Biotechnology Research and Innovation (2018) 2, 22–29

Biotechnology Research & Innovation

http://www.journals.elsevier.com/biotechnology-research-and-innovation/

RESEARCH PAPER

Identification of three robust and efficient *Saccharomyces cerevisiae* strains isolated from Brazilian's cachaça distilleries

Raquel de Sousa Paredes[a], **Igor Patrick Vasconcelos Vieira**[b], **Vinícius Mattos de Mello**[c], **Leonardo de Figueiredo Vilela**[d], **Rosane Freitas Schwan**[d], **Elis Cristina Araújo Eleutherio**[a,*]

[a] *Federal University of Rio de Janeiro (UFRJ), Biochemistry Department, Rio de Janeiro, RJ, Brazil*
[b] *University of Brasília (UnB), Department of Cellular Biology, Brasília, DF, Brazil*
[c] *Federal University of Rio de Janeiro (UFRJ), Catalysis Group (NUCAT-COPPE), Rio de Janeiro, Brazil*
[d] *Federal University of Lavras (UFLA), Biology Department, Minas Gerais, Brazil*

Received 23 October 2017; accepted 16 July 2018
Available online 30 July 2018

KEYWORDS
Saccharomyces cerevisiae;
Glucose fermentation;
Ethanol production;
Cells recycle;
Industrial fermentation

Abstract Three isolated strains from Brazilian's cachaça distilleries were tested for glucose consumption and ethanol production during the fermentation process. The ethanol productivity, cell viability and mitochondrial mutagenic rate of each strain was evaluated at the end of a 24 h-fermentation round. The strains' resistance to the fermentation process was evaluated after cell recycling followed by another round of fermentation. Among the isolated industrial strains evaluated, the strains CCA083 and CA751 has shown the best performance in terms of productivity and yield. The cachaça strain CCA083 was able to keep a high glucose consumption and ethanol productivity during the second fermentation round. This result suggests that the stress response mechanism is activated due to the heat shock pretreatment, which creates a protective effect on the cell. Therefore, these results could bring up to light a new framework for industries and researchers in order to develop strains with increased stress tolerance on first and second-generation ethanol production.

Introduction

The search for alternative renewable energy sources to replace the use of fossil fuels has become a worldwide priority. This is related with the global climate changes caused by the buildup of greenhouse gases associated with the population growth and the increasing per capita fuel consumption (Virmond, Rocha, Moreira, & José, 2013). In this context, the leading candidate to replace gasoline is ethanol due to its reduced CO_2 release after combustion (Lopes et al., 2016). Brazil is the second largest ethanol producer in the world, using sugar cane juice and residual molasses from sugar refineries (1G fuel ethanol) (Basso, Basso, & Rocha, 2011). The ethanol production in Brazil

* Corresponding author.
E-mail: eliscael@iq.ufrj.br (E.C. Eleutherio).

https://doi.org/10.1016/j.biori.2018.07.001
2452-0721/© 2018 Sociedade Brasileira de Biotecnologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

is carried out in three stages: obtaining the fermentable sugars (sugar cane pressing), sugar fermentation by microorganisms and ethanol purification (Amorim, Lopes, Oliveira, Buckeridge, & Goldman, 2011). For fuel usage, the ethanol is distilled a second to dehydrate (Mussatto et al., 2010). In this process, the glucose and other six-carbon sugars, such as fructose, present in the processed sugar cane juice are completely metabolized into ethanol by the yeast *Saccharomyces cerevisiae* (Hahn-Hägerdal, Karhumaa, Fonseca, Spencer-Martins, & Gorwa-Grauslund, 2007).

