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# Isolation and Characterization of Potential Starter Yeasts from Traditional Moroccan Sourdoughs

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**The increasing demand for baked products has given a boost to research on isolation and selection of novel yeast strains with improved leavening activity. Twelve sourdough samples were collected from several localities of the Fez region in Morocco. The pH and total titratable acidity (TTA) values of these samples varied from 3.03-4.63 and 14-17.5 ml of 0.1 N NaOH/10 g of sourdough, respectively, while yeast counts ranged from 5.3 6.77 Log CFU/g. Thirty-two yeast isolates were obtained and evaluated for their leavening ability. Out of all isolates, four yeasts molecularly identified as *Saccharomyces cerevisiae* (three strains) and *Kluyveromyces marxianus* (one strain) showed highest specific volumes of 4.69, 4.55, 4.35 and 4.1 $cm^3/g$, respectively. These strains were further assessed for their tolerance to high concentrations of salt, sugar, elevated temperatures, and low pH conditions. *K. marxianus* showed higher resistance than the *S. cerevisiae*. Thus, Moroccan sourdoughs harbor technologically relevant yeasts that could be used as potential starters for bread preparation.**

**Keywords:** **Sourdough, baker's yeast, leavening ability, bread, stress**

## Introduction

Baked and cereal based foods have been an integral part of our society's diet for thousands of years. *Saccharomyces cerevisiae*, or baker's yeast, is known to be the microorganism of choice for bread making [1, 2]. This yeast uses sugars present in the flour to mainly release carbon dioxide ($CO_2$) and ethanol as primary metabolites. Production of $CO_2$ increases dough volume by incorporation of air bubbles through dough matrix [3, 4]. Other secondary metabolites such as aldehydes, esters, and amino acids are also produced during dough fermentation and they participate to the sensory profile of the final product [5, 6]. In addition, *S. cerevisiae* is classified as safe for health and has received the GRAS (Generally Recognized as Safe) status by the US Food and Drug Administration.

Although *S. cerevisiae* has been exhaustively improved to suit different types of dough [7, 8], baking industries are always on the lookout for new and effective yeast strains that show good resistance to baking-associated stresses. The source of yeasts isolation and selection is a critical choice. Traditional sourdough is based on a spontaneous fermentation of flour and water, where lactic acid bacteria (LAB) and yeasts coexist in a complex biological system [9–11]. Generally, LAB population is dominant, with a LAB to yeasts ratios from 100:1 to 10:1 [12]. Research related to sourdoughs has focused more on LAB and their nutritional and metabolic activities [13–16]. However, yeasts also play a key role in sourdough formulation [11, 17, 18]. In addition to the leavening ability, yeasts contribute to flavor development [11], produce vitamins [19] and phytase [20], and exhibits

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nutritional characteristics [21].

Stable sourdough may contain only one or two species of yeast [11]. These yeasts are characterized by their ability to withstand stressful conditions mainly due to low pH, low oxygen levels, temperature changes and high carbohydrate concentrations [11, 16]. Generally, *S. cerevisiae* is the one of the most frequently found species identified in spontaneous sourdough. Other so-called non-conventional yeasts such as *Candida humilis, Torulaspora delbrueckii, Pichia kudriavzevii* and *Candida glabrata* are also retrieved [16]. Moreover, *Kazachstania gamospora*, *Wickerhamomyces subpelliculosus*, *T. delbrueckii* and *Saccharomyces bayanus* have been described as potential leavening agents [22–25]. The aim of the present study was the selection of promising yeast strains from sourdough samples to be used as starter cultures in the preparation of bread and leavened bakery products.

## Materials and Methods

### Sample collection

Twelve sourdough samples (S1–S12) were collected from several localities throughout Fez region, Morocco, in February 2017. All samples were brought to the laboratory in sterilized bottles, and were kept at 4℃ until analyzed.

### Determination of pH and total titratable acidity

pH and total titratable acidity (TTA) were determined as described by the Approved Methods 02-52 and 02-31 [26], respectively. Ten grams of each sourdough sample were first mixed with 90 ml of distilled water for 15 min, and then the pH was determined using a pH meter (DESKTOP PHMETER PH50, LabBox, Spain). For TTA measurements, the previous homogenized mixture was titrated against 0.1 N NaOH using phenolphthalein as color indicator (1 g/100 ml). The TTA value is expressed as the titrated amount (ml) of 0.1 N NaOH/10 g dough.

### Isolation and preservation of yeasts

Yeasts were isolated from the sourdough samples using the standard serial method. Briefly, ten grams of sourdough were aseptically suspended in 90 ml of sterile physiological water and mixed for 10 min at room temperature. Serial dilutions were prepared and 0.1 ml of each dilution was spread into YPD agar (10 g of yeast extract, 20 g of peptone, 20 g of glucose, 15 g of agar; per litter) supplemented with chloramphenicol at a concentration of 100 µg/ml. YPD plates were incubated at 30℃ for 48 h. Viable cell counts were performed in triplicate and the results are expressed in CFU (colony forming units). Colonies showing different morphologies were randomly peaked and streaked on YPD agar and further stored in glycerol broth solution (30%) at -80℃.

