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Potentialities of Yeast Strains to be Used as Freeze-Drying Starters for the Production of Traditional Sorghum Beer in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KMD and AA were responsible for study design and supervision of work. Authors WHC, FC, DMT, KMJPB, AKK and YCTB were responsible for laboratory work, data analysis and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

Freeze-drying is a well-known dehydration method widely used to preserve microorganisms. In order to produce freeze-dried yeast starter culture for the brewing purpose of African traditional sorghum beer, we tested tolerance to ethanol stress and evaluated the relative expression level of

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genes *TIF 11* and *YJL144W* encoding the hydrophilins. Among the strains tested, the best viability rate to ethanol stress (7.5% ethanol (v/v)) was found with *Saccharomyces cerevisiae* F12–7 and *Candida tropicalis* C0–7 respectively with 95% and 80%. For *Saccharomyces cerevisiae* strains, the strain F12-7, which had distinguished itself from other strains in previous tests, the *TIF11* and *YJL144W* genes were the least expressed. For *C. tropicalis* strains, the statistical analyzes of the relative expression levels from the Tukey test revealed no difference between the strains for the 2 genes (P> 0.05).

Keywords: Ethanol stress; freeze-drying starter; yeasts; hydrophilins; traditional sorghum beer.

1. INTRODUCTION

Fermentation has been applied for thousand years as a method to preserve perishable foods. It seems that fermentation appeared in the period 8000–6000 BC in Iraq with the fabrication of cheese. Later, in the period 4000–2000 BC, Egyptians discovered how to use yeasts to make leavened bread and wine [1]. Nowadays, a large variety of fermented foods are consumed around the world and appreciated for their organoleptic and sensory properties, including cheese, yoghurt, kefir, beer, kombucha, pozol, sourdough, kimchi, olives, sauerkraut, pickles, tempe, gari, fufu or sausages [2]. Sorghum beer is a traditional fermented beverage from most of West African countries where sorghum is produced. It is known as tchapalo in Côte d' Ivoire and by various other names in other African countries. The beers are consumed at various festivals and African ceremonies (e.g., marriage, birth, baptism, the handing over of a dowry, etc.) and constitute a source of economic return for women beer producers. But these fermentations are uncontrolled and the microorganisms involved come from the raw materials, equipment and local environments or from residues of previous fermentation batches. These microorganisms play an active role in the physical, nutritional, and organoleptic modification of starting materials [3]. The use of starter cultures was suggested as the appropriate approach to alleviate the problems of variations in organoleptic quality and microbiological stability of traditional beverages and foods [4-5] and resisted to inhibitors as ethanol [6]. However, these starter cultures were introduced during the fermentation in the form of fresh microbial suspensions which are difficult to maintain for extended periods. To overcome this problem, the use of dried starter cultures, active and stable during storage, is the viable and sustainable solution. The use of microorganisms for starter culture production by freeze drying requires the satisfaction of various conditions. Freeze-drying is a well-known dehydration method widely used to preserve microorganisms. It is also commonly used in food preservation and for a variety of pharmaceutical applications, including protein- based drugs [7-8]. By its ability of combining freezing and drying in a unique operation, this process can create final dried products with the highest quality, but the freeze-drying step is especially critical as it negatively affects both viability and physiological state of the yeasts [9]. The formation of ice crystals induces mechanical damage that leads to cellular death during freezing [10]. Microbial survival during this process depends on many factors, including the intrinsic resistance traits of the strains, initial concentration of microorganisms, growth conditions, the drying medium, and hydrophilin presence in yeast cytoplasm which is required for dehydration stress tolerance [11-14]. Hydrophilins are a group of highly hydrophilic and glycine-rich proteins, which are hypothesized to play a role in cellular dehydration tolerance. The yeast genome includes 12 genes encoding hydrophilins and most of them are stress-responsive [15]. They are assumed to play important roles in cellular dehydration tolerance. There are 12 genes in the yeast that encode hydrophilins and most of these genes are stress responsive [15]. In many studies, the microorganisms were used for the production of freeze-dried starter culture without known to capacities to resist to dehydration stress. The viability was enhanced by adding many protective compounds such as disaccharides, polyols, monosaccharides, skim milk, and other organic molecules [16]. According to the work of Berny and Hennebert [17], by using skim milk as a support material in combination with two compounds between honey, sodium glutamate, trehalose or raffinose, the viability of *Saccharomyces cerevisiae* cells increased from 30% to 96–98%. Abadias et al. [18]. reported a survival rate of 28.9% for *Candida sake* when 10% skim milk was used. Zhao and Zhang [19] obtained the highest viability (53.6%) after freeze-drying of *Oenococcus oeni* H-2 by using 2.5% sodium glutamate. But the adding many

protectives compounds can be of economic disadvantage in developing countries.

To date the study of yeasts intrinsic capacities to resist to dehydration and stress tolerance for the freeze-drying starter culture production for traditional sorghum remains untapped.

In this study, we aimed to select strains of *Saccharomyces cerevisiae* and *Candida tropicalis* based on their ability to tolerate ethanol toxicity and to evaluate the expression level of he hydrophillins encoding genes *TIF 11* and *YJL144W* to express encoding for hydrophilins.

2. MATERIALS AND METHODS

2.1 Yeast Strains

The yeast strains used in this study were *Candida tropicalis* (C0-7; F0-5; C8-10) and *Saccharomyces cerevisiae* (D12-3; D12-10; E4-4; A12-1; C8-5; F12-7), preserved in the culture collection of the Department of Food Technology (University of Nangui-Abrogoua, Abidjan, Côte d'Ivoire). These strains were originally isolated from traditional sorghum beer from the district of Abidjan (Southern Côte d'Ivoire) and were thereafter identified through Polymerase Chain Reaction-Restriction Fragment Length polymorphism (PCR-RFLP) analysis of the Internal Transcribed Spacer (ITS) region and sequencing of D1/D2 domains of the 26S rRNA gene [20]. Yeast strains were maintained routinely at -20°C in 20% glycerol.

2.2 Yeast Strains Tolerance to Ethanol

All yeast strains were evaluated for their ability to growth under ethanol stress. The strains were transferred from Sabouraud-chloramphenicol plates to pre-culture 10 mL YPG broth (10 g/L yeast extract, 10 g/L peptone, 10 g/L glucose) and incubated at 30°C for 24 h. Pre-cultures with OD_{600nm} = 0.2 were used to inoculate 50 mL of YPD broth supplemented with either 0%, 2.5%, 5% and 7.5% ethanol (v/v), respectively. Cultures were incubated at 30 °C for 24 h under constant agitation at 150 rpm agitation, with sampling after 0, 4, 8 and 24 h for further analyses (see below) [21].