In order to achieve higher levels of ethanol, some distilleries recycle yeast cells from fermentation multiple times. Though, the cells are pretreated before every fermentation step. The pretreatment consists on washing the yeast biomass with sulfuric acid decreasing the pH up to 1.0 (Fischer, Klein-Marcuschamer, & Stephanopoulos, 2008). This step considerably reduces bacterial contamination and reduces the cost associated with inoculums preparation (Basso et al., 2011; Della-Bianca and Gombert, 2013; Pereira, Gomes, Guimarães, Teixeira, & Domingues, 2012). Besides acid pretreatment itself, the alcoholic fermentation industrial process includes some stressful conditions such as osmotic pressure, high levels of ethanol, high temperature and low pH (Dorta, de Oliva-Neto, de Abreu-Neto, Nicolau-Junior, & Nagashima, 2006; Ivorra, Perrez-ortin, & Olmo, 1999). These agents can affect cell metabolism at different levels. The ethanol produced during fermentation can change the cellular membrane polarity and at high concentrations is able to reduce the biomass multiplication and viability (Lynd, Ahn, Hill, & Klapatch, 1991). Additionally, temperature variations are frequent due to the exothermic cell metabolism, the high volume reservoirs with inefficient cooling systems and dissolved CO_2 build up. For instance, in the hottest areas of Brazil, the temperature can go up to 40 °C inside the bioreactors (Basso et al., 2011). These stress factors exert a continuous selective pressure on yeast over the course of each fermentation cycle, allowing only the most resistant strains to survive the 8-month sugarcane crop season in Brazil (Basso, Amorim, de Oliveira, & Lopes, 2008). The cell survival to such stressful conditions depends on its ability to quickly adapt to environmental changes. Thus, it is of great interest to evaluate *S. cerevisiae* wild type strains already in use by distilleries since these cells were selected throughout the years. Therefore, choosing the best strain is not only correlated with the alcohol production efficiency but also with the stress resistance (Da Silva, Batistote, & Cereda, 2013).

Herein, the strategy of this work was to characterize the resistance and ethanol productivity of three strains isolated from a Brazilian hard liquor (cachaça) distillery. Therefore, ethanol productivity, cell viability and mitochondrial mutagenic rate of each strain were evaluated at the end of two 24 h-fermentation cycle. *S. cerevisiae* industrial strains that demonstrated a good performance on alcoholic fermentation, followed by recycle, were selected and tested for thermal stress resistance simulating industrial fermentation conditions.

Materials and methods

Yeast strains

Three distillery wild type strains UFLA CCA035, UFLA CCA083, UFLA CA751 obtained from Dr. Rosane F. Schwan (Department of Biology, Federal University of Lavras, Minas Gerais, Brazil), two reference industrial strains PE-2 (3) and CAT-1 (Fermentec, Brazil), the baker's yeast (Fleischmann®, USA) and laboratorial strain BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ) were used in this work (Table 1).

Media and growth conditions

All strains were kept on solid YPD medium (10 g/L yeast extract, 20 g/L glucose, 20 g/L peptone and 20 g/L agar) as stock. Before every experiment, cells were grown up to mid exponential phase (0.8 mg dry weight/mL) in liquid YPD medium (10 g/L yeast extract, 20 g/L glucose, 20 g/L peptone) at 28 °C and 160 rpm, with a flask volume/medium ratio of 5:1.

Fermentation conditions

Fermentation process was carried out in Erlenmeyer 125 mL flasks filled with 50 mL of fermentation medium containing 50 g/L of glucose, 4.0 g/L $(NH_4)_2SO_4$ and 4.0 g/L KH_2PO_4 and the pH was adjusted to 5.0. Initial cell concentration was set to 1.5 mg/mL (dry weight) and cells were incubated at 30 °C and 90-rpm agitation for 24 h. 1 mL samples were taken at 0 h and 24 h of fermentation. An aliquot was diluted and optical density (OD_{600}) was determined. The remaining volume was centrifuged at 4500 × *g* for 5 min. Supernatant was collected and used for the determination of glucose consumption and ethanol production. Cells in the

Table 1 Yeasts strains and origins used in this work.