### Dough preparation and determination of leavening ability

For determination of leavening ability, the isolated strains were grown separately overnight in 100 ml of YPD broth at 30℃ with shaking at 150 rpm. Yeast cells were harvested by centrifugation at 11200 ×*g* for 15 min, washed twice with sterile distilled water, and then filtered in order to obtain a yeast cake. Commercial wheat flour Anwar El Manar (13.5% moisture basis) was used for dough preparation. The following ingredients were thoroughly mixed for 15 min in a beaker glass: 100 g of flour, 1.5 g of salt, 2 g of sucrose, 2 g of yeast and 67 ml of sterile distilled water. The mixture was then divided into two samples of 50 g and each was transferred to a graduated cylinder. Fermentation was left for 240 min at 30℃ and the volume of dough was measured every 30 min. Specific leavening volume was evaluated as the difference between the volume of the dough at the end and the start of fermentation per g of dough ($cm^3/g$). Dough without yeast and dough inoculated with a commercial baker's yeast (SOMADIR, Morocco) identified as *S. cerevisiae* were used as negative and positive controls, respectively. The accession number of the control yeast sequence reported in this study is LT605145.

### Molecular identification of the selected isolates

Yeast isolates with higher leavening activities were identified at the molecular level based on the amplification of 5.8S-internal transcribed spacer (ITS) regions by the polymerase chain reaction (PCR) technique. Genomic DNA was extracted from pure cultures of isolates according to the protocol described by Harju *et al.* [27]. The amplification was carried out using the primer sets of ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3'), with a PCR reaction mixture having a final volume of 50 µl containing Taq buffer (×1) with $MgCl_2$ (15 mM), dNTP (0.2 mM),

Taq polymerase (0.04 U/ul), 0.25 uM of each primer, pure water and 1 ul of template DNA. PCR program was set under the following conditions: 30 cycles of 45 s at 94℃, 60 s at 55℃, and 60 s at 72℃, with an initial denaturation step of 5 min at 94℃ and a final extension step of 10 min at 72℃. The amplicons obtained were sequenced using Applied Biosystems® 3130 Genetic Analyzer and compared to the sequences published in GenBank NCBI database using the BLASTN program. The phylogenetic tree of the identified yeast strains was established using the Neighbor-Joining method in the MEGA X program based on ITS sequences.

### Evaluation of stress tolerance

To evaluate the response of the selected strains including control yeast towards various stress conditions the followed protocols were performed; yeast cells were grown overnight in 10 ml of YPD liquid medium at 30℃ in a rotary incubator shaker at 150 rpm. After incubation, the cells were collected by centrifugation at 11200 ×*g* for 10 min, washed with sterile deionized water and adjusted to a cell density of $OD_{600\ nm}$ 0.5. Successive dilutions were made and 0.1 ml of appropriate dilutions was spread onto a different solid media agar depending on the stress condition tested. For saline stress conditions, cells were cultured on YPD agar supplemented with 2, 4 and 6% (w/v) of NaCl and incubated at 30℃ for 48 h. For sugar stress conditions, cells were cultured on YPD agar containing sucrose, instead of glucose at concentrations of 20, 30 and 40% (w/v) and incubated at 30℃ for 48 h. For heat stress conditions, cells were cultured on YPD agar and incubated for 48 h at 37, 40 and 45℃.

Tolerance of the strains to the above stress conditions was expressed by enumeration of yeast cells (Log CFU/ml) after incubations.

In addition, the ability of the selected strains to tolerate acidity was evaluated as follows: 1 ml of the adjusted culture of yeast cells was inoculated into 100 ml of YPD broth calibrated at the following pH: 2, 3 and 4. Growth was assessed by measuring absorbance at 600 nm for 48 h.

### Statistical analyses

All experiments were conducted in triplicate. Analysis of variance (ANOVA) with Tukey test was carried out by Minitab 18 Statistical Software to determine significant ($p < 0.05$) differences among the results.

## Results and Discussion

### pH, total titratable acidity (TTA) and yeast cell counts

The pH and TTA results of the sourdoughs samples are summarized in Table 1. Overall, the pH measurements were acidic and ranged from 3.03 to 4.63, whilst those of TTA ranged from 14 to 17.5 ml of 0.1 N NaOH/10 g of sourdough. Sample S3 showed the lowest pH of 3.03 with the highest TTA of 17.5 ml of 0.1 N NaOH/10 g dough. Our findings are similar to those of Lhomme *et al.* [28], who reported that the median of pH and TTA of sixteen traditional French sourdoughs was 3.5 and 16.2 ml of 0.1 N NaOH, respectively. According to Arendt *et al.* [29], the acidity of sourdoughs depends on the method of propagation of dough and on the type and concentration of the starter cultures.

Viable counts of yeasts in S1-S12 samples are also given in Table 1, yeast cell density ranged from 5.3 to 6.77 Log CFU/g. Palla *et al.* [30] stated that the amount of yeasts in sourdoughs collected from different Tuscan bakeries varied between 5.47 ± 0.02 to 7.48 ± 0.02 Log CFU/g of dough sample. Similarly, Hammes *et al.* [31] found that in mature sourdoughs, yeasts were in a range close $10^6$ to$10^7$ CFU/g.