2.2.1 Determination of yeast viability

Cellular 'viability' was determined using the methylene blue method [22]. To 200 µL sterile solution of methylene blue (0.3 mM in 68 mM Na_3 citrate) was mixed with 200 µL of a yeast suspension diluted in order to achieve an OD_{600nm} of 0.4–0.7. The mixture was shaken and, after 5 min incubation, placed in a Thoma's counting chamber. The number of stained (non-active cells) or un-stained (active cells) and the number of buds were counted in five different fields with a total of at least 200–300 cells. The percentage of 'viable' cells ('viability') was determined as the proportion of unstained (living) cells over the total number of cells (regardless of staining).

2.2.2 Residuals glucose and ethanol contents

Glucose and ethanol were isolated and quantitated through HPLC separation (Agilent Technologies, 1200 series, UK) using a column Aminex HPX-87H, 300 mm × 7.8 mm (Biorad) coupled to a refractometer (Agilent Technologies) and a UV diode array detector (Agilent Technologies). The acquisition and processing of data were carried out using the Chromoleon software. Run parameters were as follows: oven temperature: 50°C; run duration: 35 min; mobile phase: 5 mM sulfuric acid; flow rate: 0.5 mL/min; injected sample volume: 20 µL. Concentration was determined through the analysis of peak areas at the maximum absorbance wavelength (210 nm), by comparison with calibration curves generated with known concentrations of pure standards.

2.3 Relative Expression of the *TIFF11* and *YJL144W* Genes through Quantitative Real-time PCR (qRT-PCR)

2.3.1 Yeast strains, growth conditions and sampling

The *Saccharomyces cerevisiae* strains (D12-3; D12-10; E4-4; A12-1; C8-5; F12-7) and *Candida tropicalis* strains (C0-7; F0-5; C8-10) were grown at 30°C in a synthetic minimal medium containing 0.17% (w/v) yeast nitrogen base (DIFCO), 0.5% (w/v) ammonium sulfate and 2% (w/v) galactose (YNGal) or glucose (YNGlu). Prototroph strains were used in order to prevent aminoacid complementation of the medium. The pH was adjusted to 5.0 with succinic acid and sodium hydroxide. Cell growth was monitored through periodical OD_{600nm} measurements over at least 10 days. Replicate culture flasks were generated independently at different times from distinct inocula.

Yeast samples for real-time PCR analysis (approx. 10^8 cells) were immediately centrifuged,

Table 1. List of candidate reference gene and genes of interest

Primers	Primers sequence
TIF11 Forward	5'- GGGTAAGAAAAACACTAAAGGTGGT-3'
TIF11 Reverse	5'- CAAGTTCTTCATCTTCACCTTCCTC -3'
YJL144w Forward	5'- GGAGGGAAACTTCAACAATATACAGGACAC-3'
YJL144w Reverse	5'-TCATGAACAACGGCGAGAGTGAACGTCT-3'
TAF10 Forward	5'-ATATTCCAGGATCAGGTCTTCCGTAGC-3'
TAF10 Reverse	5'-GTAGTCTTCTCATTCTGTTGATGTTGTTGTTG-3'

TAF10: Reference gene

then cell pellets were flash-frozen in liquid nitrogen and stored at -80°C until used for RNA extractions.

2.3.2 Total RNA extraction

Frozen cells were mechanically disrupted using a ball mill (Mikro-Dismembrator S; B. Braun Biotech International). Total RNA was extracted using the RNeasy mini kit (Qiagen) according to the manufacturer's instruction. The purity and concentration of the extracted RNA were assessed spectrophotometrically using the ND-1000 UV-visible light spectrophotometer (NanoDrop Technologies) and its integrity was checked with the Bioanalyzer 2100 with the RNA 6000 Nano LabChip kit (Agilent).

2.3.3 Quantitative RT-PCR

Oligonucleotides for real-time PCR (Table 1) were designed using Beacon Designer 2.0 software (PREMIER Biosoft International), which included a BLAST analysis against *Saccharomyces cerevisiae and Candida tropicalis* Genome sequence for specificity confidence, and analysis using the Mfold server to avoid positioning on unfavorable secondary structures.

One microgram of total RNA was reverse-transcribed into cDNA in a 20 µL reaction mixture using the iScript cDNA synthesis kit (Bio-Rad). This experience was repeated three times. The cDNA levels were then analyzed using the MyIQ real-time PCR system from Bio-Rad. Each sample was tested in duplicate in a 96-well plate (Bio-Rad, CA). The reaction mix (25 µL final volume) consisted of 12.5 µL of iQ SYBR Green Supermix (BioRad), 2.5 µL of each primer (250 nM final concentration), 2.5 µL of H2O, and 5 µL of a 1/10 dilution of the cDNA preparation. The absence of genomic DNA in RNA samples was checked by real-time PCR before cDNA synthesis (minus RT control). A blank (No Template Control) was also incorporated in each assay. The thermocycling program consisted of one hold at 95°C for 4 min, followed by 40 cycles of 10 s at 95°C and 45 s at 56°C. After completion of these cycles, melting-curve data were then collected to verify PCR specificity, contamination and the absence of primer dimers.

The PCR efficiency of each primer pair (Eff) was evaluated by the dilution series method using a mix of sample cDNAs as the template. It was determined from standard curves using the formula $10^{(-1/slope)}$. For the calculations, the base of the exponential amplification function was used (e.g. 1.94 means 94% amplification efficiency). Relative expression levels were determined with efficiency correction, which considers differences in primer pair amplification efficiencies between target and reference gene [23-24]. Expression data and associated technical errors on duplicates were calculated using the gene expression module of the BIORAD iQ5 software, which follows models and error propagation rules outlined in the geNorm manual.

2.4 Statistical Analyzes

Relative expression levels were compared between yeast strains through analysis of variance (ANOVA) and significant differences were detected using the test of Tukey with a 0.05 threshold, using the STATISTICA software (99^{th} Edition; StatSoft).

3. RESULTS AND DISCUSSION

3.1 Strains Viability

The evolution of strain viability of *Saccharomyces cerevisiae* during culture with different initial concentrations of ethanol (0%; 2.5%; 5%; 7.5%) is shown in Fig. 1. Globally, all strains show a relatively important growth over time. With an initial ethanol concentration of 0%, the viability rate ranges from 37.40% to 100%, whereas it is comprised between 55.16% and 99.17% with 2.5% ethanol and between 55.55%

and 99.24% with 5% ethanol in the culture medium, respectively. Similar observations are obtained with *Candida tropicalis* strains: the percentage of viable yeasts ranges from 83.6 to 99.49% under 0% ethanol; 77.19 to 91.07% with 2.5% ethanol; 39.23 and 93.07% with 5% ethanol, respectively (Fig. 2). This result is in accordance with the work of Lyumugabe et al. [21], in which authors reported that yeasts strains displayed resistance to an ethanol concentration of 5%. The phenomenon to ethanol tolerance is particularly difficult to study since there is no universal technique for its characterization. Some authors rely on cell growth values [25-26], the specific rate of ethanol production [25], or the viability [27-28]. It is also possible to study the flux of protons across the plasma membrane [29].