Strain	Group	Precedence	Reference
BY4741	Laboratory	Dr. E.C.A.	EUROSCARF (Germany)
Fleischmann	Industrial (Baking)	Eleutherio	Fleischmann® (USA)
CAT-1	Industrial (Fuel		Fermentec Ltda (Brazil)
PE-2	Ethanol)		Basso et al. (2008)
UFLA CCA035	Industrial	Dr. R.F. Schwan	Culture Collection of Agricultural Microbiology (CCMA, Brazil)[a]
UFLA CCA083	(Cachaça		Culture Collection of Agricultural Microbiology (CCMA, Brazil)[a]
UFLA CA751	Distillery)		Culture Collection of Agricultural Microbiology (CCMA, Brazil)[a]

[a] Culture Collection of Agricultural Microbiology of the Federal University of Lavras, Minas Gerais, Brazil. (http://www.wfcc.info/ccinfo/index.php/collection/by_id/1083/).

fermentation flask were washed twice with ice-cold deionized water and re-inoculated in fresh fermentation medium (50 g/L of glucose, 4.0 g/L $(NH_4)_2SO_4$ and 4.0 g/L KH_2PO_4) for a second fermentation round. After cell recycling, the fermentation medium was centrifuged and the supernatant was collected for glucose and ethanol assessment.

Cell viability and mitochondrion mutagenic rate

Viability is defined as the ability of a single organism to reproduce and form a colony (colony forming units or CFU) within approximately 72 h (Baron, Laws, Chen, & Culotta, 2013). To determine the yeast viability, 1×10^6 cells were collected and washed twice with sterile distilled water. Then, cells were serially diluted down to 1×10^3 cells/mL and a 100 μL aliquot was plated in agar-YPD medium. The plates were incubated at 28 °C for 72 h and the colonies were counted. Cell growth was also analyzed in agar-YPGly medium (40 g/L glycerol, 10 g/L yeast extract, 20 g/L peptone) to test strains for petite formation (Adamis, Panek, & Eleutherio, 2007; Mannarino et al., 2008). Petite formation is measured as the percentage of colonies on YPGly in relation to YPD plates, according to the following formula:

$$\text{Viability (\%)} = \frac{\text{number of cells after fermentation}}{\text{number of cells before fermentation}} \times 100$$

$$\text{Petite yeast cells (\%)} = \frac{\text{number of cells after fermentation on glycerol medium}}{\text{number of cells after fermentation on glucose medium}} \times 100$$

$$\text{Non-petite yeast cells (\%)} = (\text{total viable cells} - \text{petite yeast cells}) \times 100$$

Heat stress and fermentation conditions

To verify the industrial yeast robustness after 24 h of fermentation process the CCA083 and CA751 cells were incubated before the fermentation at 51 °C for 8 min under agitation (160 rpm). Immediately after heat treatment, cells were collected by centrifugation ($4800 \times g$ for 5 min at room temperature), washed with sterile distilled water and transferred to fermentation medium. The fermentation process was carried out at 35 °C, 90 rpm, for 24 h. The cell viability and petite formation was analyzed in the end of fermentation and recycle process.

Glucose assessment

Glucose consumption was analyzed using a high performance liquid chromatography (HPLC) system equipped with a refractive index detector (RID-10A Shimadzu Corporation, Kyoto, Japan) and a LiChrospher® 5 μm NH_2 100 Å column (250 × 4 mm, Merck Millipore, Darmstadt, Germany). Mobile phase contains acetronitrile/water 4:1 (v/v) ratio and the flow rate was set to 1.0 mL/min with the column temperature adjusted to 40 °C.

Ethanol assessment

After each fermentation cycle, cells were removed from media by centrifugation ($4800 \times g$ for 5 min at room temperature). The ethanol in the supernatant was separated by distillation at 100 °C and collected by condensation. Ethanol concentration was measured by dichromate oxidation method (Seo et al., 2009).

Statistical analysis

All assays were performed in two independent replicates. Results were analyzed using GraphPad Prism 6 multiple t tests and two-way ANOVA with a 95% confidence level. Significance values are represented on each figure legend. All data was considered statistically significant between each strain and tested conditions.

