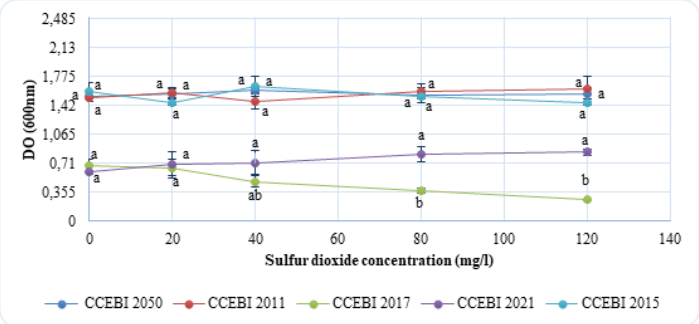

Fig. 3- Growth of the yeast strains at different sulfur dioxide concentrations (YPD medium plus sulfur dioxide; 24 h of incubation)

Production of hydrogen sulfide (H_2S)

Hydrogen sulfide is one of the volatile sulfur compounds with the greatest impact on the sensory profile of wine, as it is associated with the so-called aromas of reduction (due to its rotten egg smell) and is capable of nullifying the fruity and floral attributes of wine. Furthermore, it is a very reactive chemical compound: its combination with wine must molecules can lead to the synthesis of other volatile sulfur compounds such as ethanethiol, also known to be harmful to wine quality.[28] Hence, the vital importance of determining whether or not a yeast strain is capable of producing this metabolite and to what extent.

In this study *Candida intermedia* CCEBI 2034 strain was used as a positive control of hydrogen sulfide production. This strain developed dark brown colonies (Figure 4), confirming that it is a yeast with high levels of hydrogen sulfide production. A similar result was obtained with the *S. cerevisiae* CCEBI 2021 strain, while *K. marxianus* CCEBI 2011 developed light brown colonies, which corresponds to a production of H_2S at low concentrations. The strains of the species *S. cerevisiae* CCEBI 2015 and CCEBI 2050 developed cream-colored colonies, indicating very low or no sulfide production. *S. cerevisiae* CCEBI 2017 had small, brown colonies, so it is presumably a medium to high sulfide producer (Figure 4).

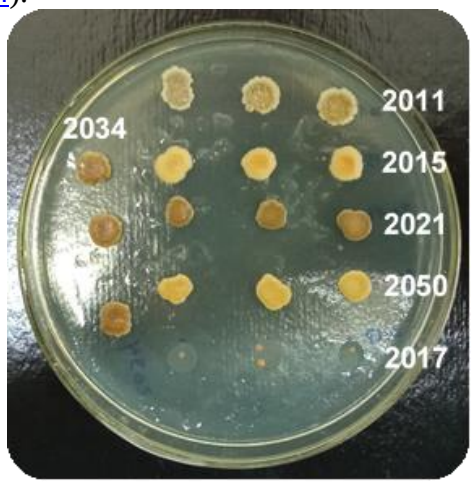

Fig. 4- Hydrogen sulfide production assay on modified BiGGY agar medium (four days of incubation at 28 °C). The numbers correspond to the strain code

In yeasts, the main metabolic pathway that produces H_2S is the sulfate assimilation pathway; the H_2S formed is used in the synthesis of sulfur-containing amino acids.[29] *S. cerevisiae* is a yeast that, as mentioned above, presents a wide genetic variability; hence, it is not surprising to find strains that are sulfide producers and others that are not, as occurred in this research.[29]

According to Li *et al.*, [29] numerous investigations have been made during the last decade to identify relevant genetic factors in *S. cerevisiae* during wine fermentation. It has been found that interstrain variations in H_2S production are caused by different expression levels and mutations of genes involved in the sulfate assimilation pathway.

In the case of *K. marxianus*, authors such as Erasmus and Divol [9] they carried out an evaluation of H_2S

production in seven *K. marxianus* strains employing a medium of similar composition to that used in our study. The results obtained for this researchers led to grouping the strains into three groups according to different production levels (low, intermediate and high), which demonstrates the variability between strains. In our study, although the *K. marxianus* CCEBI 2011 strain tested positive for H_2S production, it does not produce it in high concentrations, so its potential use in winemaking processes is not ruled out.