**Table 1. pH, TTA and yeasts counts of the twelve sourdoughs samples.**

| Samples | pH | TTA (ml of 0.1 N NaOH/10 g of sourdough) | Yeast counts (Log CFU/g) |
|---|---|---|---|
| S1 | 3.5 ± 0.01 | 16.5 ± 0.01 | 5.3 ± 0.01 |
| S2 | 3.15 ± 0.03 | 17.5 ± 0.00 | 5.7 ± 0.02 |
| S3 | 3.03 ± 0.1 | 17.5 ± 0.06 | 5.96 ± 0.04 |
| S4 | 4.18 ± 0.06 | 16.5 ± 0.01 | 6.77 ± 0.01 |
| S5 | 4.63 ± 0.01 | 14 ± 0.00 | 6.51 ± 0.11 |
| S6 | 3.92 ± 0.03 | 15 ± 0.01 | 5.77 ± 0.02 |
| S7 | 4.3 ± 0.01 | 14.5 ± 0.00 | 6.53 ± 0.10 |
| S8 | 4.17 ± 0.02 | 16.3 ± 0.01 | 5.9 ± 0.2 |
| S9 | 3.33 ± 0.05 | 17 ± 0.01 | 6.05 ± 0.2 |
| S10 | 3.75 ± 0.01 | 16.5 ± 0.01 | 5.84 ± 0.21 |
| S11 | 3.6 ± 0.02 | 16 ± 0.01 | 5.76 ± 0.30 |
| S12 | 4 ± 0.06 | 15.5 ± 0.01 | 6.13 ± 0.14 |

**Table 2. Leavening performance of the isolated yeasts.**

| Isolates | Dough expansion in $cm^3/g$ | | | | |
|---|---|---|---|---|---|
| | 30 min | 60 min | 90 min | 120 min | 180 min |
| YS1 | 0.53 ± 0.01 | 1.16 ± 0.00 | 1.16 ± 0.00 | 1.16 ± 0.00 | 1.16 ± 0.00 |
| YS2 | 1.14 ± 0.00 | 2.23 ± 0.10 | 2.99 ± 0.00 | 2.99 ± 0.00 | 2.99 ± 0.00 |
| YS3 | 1.19 ± 0.00 | 1.91 ± 0.02 | 2.66 ± 0.00 | 2.66 ± 0.00 | 2.66 ± 0.00 |
| YS4 | 0 ± 0.28 | 0.95 ± 0.07 | 1.5 ± 0.00 | 2.47 ± 0.10 | 3.1 ± 0.14 |
| YS5 | 0 ± 0 | 0.59 ± 0.13 | 1.37 ± 0.04 | 2.24 ± 0.06 | 2.7 ± 0.14 |
| YS6 | 1.14 ± 0.02 | 0.71 ± 0.01 | 1.23 ± 0.04 | 1.90 ± 0.02 | 2.5 ± 0.02 |
| YS7 | 2.95 ± 0.00 | 3.7 ± 0.2 | 3.7 ± 0.05 | 3.7 ± 0.05 | 3.7 ± 0.05 |
| YS8 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| YS9 | 0 ± 0.00 | 0.8 ± 0.12 | 1.3 ± 0.00 | 1.3 ± 0.00 | 1.3 ± 0.00 |
| YS10 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| YS11 | 3.71 ± 0.00 | 4.69 ± 0.00 | 4.69 ± 0.00 | 4.69 ± 0.00 | 4.69 ± 0.00 |
| YS12 | 0.49 ± 0.00 | 0.5 ± 0.00 | 1.2 ± 0.00 | 1.16 ± 0.05 | 1.19 ± 0.09 |
| YS13 | 0.31 ± 0.01 | 0.65 ± 0.02 | 1.31 ± 0.02 | 1.36 ± 0.00 | 1.36 ± 0.00 |
| YS14 | 0.99 ± 0.00 | 1.65 ± 0.00 | 1.65 ± 0.00 | 1.65 ± 0.00 | 1.65 ± 0.00 |
| YS15 | 2.50 ± 0.00 | 3.86 ± 0.01 | 4.06 ± 0.00 | 4.06 ± 0.00 | 4.06 ± 0.00 |
| YS16 | 1.19 ± 0.00 | 1.19 ± 0.00 | 2.51 ± 0.03 | 2.51 ± 0.03 | 2.52 ± 0.02 |
| YS17 | 1.94 ± 0.06 | 2.1 ± 0.04 | 3.07 ± 0.03 | 3.1 ± 0.07 | 3.1 ± 0.07 |
| YS18 | 1.27 ± 0.02 | 1.27 ± 0.02 | 2.96 ± 0.07 | 2.96 ± 0.07 | 2.96 ± 0.07 |
| YS19 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| YS20 | 0.81 ± 0.01 | 0.81 ± 0.02 | 2.53 ± 0.02 | 3.10 ± 0.12 | 3.11 ± 0.11 |
| YS21 | 1.21 ± 0.01 | 2.4 ± 0.01 | 2.4 ± 0.01 | 2.4 ± 0.01 | 2.4 ± 0.01 |
| YS22 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| YS23 | 1.16 ± 0.00 | 1.17 ± 0.00 | 2.29 ± 0.00 | 2.29 ± 0.00 | 2.29 ± 0.00 |
| YS24 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| YS25 | 0 ± 0.00 | 0 ± 0.00 | 0.5 ± 0.00 | 0.9 ± 0.15 | 0.9 ± 0.06 |
| YS26 | 3.6 ± 0.28 | 4.15 ± 0.00 | 4.15 ± 0.00 | 4.36 ± 0.00 | 4.36 ± 0.00 |
| YS27 | 0 ± 0.00 | 0.70 ± 0.00 | 1.24 ± 0.00 | 1.71 ± 0.01 | 1.99 ± 0.01 |
| YS28 | 3.1 ± 0.14 | 3.95 ± 0.00 | 4.05 ± 0.07 | 4.1 ± 0.05 | 4.25 ± 0.05 |
| YS29 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0.5 ± 0.32 | 0.5 ± 0.45 |
| YS30 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| YS31 | 0.73 ± 0.05 | 0.785 ± 0.02 | 0.785 ± 0.02 | 0.785 ± 0.02 | 0.78 ± 0.02 |
| YS32 | 3.55 ± 0.00 | 4.55 ± 0.00 | 4.55 ± 0.00 | 4.55 ± 0.01 | 4.55 ± 0.01 |
| Negative control | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| Positive control | 2.65 ± 0.02 | 3.7 ± 0.05 | 3.9 ± 0.00 | 3.9 ± 0.00 | 3.9 ± 0.46 |