Results

Fermentation performance

Three cachaça distillery strains were tested for glucose consumption and ethanol production under fermentation conditions. After the first 24 h of fermentation, the cachaça strains CCA083 and CA751 had a similar glucose consumption compared to the well-known industrial strains PE-2 and CAT-1. However, the strain CCA035 glucose consumption was lower when compared to the other two cachaça strains, reaching the same percentage as laboratorial strain BY4741 and industrial baker's yeast strain (Fig. 1A). The ethanol production of the strains CCA083, CAT-1 and PE-2 reached the highest levels. On the other hand, the strains CCA035 and CA751 had a lower ethanol production, as shown in Fig. 1B. All cachaça strains presented high ethanol productivity during the first fermentation, reaching 0.50 g L^{-1} h^{-1} for CCA035, 0.60 g L^{-1} h^{-1} for CCA083 and 0.48 g L^{-1} h^{-1} for CA751 (Table 2). In contrast, BY4741 strain and baker's yeast achieved an ethanol productivity of 0.29 g L^{-1} h^{-1} and 0.38 g L^{-1} h^{-1}, respectively. The lower performance of BY4741 and the baker's yeast was expected, since those strains were not selected for their ethanol productivity traits. The yielding of cachaça strains were comparable to the results obtained from PE-2 and CAT-1 strains, where CCA0883 reached 45% and CA751 reached 41% (Fig. 1C). Nonetheless, the laboratorial strain BY4741 could not reach yields higher than 40% of the theoretical maximum and baker's yeast reached 41% of yield during fermentation process. These results show that the ethanol industrial strains are naturally adapted to the fermentation process after years of selective pressure. Although the baker's yeast is also an industrial strain, it was not able of keeping high ethanol productivities on the first fermentation round. During the second fermentation process, after cell recycle, CCA083 and CA751 strains had a higher productivity, achieving 0.25 g L^{-1} h^{-1}, when compared with laboratorial

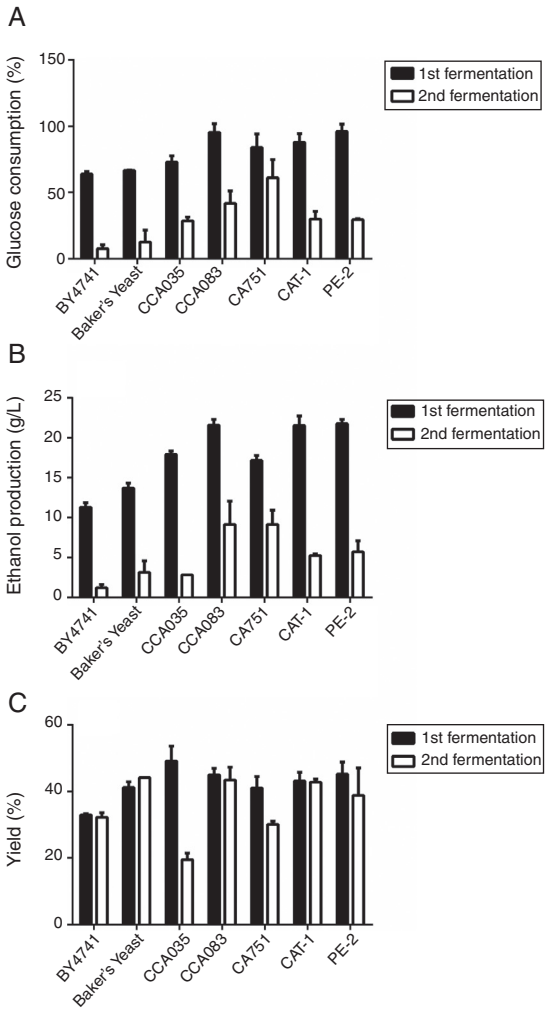

Figure 1 Fermentation performance of three cachaça distillery strains and four common industrial and laboratorial strains. Glucose consumption (A), ethanol production (B) and fermentation yield (C) after two fermentation rounds. Black bars – first fermentation; white bars – second fermentation (cells recycle). *p*-Value <0.0136.

strain BY4741 and baker's yeast, reaching, respectively, 0.03 g L^{-1} h^{-1} and 0.09 g L^{-1} h^{-1}. The CCA035 strain obtained the lowest productivity (0.08 g L^{-1} h^{-1}) when compared with CCA083 and CA751. When comparing the fermentation yield among the cachaça strains, it was possible to identify that the strain CCA083 presented the best fermentation yield reaching 43% of the theoretical maximum during the second fermentation process. The CCA035 and CA751 strains presented, respectively, 20% and 30% of yield during the second fermentation process. It is believed that this phenomenon is due to accumulated stress over the process. Therefore, it is necessary to investigate the physiological responses to stress and its correlation with ethanol production.