Antagonism between *K. marxianus* CCEBI 2011 and *S. cerevisiae* strains

Certain species of yeast, called killer yeasts, have the ability to produce killer toxins (mycotoxins), which can prevent the growth of microorganisms sensitive. These toxins give them strains that produce them the ability to compete successfully with their cohabitants. In addition to *S. cerevisiae*, strains of *Kluyveromyces* have been identified producers of killer toxins.[30]

The ability of a yeast strain to inhibit other strains is both an advantage and a disadvantage when designing a fermentation process for wine production. On the one hand, a strain with inhibitory capacity serves as a biological control for microorganisms that can compromise the quality of the final product; on the other hand, this capacity can limit the use of the strain in mixed cultures, whether sequential or in co-inoculation, to obtain wines with greater organoleptic diversity. Therefore, evaluating the inhibitory capacity of strains is of utmost interest when selecting them as starter cultures and defining their use.

In this investigation, no antagonistic effect was detected between *K. marxianus* CCEBI 2011 and *S. cerevisiae* strains under study, evidenced by the absence of an inhibition zone in the contact zone between streaks (Figure 5). These results suggest that *S. cerevisiae* strains under study can coexist with *K. marxianus* CCEBI 2011 during fermentation without inhibiting each other.

The lack of antagonism represents an advantage for their use in mixed cultures, allowing the advantageous qualities of strains of species *Saccharomyces* and not-*Saccharomyces*, to be combined without compromising the efficiency of the fermentation process.

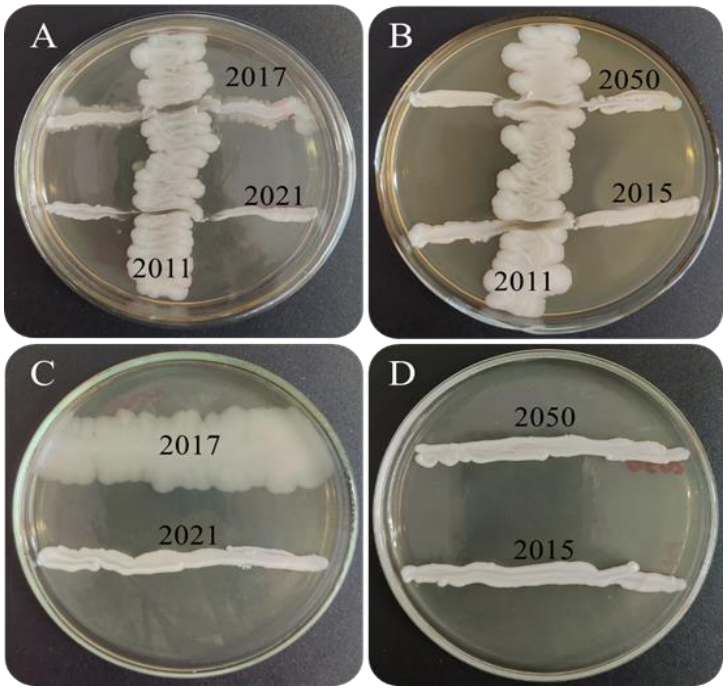

Fig. 5- Antagonism test between *K. marxianus* CCEBI 2011 and the strains *S. cerevisiae* CCEBI 2017 and CCEBI 2021 (A); *S. cerevisiae* CCEBI 2050 and CCEBI 2015 (B). Negative control *S. cerevisiae* CCEBI 2017 and CCEBI 2021 (C); *S. cerevisiae* CCEBI 2050 and CCEBI 2015 (D) on YPD agar medium after 48 hours of incubation. The numbers correspond to the strain codes

CONCLUSIONS

The CCEBI 2015, CCEBI 2050 and CCEBI 2011 strains present oenological characteristics favorable for use as starter cultures, in the form of mixed cultures, in the wine industry. Their high resistance to SO_2, ethanol tolerance, osmotolerance, and low/zero H_2S production make them ideal candidates for achieving efficient fermentations and wines with improved sensory properties. Furthermore, the absence of antagonism between *K. marxianus* and *S. cerevisiae* strains suggests the possibility of using these strains in combination to obtain a final product of higher quality and distinctive organoleptic characteristics. Future research should focus on testing these strains in wine fermentations, using sequential inoculation or coinoculation variants, in order to evaluate their impact on the wine's sensory profile.

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INTEREST CONFLICT

The authors declare that there are no conflicts of interest relevant to the content of this article.

AUTHOR'S CONTRIBUTION

Rolando Carrazana Isaac: research conceptualization and design, data collection, analysisand interpretation of results, writing the original draft.

Manuel de J. Serrat Díaz: research conceptualization and design, analysis and interpretation of results, validation, supervision, writing and revision of the manuscript.