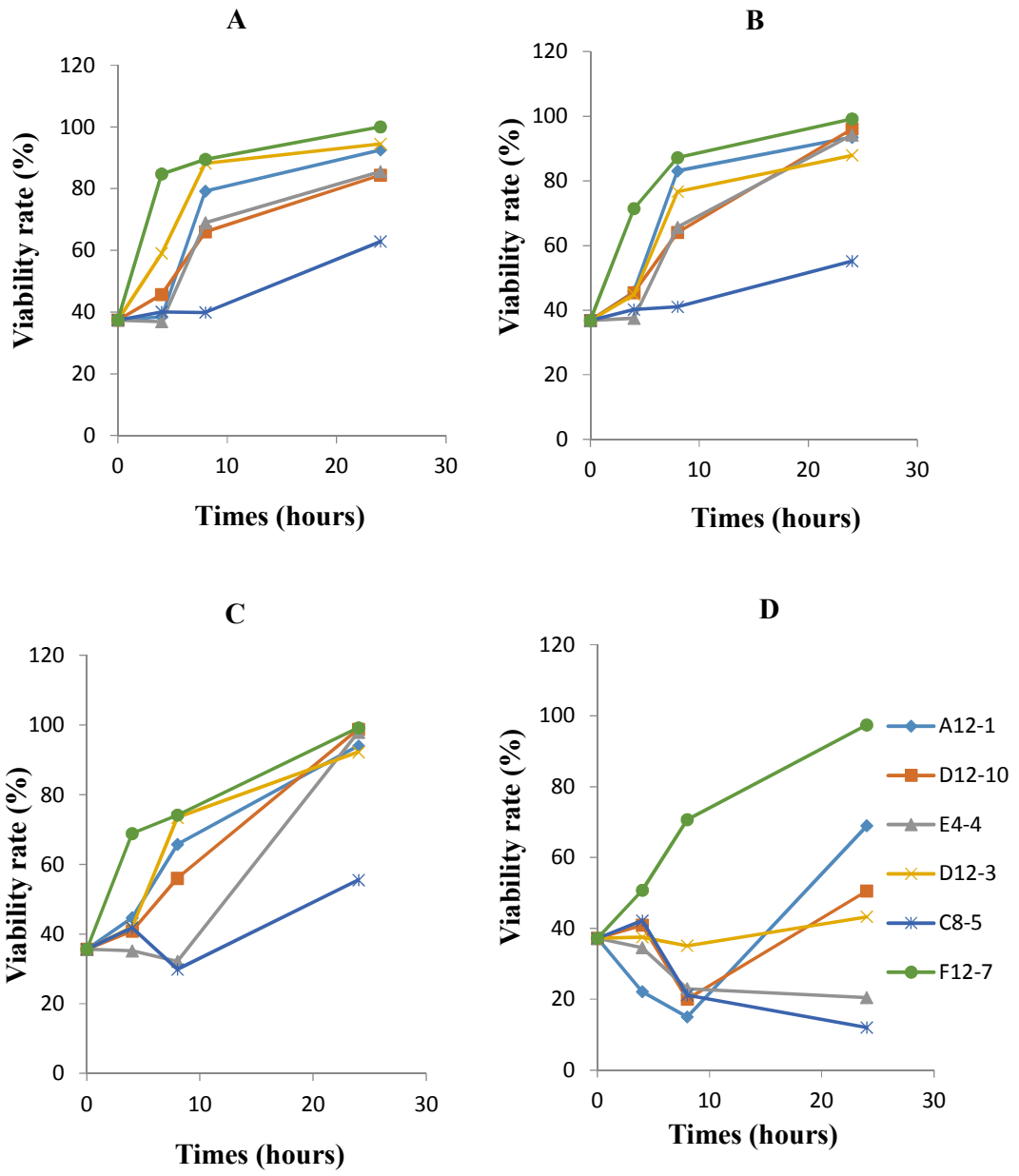

Fig. 1. *S. cerevisiae* strains viability in presence of 0% (A); 2.5% (B); 5% (C); 7.5% (D) initial rate ethanol