### Isolation and evaluation of leavening ability

From the twelve sourdoughs samples analyzed, approximately 32 yeast isolates were obtained and designated as YS1-YS32. All the isolates were screened for their ability to leaven dough. The results showed that the specific volume varied from 0 to 4.69 $cm^3/g$ after 180 min of fermentation (Table 2). Doughs inoculated with YS11, YS32, YS26 and YS28 exhibited the highest leavening capacity, significantly ($p < 0.05$) higher of that of control yeast, with the specific volumes of 4.69, 4.55, 4.35 and 4.1 $cm^3/g$, respectively. Isolate YS7 also presented a good leavening profile, showing a specific


**Fig. 1. Phylogenetic tree reconstruction of yeast strains studied using the Neighbor-Joining method based on ITS sequences in MEGA X program.** Numbers on the branches indicate bootstrap percentage after 1000 replications.

volume of 2.95 $cm^3/g$, significantly ($p < 0.05$) higher of that of control yeast in the first half hour of fermentation. The five yeast cultures were identified by sequencing the ITS regions. The analysis of similarity percentages on the Gene Bank BLASTN database showed that the isolates YS11, YS32, YS26, YS28 and YS7 were identified as *S. cerevisiae* (KX029124), *K. marxianus* (KY103801), *S. cerevisiae* (KY105140), *Saccharomyces cerevisiae* (MN585904) and *Pichia kudriavzevii* (LC389012), with a similarity percentage of 99.42%, 100.00%, 99.26%, 99.45% and 99.80% respectively. The molecular evolutionary relationship among the yeast species is illustrated on the phylogenetic tree constructed using the Neighbor-Joining method based on ITS sequences (Fig. 1). Three of the five performing species belonged to the genus *Saccharomyces* (YS11, YS26 and YS28). These results are consistent with numerous studies that describe *S. cerevisiae* as a suitable leavening agent for the preparation of baked products [32]. *K. marxianus* YS32 showed a significant increase in dough volume, confirming a previous report [33], where *K. marxianus* NRRL-Y-1109 and *K. marxianus* NRRL-Y-2415 showed comparable proofing activity in lean dough with two strains of *S. cerevisiae* isolated from active and dry baker's yeast. The use of *K. marxianus* strain seems to be interesting in bread making, especially that it has been granted the status of Qualified Presumption of Safety (QPS) and GRAS in the European Union and the United States, respectively [34, 35]. To confirm the safety of *K. marxianus* YS32, a test evaluating the production of biogenic amines was conducted according to adapted version of the method explained by Joosten and Northholt [36, 37], and the results (Data not shown) revealed any production of biogenic amines by the studied strain. Regarding *P. kudriavzevii* YS7, there is no study in literature describing the use of this strain as the only leavening microorganism for bread preparation. For this reason, this strain was a subject of a patent project and it has been deposited with the Moroccan Coordinated Collections of Microorganisms CCMM under the number CCMMP5.

As widely known, volume is considered as an import-

**Table 3. Sugars assimilation by the selected yeast strains.**

| Sugars | *S. cerevisiae* YS11 | *S. cerevisiae* YS26 | *S. cerevisiae* YS28 | *K. marxianus* YS32 | *P. kudriavzevii* YS7 |
|---|---|---|---|---|---|
| D-Glucose | + | + | + | + | + |
| Sucrose | + | + | + | + | + |
| Fructose | + | + | + | + | + |
| Lactose | - | - | - | + | - |
| Maltose | + | + | + | - | - |

(+) assimilated, (-) not assimilated

ant organoleptic characteristic of bread [18]. Therefore, yeast capable of hydrolyzing sugars present in dough will release $CO_2$, the responsible of dough rising. Table 3 shows the consumption of sugars (glucose, sucrose, fructose, lactose and maltose) by the five isolated yeasts. The results showed that all strains of *S. cerevisiae* (YS11, YS26 and YS28) were able to assimilate all the sugars tested except lactose, while *K. marxianus* YS32 did not assimilate only maltose, and *P. kudriavzevii* YS7 assimilated glucose, sucrose and fructose. In general, the conventional baker's yeast *S. cerevisiae* ferments the sugars present in the dough in the following order: glucose, sucrose, fructose and lastly maltose [38]. The study conducted by Caballero *et al.* [33] mentioned that although *S. cerevisiae* strains have the maltose-uptake implicated enzymes they begin maltose consumption late, after 300 min. *K. marxianus* YS32 and *P. kudriavzevii* YS7 were not able to ferment maltose, but their leavening performance was not different from other *S. cerevisiae* strains. Other sugars presented in dough would be responsible for this fermentation. In fact, flour from wheat contains mostly starch as well as maltose, sucrose, fructose, glucose, and other oligosaccharides [39–41]. Furthermore, 2 g of sucrose was used as an ingredient in the preparation of the dough, which improved the leavening performance. Overall, the leavening properties of these five yeast strains classiest them as probable active starter for dough fermentation.