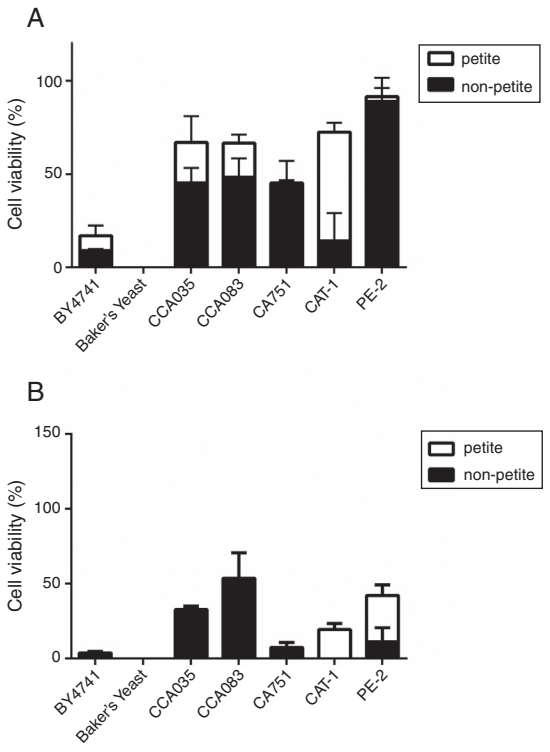

Figure 2 Cell viability of three cachaça strains and four industrial and laboratorial strains after two fermentation rounds. (A) Ratio of petite and non-petite formation after 24 h of fermentation. (B) Ratio of petite and non-petite formation after cell recycle. Black bars – non-petite; white bars – petite. *p*-Value <0.0001.

Ethanol performance and accumulated oxidative stress damage

One way to evaluate oxidative stress damage is to determine mitochondrion mutagenesis (Adamis et al., 2007; Mannarino et al., 2008). The percentage of non-petite and petite cells among viable cells was obtained by plating the yeast in both glucose and glycerol solid media. After the first fermentation (Fig. 2A), only the industrial strain PE-2 showed high viability (91% viable cells being 89% non-petite cells), while CAT-1 showed 72% viable cells but only 14% non-petite. On the other hand, CCA035, CCA083 and CA751 has shown a viability between 44–67% while approximately 45% of the cells were non-petite (Table 3). The baker's yeast strain did not show a viability profile even though it was able to consume glucose and produce ethanol. This result could be related to a lower resistance to the fermentation process stress. During the second fermentation process, the CCA083, CCA035 and CA751 strains showed, respectively, 53%, 32% and 7% of viability containing only non-petite cells. It is noteworthy that the isolated cachaça strains have lower levels of accumulated stress during the first fermentation (Fig. 2B). Based on these results, the CCA083 and CA751 strains were chosen to be tested for heat shock pretreatment. Cells were prior incubated at 51 °C for 8 min in similar conditions to industrial process. Then, fermentation performance was evaluated after 24 h. The results of

Table 2 Summary table of results presented in Fig. 1. Fermentation yield rate, specific glucose consumption and productivity of three distillery strains, typical industrial and laboratorial strains after 24 h of fermentation process.[a]