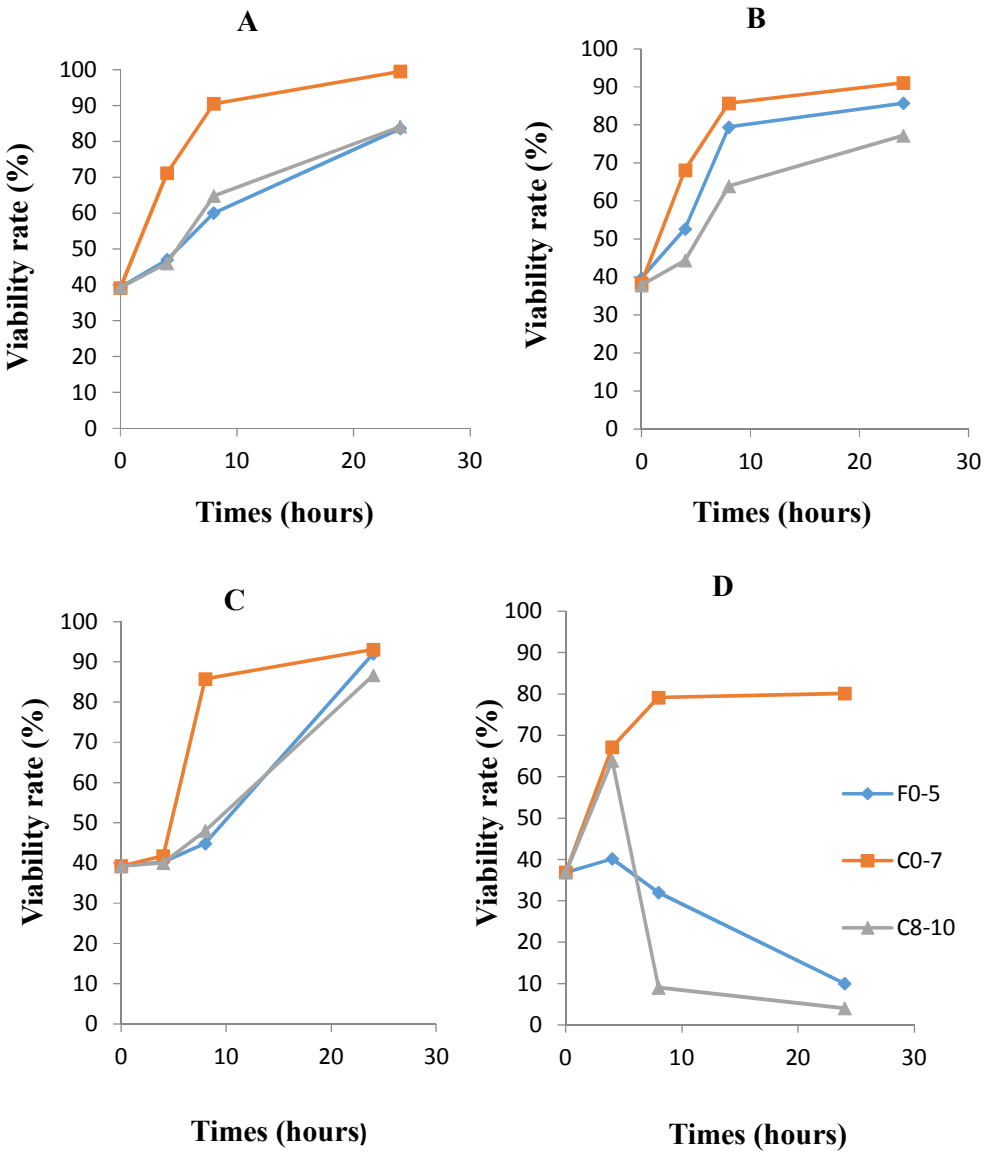

Fig. 2. *C. tropicalis* strains viability in presence of 0% (A); 2.5% (B); 5% (C); 7.5% (D) initial rate ethanol

For both *Saccharomyces cerevisiae* and *Candida tropicalis* strains, the major difference in yeast viability is detected with an initial ethanol concentration of 7.5%, under which only the F12-7 and C0-7 strains, respectively, are able to grow, with viability rates of 95% and 80%. Such an important viability rate for *S. cerevisiae* F12-7 has been previously reported by Le Marois [6]. In fact, this species was able to tolerate high ethanol concentrations up to 10% (v/v) [30-31]. Ethanol is known to be an inhibitor of yeast growth and is also a limiting factor for alcoholic fermentation [32]. The mechanism of toxicity mainly affects the plasma membrane, strongly modifying its structure and its properties. In fact, from a macrokinetic point of view, the increase in ethanol concentration in the medium causes a decrease in cell viability [33-36]. Ethanol modifies the structure of the plasma membrane from a lipid point of view [37]. The permeability and fluidity of the membrane increase sometimes to cell lysis [38-39]. However, a cell with permeable membrane is subject to leakage of compounds as cofactors and coenzymes essential to its

survival [40]. The mechanism of resistance of yeast to ethanol was due to modification of their lipids compounds particularly fatty acids unsaturated [41-42]. Thus, the resistance of *Saccharomyces cerevisiae* F12-7 and *Candida tropicalis* C0-7 to 7.5% ethanol initial rate should be probably due to synthesis or modification of fatty acids unsaturated but the assays of lipids compounds in the yeasts membrane would be confirm this affirmation.

However; more studies are necessary in order to discriminate between these different mechanisms possibly underlying ethanol tolerance in these two yeast strains. In sum, the strains *Saccharomyces cerevisiae* F12-7 and *Candida tropicalis* C0-7 seemed to be appropriate to useb as starter for traditional sorghum beer production.

3.2 Residual Ethanol and Glucose Contents

3.2.1 Residual glucose contents

Changes in residual glucose concentrations over time are shown in Figs. 3 and 4. Overall, a decrease in glucose contents is observed with all strains for both species. In the absence of ethanol, residual glucose are comprised between 2.67 and 0.5 g/L for *Saccharomyces cerevisiae* strains and between 1.78 g/L and 3.83 g/L for *Candida tropicalis* strains. In the presence of ethanol (2.5%; 5% 7.5%), residuals glucose contents are globally higher than in those observed in ethanol-free medium. For *Saccharomyces cerevisiae* strains, at 2.5% ethanol, these concentrations are 2.47 and 5.9 g/L. At the 5% ethanol level, residuals glucose concentrations increased relatively (between 3.69 and 5.27 g/L) for all strains excepted strains A12-1 and D12-10 where one decrease is rather observed compared to the rate of 2.5% of ethanol. At 7.5% initial rate ethanol, the lowest residual glucose concentration (2.86 g/L) was obtained with F12-7. For the other strains, they are between 3.20 and 8.60 g/L.

For *Candida tropicalis* strains, at 2.5% initial rate ethanol, the residuals concentrations of glucose were comprised between 2.74 and 5.81 g/L and at 5% % initial rate ethanol, the residuals concentrations were between 0.8 and 1.19 g/L. With an initial ethanol level of 7.5% in the culture medium, only the C0-7 strain allowed a greater use of glucose. Contents ranged from 10 g/L to 6.19 g/L (ie, consumption of 3.81 g/L) as they increased from 10 g/L to 8 g/L (ie, consumption of 2 g/L) with the other two strains. In general, a decrease in the glucose concentration was observed for all strains regardless of the ethanol level. This decline reflects the use of glucose by the strains for their growth. However, the residual glucose concentration of the medium was influenced by the initial ethanol level. In general, the higher the initial ethanol content, the less glucose is used by the yeasts, resulting in higher residual glucose concentrations. Thus, high contents of ethanol would inhibit the use of glucose by yeast strains. The residual glucose content is most low with *Saccharomyces cerevisiae* F12-7 than *Candida tropicalis* C0-7. So, the *Saccharomyces cerevisiae* resist more to ethanol than *Candaida tropicalis*.

3.2.2 Residual ethanol contents

During the culture of strains of *Saccharomyces cerevisiae*, the ethanol contents decreased regardless the strain and the initial rate ethanol (Fig. 5). Cultures with strain F12-7 were the media where the largest decreases were observed. Thus, rates ranged from 7.5% to 4% after 24 h of culture while rates with other strains declined only from 7.5% to about 6%.