For the rest of the study, only the four yeast strains, which had a specific volume higher of that of control yeast, were retained.

#### Growth under stress conditions

The ability of baker's yeast to withstand various environmental stresses during biomass production and bread- making process is a very important trait of successful baker's yeast [23, 42]. It's well-known that sourdough yeasts cope with harsh conditions during their growth, conditions caused by high carbohydrate concentrations, low pH, nutritional deficiency, temperature fluctuations and interactions with other microorganisms (e.g. especially LAB) [11]. This attribute has an important technological value. For this reason, the growth of the five selected yeast strains, including control yeast under different stress conditions was evaluated.

Sodium chloride (NaCl) is an essential ingredient that goes into the preparation of bread and many baked goods. Its addition to dough develops the structures of gluten, prolongs shelf life and improves flavor and sensory properties [43, 44]. High concentrations of NaCl affect strongly the fermentation rate of baker yeast [42]. Therefore, an assessment of salt tolerance is needed. Fig. 2A displayed the response of yeast cells to high salinity (2, 4 and 6% (w/v) of NaCl): the growth of all the yeast strains decreased with increased concentrations of NaCl added to the medium. At the concentration of 6%, the control yeast showed poor growth on agar plates. Generally, the isolated strains from sourdoughs samples revealed significantly better salt-tolerance than the control yeast. This could be related to their sources of isolation. Zhou *et al.* [22] found that the yeasts *Wickerhamomyces subpelliculosus* and *Barnettozyma californica* isolated from high osmotic foods were more resistant to 6% of NaCl than the commercial baker's yeast.

Sweetbreads are made with high concentrations of sugar reaching in some recipes 40% of sucrose per weight of flour [45]. However, a high dose of sucrose exerts severe osmotic stress on baker's yeast resulting in cell damage and reduced fermentation capacity [46–48]. To avoid harmful injury, baker's yeast should have the appropriate resistance tools. Oda and Ouchi [38] reported that the performance of yeast starters in sweeten doughs is more related to tolerance capacity than to invertase activity. Fig. 2B represents the tolerance of the five strains to increasing sugar concentrations (20, 30 and 40% (w/v) of sucrose). All strains tested in this study were able to cope with high sucrose concentrations of up to 40%, with an optimal growth at 30%. *K. marxianus* YS32 showed significantly the highest tolerance toward sucrose stress. Consistently with our findings, Saini *et al.* [49] demonstrated that *K. marxianus* was more resistant and more robust to osmotic stress caused by high lactose concentration (200 g/l) compared to two strains of *S. cerevisiae* which were cultivated at the same concentration of hydrolyzed lactose.

Temperature is another factor affecting cell viability [50]. Few researches have focused on this parameter and its influence on behavior of yeasts during fermentation [51]. Fig. 2C illustrates the growth profile of the five strains on YPD agar at different temperatures (37, 40 and 45℃). In this study, all the strains were able to grow at 37 and 40℃. At the highest temperature of the test

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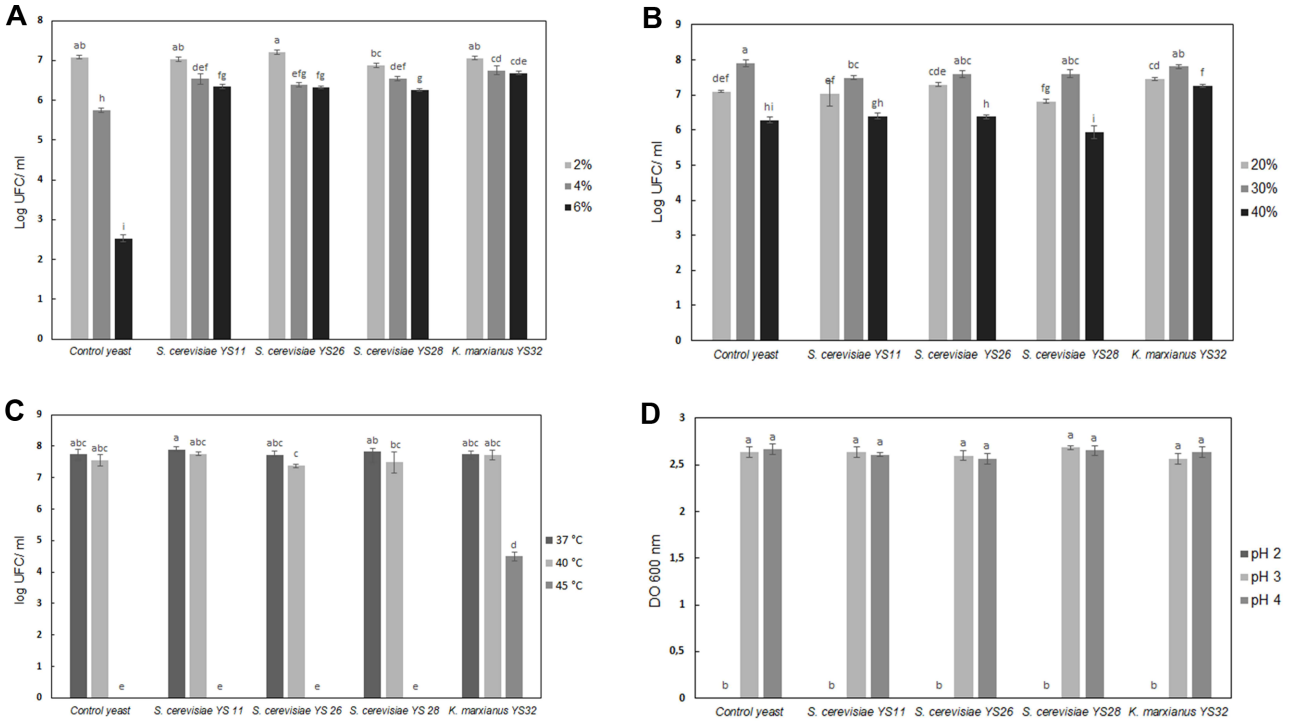