Ethanol contents decreased during culture for all strains of *Candida tropicalis* (Fig. 6). However, these decreased vary across strains. The C0-7 strain was the one that makes it possible to obtain the largest decreases whereas the strains F0-5 and C8-10 showed similar decreases. Regardless of the initial ethanol contents, the lowest residual ethanol concentrations were observed in the C0-7 strain. Thus, at 2.5% initial rate ethanol, residual ethanol contents were 1.9% (v/v), 2.07% (v/v) and 1.15% (v/v) respectively for strains F0-5; C8-10 and C0-7. At 5% initial rate ethanol, the residual concentration was 3.07% (v/v) for the C0-7 strain, whereas those of strains F0-5 and C8-10 were respectively 3.75 and 4.07% (v/v). At the initial rate of 7.5% ethanol, it was still the C0-7 strain that separated from the other strains with a lower residual ethanol content (4.5% (v/v)) compared to 5.95 and 6.53% (v/v) respectively for strains F0-5 and C8-10. These results confirmed the resistance of *Saccharomyces cerevisiae* F12-7 and *Candida tropicalis* C0-7 to 7.5% initials rate ethanol and the link between the viability and residuals ethanol concentrations.

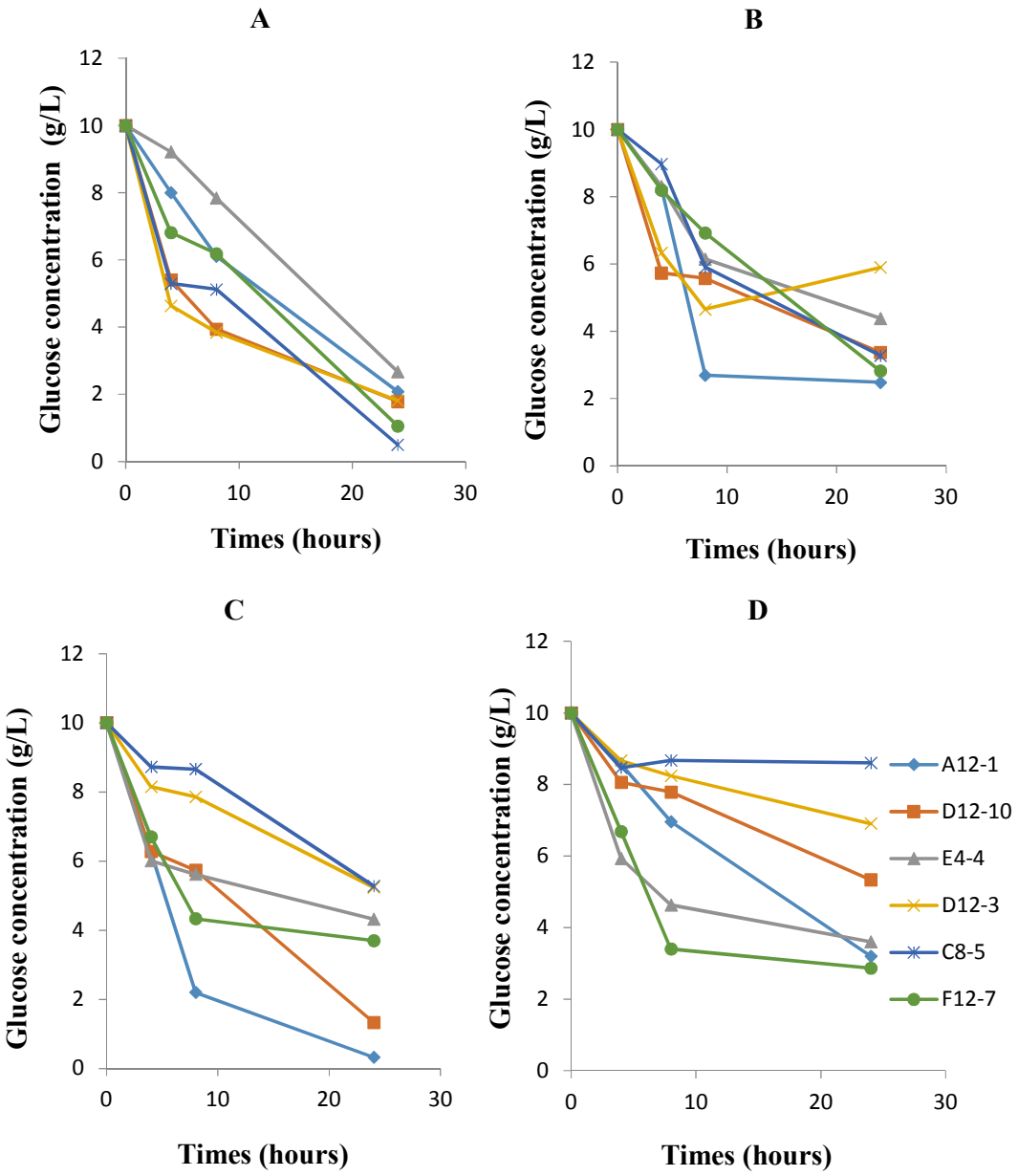

Fig. 3. Evolution of glucose concentrations in medium culture of *S. cerevisiae* strains supplemented of 0% (A); 2.5% (B); 5% (C); 7.5% (D) initial rate ethanol

3.3 Relatives Expressions Levels of *TIF11* and *YJL144W* Genes

Cellular dehydration due to desiccation or drought is a common and potentially fatal stress encountered by many organisms including plants, animals and micro-organisms. Although adaptations to these constraints have been described physiologically and at the molecular level in many species, the functional significance of most adaptations was still uncertain. In this respect, the genes coding for hydrophilins, proteins linked to the stress due to the dehydration of the microorganisms, have been of particular interest. In recent years, they have been the subject of some studies to determine the levels of expression of these genes [14-15]. The ability of micro-organisms to adapt rapidly to changing environmental conditions is essential for their survival. Thus, in order to determine the suitability of yeast strains for the use in preparation of freeze-dried starters cultures, a

study of genes involved in resistance to dehydration is necessary. In all the strains tested, the genes studied were expressed. Figs. 7 and 8 showed the relative expression level of the *TIF 11* and *YJL144W* genes respectively for *Saccharomyces cerevisiae* and *Candida tropicalis* strains compared to that of the reference gene of *TAF 10*. Generally, the *TIF11* gene was more expressed than the *YJL144W* gene and this for all the strains of the two yeast species studied. For *Saccharomyces cerevisiae* strains, the expression level of the *TIF11* gene with respect to the highest internal *TAF10* reference gene was obtained with strain D12-3 with an expression level of 5.56 ± 0.89 times greater. The lowest expression was observed with strain F12-7 where the *TIF11* gene was 1.59 ± 0.35 times expressed with respect to the reference internal gene *TAF 10*. For the *YJL144W* gene, the highest expression relative

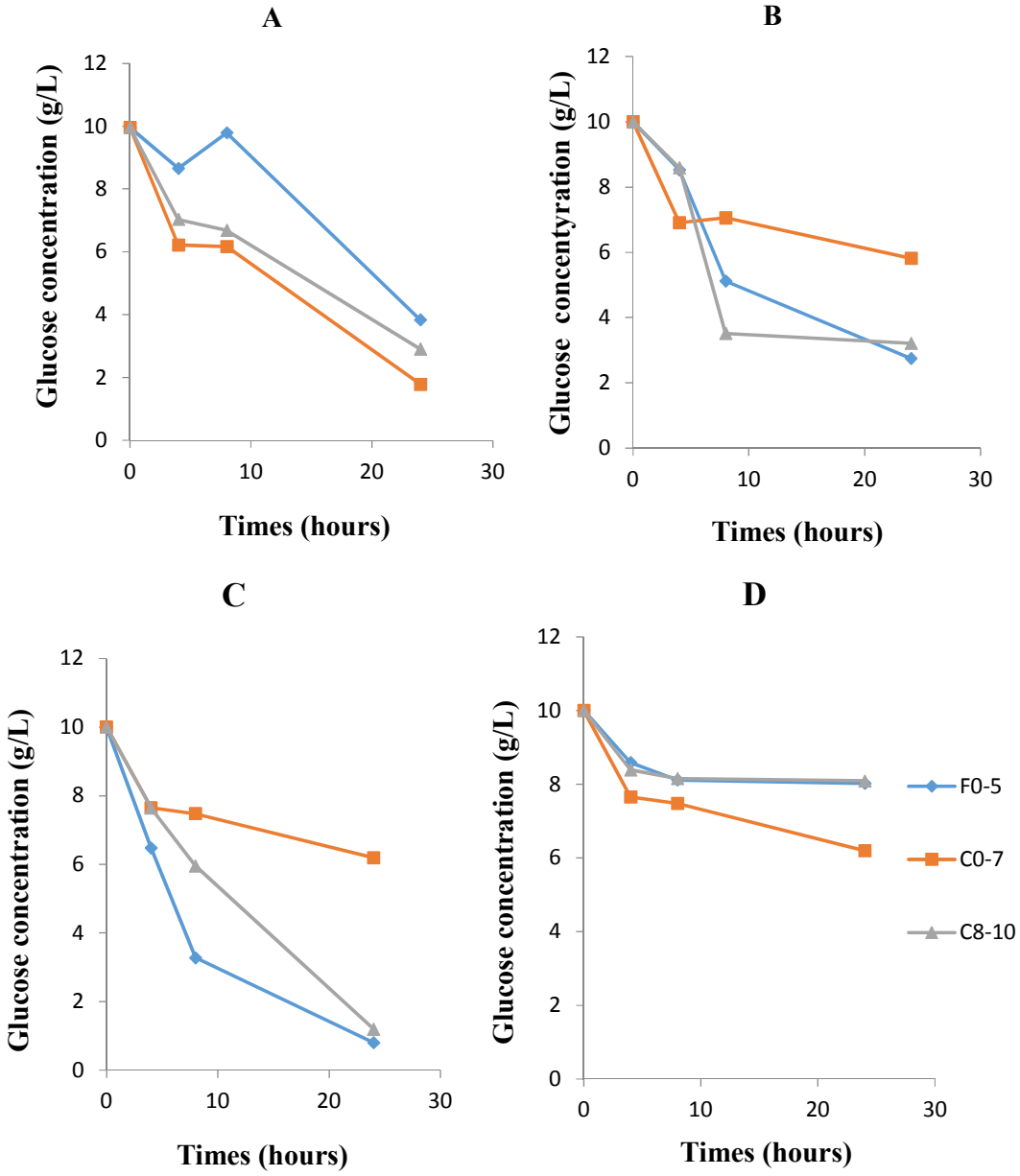

Fig. 4. Evolution of glucose concentrations in medium culture of *C. tropicalis* strains supplemented of 0% (A); 2.5% (B); 5% (C); 7.5% (D) initial rate ethanol

to the *TAF 10* internal gene was observed with the E4-4 strain with a 3.37 ± 0.48-fold increase in expression relative to that of the *TAF 10* gene. For this same gene, strain F12-7 was the one presenting the smallest expression 1.17 ± 0.33 times relative to the internal reference.