**Fig. 2. Stress Tolerance.** The growth of the four yeast strains and the control yeast was investigated at defined unfavorable conditions. (A) NaCl, 2-6%, (B) Sucrose 20-40%, (C) Temperature, 37-45℃, and (D) pH, 2-4. Results were expressed in Log CFU/ml for NaCl, sucrose and temperature, while for pH in absorbance at 600 nm. Vertical bars represent ± SD, Data average of three replicates. Values with different letters are significantly different ($p < 0.05$).

(45℃), only *K. marxianus* YS32 resisted. These results are in agreement with several studies that describe *K. marxianus* as a thermotolerant yeast that can grow in temperatures up to 52℃ [34, 49, 52]. The survival of the yeast strains under high heat conditions indicates that they can handle fermentation over a wide range of temperatures. This could have a positive impact on increasing the production of carbon dioxide, accelerating of dough proofing and the development of new flavors and aroma [53].

Sourdough bread is known to have more nutritional value than traditional bread. Today, many types of sourdoughs (liquid, dried or lyophilized) are commercialized by baking industries [54, 55]. However, dough leavened with sourdough undergoes a remarkable drop in pH values due to LAB metabolism. These acidic conditions could apply a selective pressure on the propagation of yeast cells. Therefore, the resistance of yeast cells to low pH is essential to select the most competitive starter to be used in sourdough formulation. Fig. 2D presents the effect of acidic pH (2, 3 and 4) on the growth of the five strains. The results indicate that all the yeast strains grew well at pH 3 and 4 while their growth was completely inhibited at pH 2. The pH of mature sourdoughs varies from 3.8 to 4.5 [56]. Therefore, our strains may be used for sourdough production based on their ability to survive at acidic conditions.

## Conclusion

The study reported in this paper indicates that traditional sourdoughs are an excellent source for isolation and selection of yeasts with potentials leavening ability. Three yeasts identified as *S. cerevisiae* (YS11, YS26 and YS28), and one as *K. marxianus* YS32 had the best performances in dough proofing and stress tolerance compared to commercial baker's yeast. Our study also describes for the first time the use of the species *P. kudriavzevii* as a leavening agent for bread preparation. Further research is planned to focus on the on flavors and aroma development, biomass optimization and culture preservation.

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## Conflict of Interest

The authors have no financial conflict of interest to declare.

## References

1. Newberry MP, Phan-Thien N, Larroque OR, Tanner RI, Larsen NG. 2002. Dynamic and elongation rheology of yeasted bread doughs. *Cereal Chem.* **79**: 874-879.
2. Liao Y, Miller RA, Hoseney RC. 1998. Role of hydrogen peroxide produced by baker's yeast on dough rheology. *Cereal Chem.* **75**: 612-616.
3. Romano A, Toraldo G, Cavella S, Masi P. 2007. Description of leavening of bread dough with mathematical modelling. *J. Food Eng.* **83**: 142-148.
4. Poitrenaud B. 2004. Baker's yeast, pp. 695-719, *Handbook of food and beverage fermentation technology*. Marcel Dekker, USA.
5. Gélinas P. 2012. In search of perfect growth media for Baker's yeast production: Mapping patents. *Compr. Rev. Food Sci. Food Saf.* **11**: 13-33.
6. Phaff HJ, Miller MW, Mrak EM. 2013. *The life of yeasts*. 2th Ed. Harvard University Press, Cambridge, USA.
7. Dequin S. 2001. The potential of genetic engineering for improving brewing, wine-making and baking yeasts. *Appl. Microbiol. Biotechnol.* **56**: 577-588.
8. Ostergaard S, Olsson L, Nielsen J. 2000. Metabolic engineering of *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **64**: 34-50.
9. Li H, Li Z, Qu J, Wang J. 2017. Bacterial diversity in traditional Jiaozi and sourdough revealed by high-throughput sequencing of 16S rRNA amplicons. *LWT-Food Sci. Technol.* **81**: 319-325.
10. Gänzle M, Ripari V. 2016. Composition and function of sourdough microbiota: From ecological theory to bread quality. *Int. J. Food Microbiol.* **239**: 19-25.
11. De Vuyst L, Harth H, Van Kerrebroeck S, Leroy F. 2016. Yeast diversity of sourdoughs and associated metabolic properties and functionalities. *Int. J. Food Microbiol.* **239**: 26-34.
12. Minervini F, Di Cagno R, Lattanzi A, De Angelis M, Antonielli L, Cardinali G, *et al.* 2012. Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: interactions between ingredients and microbial species diversity. *Appl. Environ. Microbiol.* **78**: 1251-1264.
13. Alfonzo A, Urso V, Corona O, Francesca N, Amato G, Settanni L, *et al.* 2016. Development of a method for the direct fermentation of semolina by selected sourdough lactic acid bacteria. *Int. J. Food Microbiol.* **239**: 65-78.
14. Gobbetti M, Minervini F, Pontonio E, Di Cagno R, De Angelis M. 2016. Drivers for the establishment and composition of the sourdough lactic acid bacteria biota. *Int. J. Food Microbiol.* **239**: 3-18.
15. Gänzle M, Gobbetti M. 2013. Physiology and Biochemistry of Lactic Acid Bacteria, pp. 183-216, *In* Gobbetti M, Gänzle M (eds.), *Handbook on Sourdough Biotechnology*. Springer US, Boston, MA, USA.
16. Huys G, Daniel H-M, De Vuyst L. 2013. Taxonomy and biodiversity of sourdough yeasts and lactic acid bacteria, pp. 105-154. *In* Gobbetti M, Gänzle M (eds.), *Handbook on Sourdough Biotechnology*. Springer US, Boston, MA., USA.
17. Perricone M, Bevilacqua A, Corbo MR, Sinigaglia M. 2014. Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products. *Food Microbiol.* **38**: 26-35.
18. Katina K, Salmenkallio-Marttila M, Partanen R, Forssell P, Autio K. 2006. Effects of sourdough and enzymes on staling of high-fibre wheat bread. *LWT-Food Sci. Technol.* **39**: 479-491.
19. Kariluoto S, Edelmann M, Nyström L, Sontag-Strohm T, Salovaara H, Kivelä R, *et al.* 2014. In situ enrichment of folate by microorganisms in beta-glucan rich oat and barley matrices. *Int. J. Food Microbiol.* **176**: 38-48.
20. Türk M, Sandberg A-S, Carlsson N-G, Andlid T. 2000. Inositol hexaphosphate hydrolysis by Baker's yeast. Capacity, kinetics, and degradation products. *J. Agric. Food Chem.* **48**: 100-104.
21. Moslehi-Jenabian S, Lindegaard L, Jespersen L. 2010. Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients* **2**: 449-473.
22. Zhou N, Schifferdecker AJ, Gamero A, Compagno C, Boekhout T, Piškur J, *et al.* 2017. Kazachstania gamospora and *Wickerhamomyces subpelliculosus*: Two alternative baker's yeasts in the modern bakery. *Int. J. Food Microbiol.* **250**: 45-58.
23. Aslankoohi E, Herrera-Malaver B, Rezaei MN, Steensels J, Courtin CM, Verstrepen KJ. 2016. Non-conventional yeast strains increase the aroma complexity of bread. *PLoS One* **11**: e0165126.
24. Pacheco A, Santos J, Chaves S, Almeida J, Leão C, Sousa MJ. 2012. The emerging role of the yeast *Torulaspora delbrueckii* in bread and wine production: using genetic manipulation to study molecular basis of physiological responses. *Struct. Funct. Food Eng. Rij. InTech* 339-370.
25. Almeida MJ, Pais C. 1996. Leavening ability and freeze tolerance of yeasts isolated from traditional corn and rye bread doughs. *Appl. Environ. Microbiol.* **62**: 4401-4404.
26. AACC. *Approved methods of the AACC. Titratable Acidity. Method 02-31.01*. 11th Ed. American Association of Cereal Chemists, St. Paul, USA.
27. Harju S, Fedosyuk H, Peterson KR. 2004. Rapid isolation of yeast genomic DNA: Bust n'Grab. *BMC Biotechnol.* **4**: 1-6.
28. Lhomme E, Lattanzi A, Dousset X, Minervini F, De Angelis M, Lacaze G, *et al.* 2015. Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. *Int. J. Food Microbiol.* **215**: 161-170.
29. Arendt EK, Ryan LA, Dal Bello F. 2007. Impact of sourdough on the texture of bread. *Food Microbiol.* **24**: 165-174.