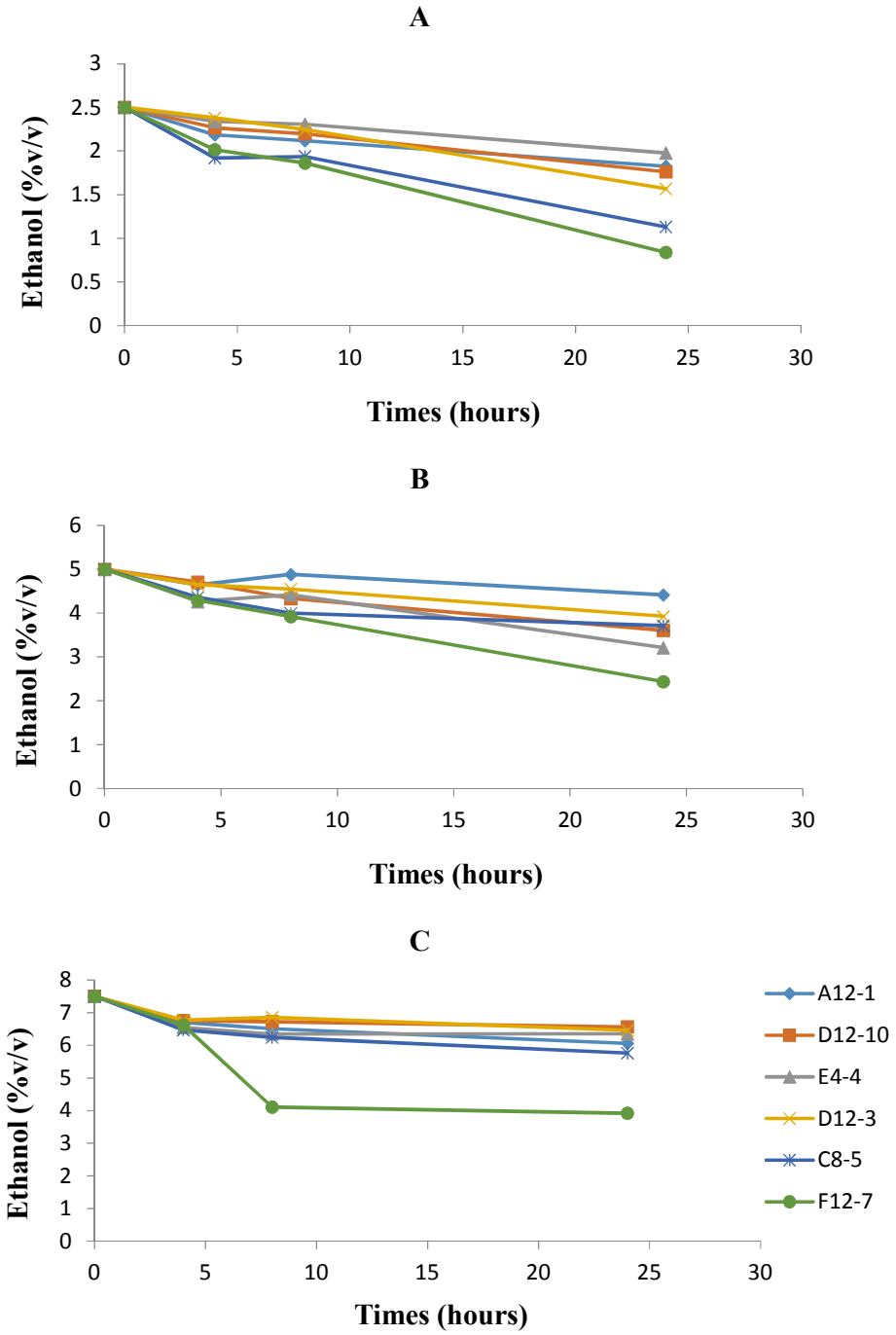

Fig. 5. Evolution of ethanol concentrations during culture of *S. cerevisiae* strains in medium supplemented of 2.5% (A); 5% (B); 7.5% (C) initial rate ethanol

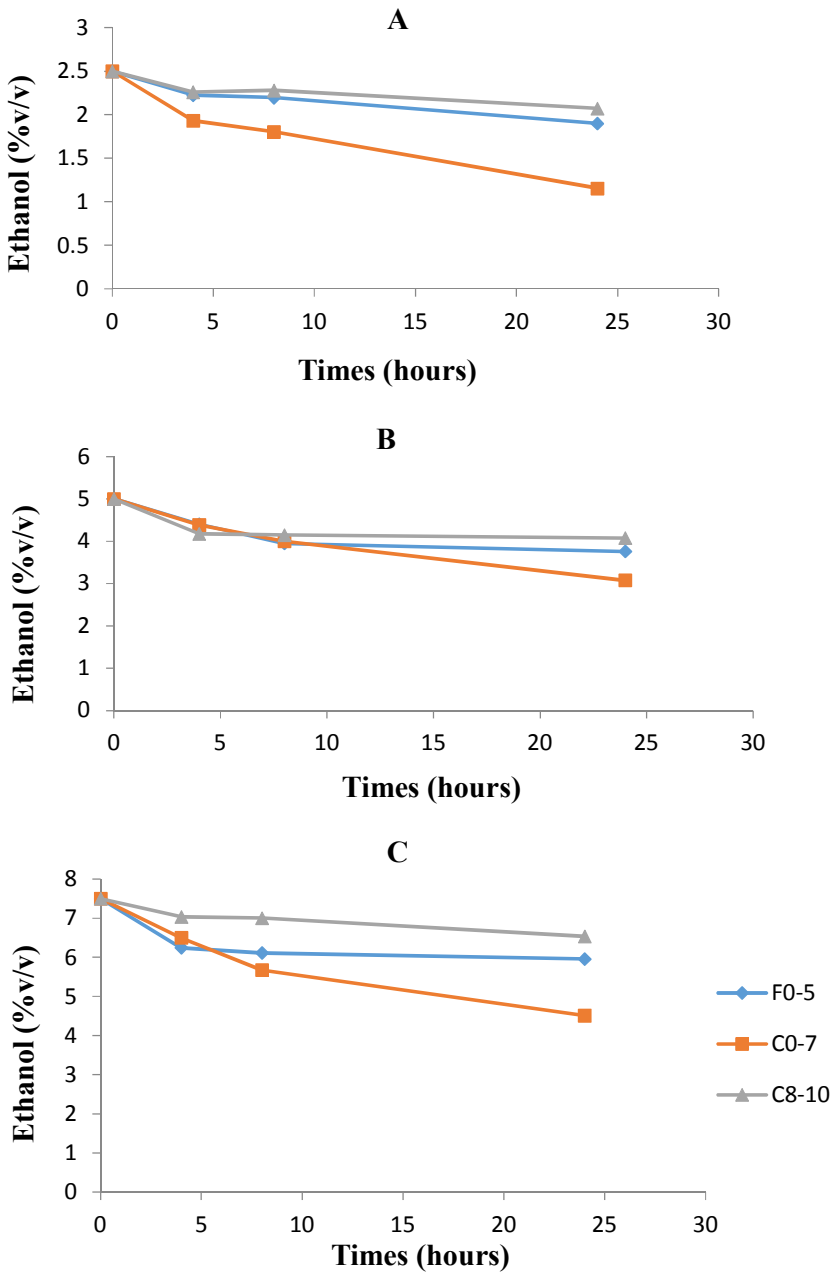

Fig. 6. Evolution of ethanol concentrations during culture of *C. tropicalis* strains in medium supplemented of 2.5% (A); 5% (B); 7.5% (C) initial rate ethanol

For strains of *Candida tropicalis*, the *TIF11* gene was 2.95 ± 0.12 times expressed in relation to the internal reference *TAF 10* for the F0-5 strain. For the 2 other strains of the same species the expression was substantially similar with 2.59 ± 0.09 and 2.58 ± 0.31 times that of *TAF 10* for C0-7 and C8-10 strains, respectively. The *YJL144W* gene was more expressed in the C8-10 strain with an expression 2.23 ± 0.70 times greater than the *TAF10* gene. For C0-7 and F0-5 strains, the expression levels of *TIFF11* and *YJL144W* are respectively 1.81 ± 0.06 and 1.78 ± 0.48 times higher than that of the *TAF 10* gene.

However, the statistical comparison of the relative expression levels using the Tukey test

revealed no difference between the strains for the 2 genes (P> 0.05).

For strains of *Saccharomyces cerevisaie*, the *TIF11* gene is more expressed in the D12-3 strain, whereas for the *YJL144W* gene the expression is greater in the strain E4-4. In strain F12-7, the *TIF11* and *YJL144W* genes were the least expressed.