30. Palla M, Agnolucci M, Calzone A, Giovannetti M, Di Cagno R, Gobbetti M, *et al.* 2019. Exploitation of autochthonous Tuscan sourdough yeasts as potential starters. *Int. J. Food Microbiol.* **302**: 59-68.
31. Hammes WP, Brandt MJ, Francis KL, Rosenheim J, Seitter MF, Vogelmann SA. 2005. Microbial ecology of cereal fermentations. *Trends Food Sci. Technol.* **16**: 4-11.
32. Asyikeen ZN, Ma'aruf AG, Sahilah AM, Khan AM, Aida WW. 2013. A new source of *Saccharomyces cerevisiae* as a leavening agent in bread making. *Int. Food Res. J.* **20**: 967.
33. Caballero R, Olguín P, Cruz-Guerrero A, Gallardo F, García-Garibay M, Gómez-Ruiz L. 1995. Evaluation of *Kluyveromyces marxianus* as baker's yeast. *Food Res. Int.* **28**: 37-41.
34. Karim A, Gerliani N, Aïder M. 2020. *Kluyveromyces marxianus*: An emerging yeast cell factory for applications in food and biotechnology. *Int. J. Food Microbiol.* **333**: 108818.
35. Bonekamp FJ, Oosterom J. 1994. On the safety of *Kluyveromyces* lactis—a review. *Appl. Microbiol. Biotechnol.* **41**: 1-3.
36. Nikolaou E, Soufleros EH, Bouloumpasi E, Tzanetakis N. 2006. Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. *Food Microbiol.* **23**: 205-211.
37. Joosten H, Northolt MD. 1989. Detection, growth, and amine-producing capacity of lactobacilli in cheese. *Appl. Environ. Microbiol.* **55**: 2356-2359.
38. Oda Y, Ouchi K. 1990. Hybridization of bakers' yeast by the rare-mating method to improve leavening ability in dough. *Enzyme Microb. Technol.* **12**: 989-993.
39. Randez-Gil F, Córcoles-Sáez I, Prieto JA. 2013. Genetic and phenotypic characteristics of baker's yeast: relevance to baking. *Annu. Rev. Food Sci. Technol.* **4**: 191-214.
40. Donalies UE, Nguyen HT, Stahl U, Nevoigt E. 2008. Improvement of *Saccharomyces* yeast strains used in brewing, wine making and baking. *Adv. Biochem. Eng. Biotechnol.* **111**: 67-98.
41. Vaisey M, Unrau AM. 1964. Flour composition, chemical constituents of flour from cytologically synthesized and natural cereal species. *J. Agric. Food Chem.* **12**: 84-86.
42. Kurtzman CP, Mateo RQ, Kolecka A, Theelen B, Robert V, Boekhout T. 2015. Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways. *FEMS Yeast Res.* **15**: fov050.
43. Noort MW, Bult JH, Stieger M, Hamer RJ. 2010. Saltiness enhancement in bread by inhomogeneous spatial distribution of sodium chloride. *J. Cereal Sci.* **52**: 378-386.
44. Quílez J, Ruiz JA, Romero MP. 2006. Relationships between sensory flavor evaluation and volatile and nonvolatile compounds in commercial wheat bread type baguette. *J. Food Sci.* **71**: S423-S427.
45. Takagi H, Shima J. 2015. Stress Tolerance of Baker's Yeast During Bread-Making Processes, pp. 23-42, *In* Takagi H, Kitagaki H (eds.), *Stress Biology of Yeasts and Fungi: Applications for Industrial Brewing and Fermentation*. Springer Japan, Tokyo.
46. Struyf N, Van der Maelen E, Hemdane S, Verspreet J, Verstrepen KJ, Courtin CM. 2017. Bread dough and baker's yeast: An uplifting synergy. *Compr. Rev. Food Sci. Food Saf.* **16**: 850-867.
47. Zhang J, Lv C, Tong J, Liu J, Liu J, Yu D, *et al.* 2016. Optimization and microbial community analysis of anaerobic co-digestion of food waste and sewage sludge based on microwave pretreatment. *Bioresour. Technol.* **200**: 253-261.
48. Verstrepen KJ, Iserentant D, Malcorps P, Derdelinckx G, Van Dijck P, Winderickx J, *et al.* 2004. Glucose and sucrose: hazardous fast-food for industrial yeast? *Trends Biotechnol.* **22**: 531-537.
49. Saini P, Beniwal A, Vij S. 2017. Physiological response of *Kluyveromyces marxianus* during oxidative and osmotic stress. *Process Biochem.* **56**: 21-29.
50. Aldiguier AS, Alfenore S, Cameleyre X, Goma G, Uribelarrea JL, Guillouet SE, *et al.* 2004. Synergistic temperature and ethanol effect on *Saccharomyces cerevisiae* dynamic behaviour in ethanol bio-fuel production. *Bioprocess Biosyst. Eng.* **26**: 217-222.
51. Torija MJ, Rozes N, Poblet M, Guillamón JM, Mas A. 2003. Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae*. *Int. J. Food Microbiol.* **80**: 47-53.
52. Fonseca GG, Heinzle E, Wittmann C, Gombert AK. 2008. The yeast *Kluyveromyces marxianus* and its biotechnological potential. *Appl. Microbiol. Biotechnol.* **79**: 339-354.
53. Maaruf A, Sahilah A, Khan MA. 2011. Leavening ability of yeast isolated from different local fruits in bakery product. *Sains Malays.* **40**: 1413-1419.
54. Reale A, Di Renzo T, Preziuso M, Panfili G, Cipriano L, Messia MC. 2019. Stabilization of sourdough starter by spray drying technique: New breadmaking perspective. *LWT* **99**: 468-475.
55. De Vuyst L, Neysens P. 2005. The sourdough microflora: biodiversity and metabolic interactions. *Trends Food Sci. Technol.* **16**: 43-56.
56. Catzeddu P. 2019. Chapter 14 - Sourdough Breads, pp. 177-188. *In* Preedy VR, Watson RR (eds.), *Flour and Breads and their Fortification in Health and Disease Prevention (Second Edition)*. Academic Press.