The *TIF11* and *YJL144W* genes have been reported to be important in strengthening the ability of yeasts to resist water stress [14-15]. Our result was in line with those of Cordero-Otero et al. [14]. Indeed, these authors emphasized that among 12 genes tested only the genes *TIF11* and *RPL42*, were overexpressed in the strain of *Saccharomyces cerevisiae* BY4742. Moreover, these genes are essential in the

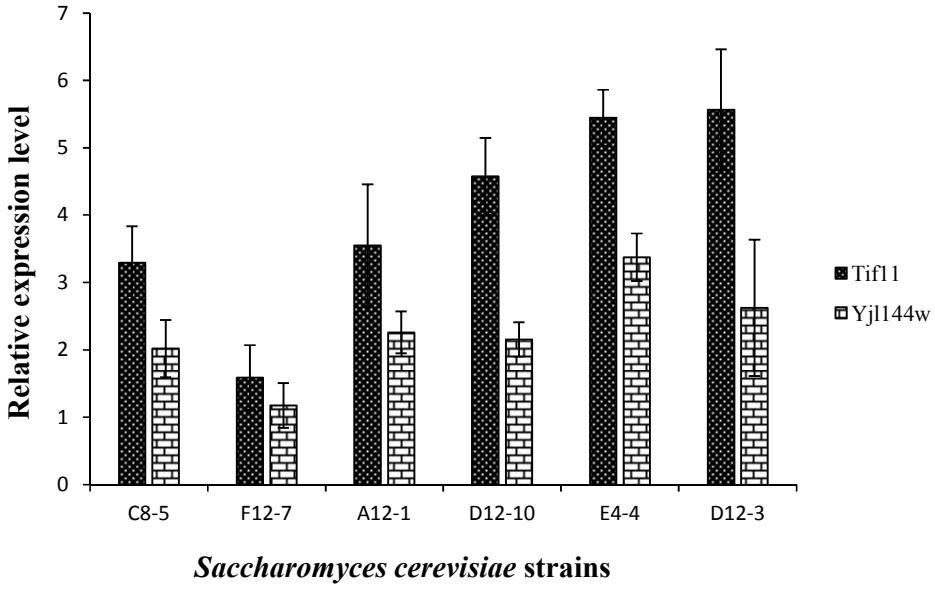

Fig. 7. Relative level expression of genes *TIF 11* and *YJL144W* in *S. cerevisiae* strain
Mean ± S.E.M = Mean values ± Standard error of means of three experiments

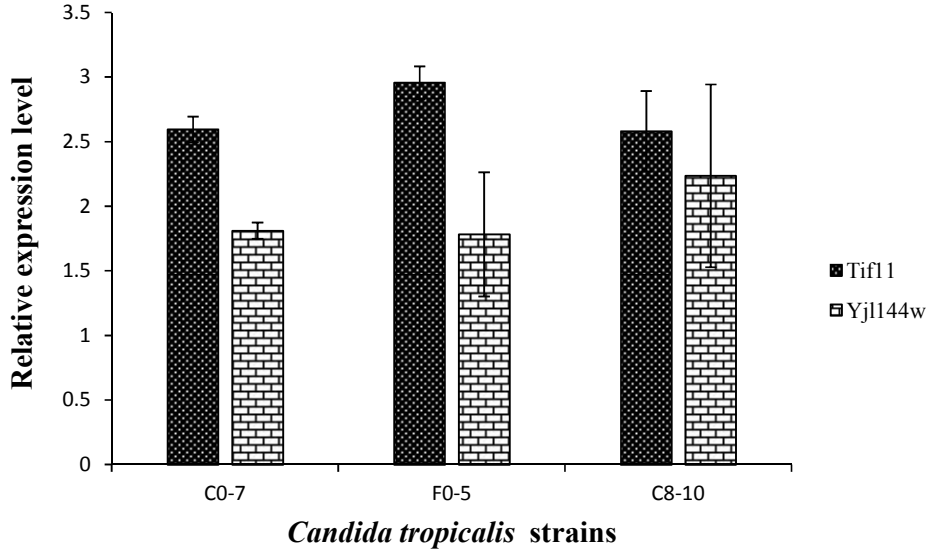

Fig. 8. Relative level expression of genes *TIF 11* and *YJL144W* in *C. tropicalis* strains
Mean ± S.E.M = Mean values ± Standard error of means of three experiments

coding of the corresponding proteins TIF11p and RPL42p involved in maintaining cell viability. The work of Dang and Hincha, [15] showed that *Saccharomyces cerevisiae* cells in which the *YJL144W* and *YMR175W* genes were overexpressed were more tolerant to desiccation; which confirms the role of the two corresponding hydrophilins of the yeast in stress tolerance due to dehydration. Although the functional role of most hydrophilins has remained speculative, there was evidence to support their participation in acclimatization or adaptive response [43]. The ectopic expression of certain yeast hydrophilins confers tolerance to water deficiency conditions [44-46]. Also, as well as the genes have been expressed, the hydrophilins may not translated. However, the hydrophilin assay could give us more informations about their functions in yeasts.

Despite the low gene expression of F12-7, this strain could be used for the starter culture freeze-drying production through ethanol stress tolerance. This deficiency could be improved by adding the protectives agents against dehydration. Face to the economic level low of Africa countries, it will be appropriate to use the protectives agents with the lowest cost and easily affordable by traditional sorghum beer. Also, the genes overexpression *TIF 11* and *YJL144W* for *Saccharomyces cerevisiae* F12-7 could be a solution to resist for freeze-drying stress. For *Candida tropicalis* strains, the statistical analyzes of the relative expression levels from the Tukey test revealed no difference between the strains for the 2 genes (*P> 0.05*). So, among *Candida tropicalis* strains, C0-7 seemed the best choice to be use for the freeze-drying starter culture production.

4. CONCLUSION

Our study was focused on the potentialities of yeasts strains to be used for freeze-drying starter production for traditional sorghum beer. Two criteria have been studied: the tolerance to ethanol stress and gene expression level encoding the hydrophilins. Among all strains were tested to ethanol stress, only *Saccharomyces cerevisiae* F12-7 and *Candida tropicalis* C0-7 have the viability rate high. Gene expression revealed that for the strain F12-7, the *TIF11* and *YJL144W* genes were the least expressed. This deficiency could be enhanced by adding the protectives agents against dehydration. For the *Candida tropicalis* strains, no significant difference we found between the strains for the 2 genes. In sum, *Saccharomyces cerevisiae* F12-7 and *Candida tropicalis* C0-7 could be used to produce the freeze-drying starter for traditional sorghum beer. The tolerance to ethanol stress remains a fundamental criterion in the strains selection for the freeze-drying starter production for the traditional sorghum beer. However, before freeze-drying starter production other investigations are needed in order to evaluate yeast performance. Also, the pathogenicity of *Candida tropicalis* strains will be studied for the using potentially non-pathogen strains.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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