	Strain	Glucose consumption (g/L)	Ethanol production (g/L)	Productivity ($g L^{-1} h^{-1}$)	Yield (%)
1st fermentation	BY4741	32 ± 1.00	11 ± 0.60	0.29 ± 0.02	33 ± 2.80
	Baker's yeast	33 ± 0.07	14 ± 0.61	0.38 ± 0.02	41 ± 1.75
	CAT-1	44 ± 0.86	21 ± 1.20	0.87 ± 0.03	43 ± 2.56
	PE-2	48 ± 2.94	22 ± 1.56	0.91 ± 0.02	45 ± 3.56
	CCA035	37 ± 2.40	18 ± 0.40	0.50 ± 0.01	49 ± 4.40
	CCA083	48 ± 3.70	22 ± 0.70	0.60 ± 0.02	45 ± 2.00
	CA751	42 ± 5.10	17 ± 0.60	0.48 ± 0.02	41 ± 3.50
2nd fermentation	BY4741	4 ± 1.5	1 ± 0.4	0.03 ± 0.01	32.3 ± 1.5
	Baker's yeast	6 ± 1.50	3 ± 0.40	0.09 ± 0.004	44 ± 0.1
	CAT-1	15 ± 2.97	5 ± 1.25	0.25 ± 0.02	43 ± 0.92
	PE-2	15 ± 0.42	6 ± 1.78	0.29 ± 0.02	32 ± 0.50
	CCA035	14 ± 2.40	3 ± 0.60	0.08 ± 0.001	20 ± 1.90
	CCA083	21 ± 4.80	9 ± 2.90	0.25 ± 0.08	43 ± 3.90
	CA751	31 ± 6.90	9 ± 1.80	0.25 ± 0.05	30 ± 1.00

[a] Average values from duplicate experiments ± standard deviation.

Table 3 Summary table of results presented in Fig. 2. Cell viability comparison of three distillery strains, typical industrial and laboratorial strains after 24 h of fermentation.[a]

	Strains	Viability (%)	Non-petite (%)	Petite (%)
1st fermentation	BY4741	17 ± 6	9 ± 1	8 ± 5
	Baker's yeast	–	–	–
	CAT-1	73 ± 23	14 ± 5	59 ± 23
	PE-2	91 ± 15	89 ± 20	2 ± 1
	CA035	70 ± 7	45 ± 8	15 ± 5
	CCA083	66 ± 10	48 ± 10	18 ± 5
	CA751	44 ± 12	44 ± 13	–
2nd fermentation	BY4741	4 ± 1	3 ± 0.6	1 ± 0.9
	Baker's yeast	–	–	–
	CAT-1	19 ± 4	–	19 ± 4
	PE-2	42 ± 18	11 ± 1	31 ± 12
	CCA035	33 ± 2	33 ± 2	–
	CCA083	54 ± 17	54 ± 3.1	–
	CA751	7 ± 3	7 ± 3	–

[a] Average values from duplicate experiments ± standard deviation.

Table 4 Summary table of results presented in Fig. 3. Fermentation yield rate, specific glucose consumption and productivity after 24 h of fermentation process.[a]

	Strains	Glucose consumption (g/L)	Ethanol production (g/L)	Productivity ($g L^{-1} h^{-1}$)	Yield (%)
1st fermentation	CCA083	33.6 ± 3.14	12.7 ± 1.25	0.35 ± 0.03	38 ± 0.18
	CA751	43.7 ± 1.06	16.7 ± 0.15	0.46 ± 0.004	38 ± 0.69
2nd fermentation	CCA083	32.6 ± 4.54	13.7 ± 2.61	0.38 ± 0.07	42 ± 2.16
	CA751	12.3 ± 1.45	5.3 ± 0.33	0.15 ± 0.01	43 ± 2.41

[a] Average values from duplicate experiments ± standard deviation.

glucose consumption, ethanol production, ethanol productivity and yield are presented in Table 4.

It is noteworthy that CA751 strain showed higher glucose consumption and ethanol production during the first fermentation, reaching 0.46 $g L^{-1} h^{-1}$ of productivity when compared with CCA083 strain obtaining 0.35 $g L^{-1} h^{-1}$ of productivity (Fig. 3A). Both strains were able to achieve a yield of 38% on the first fermentation. In contrast, after the heat

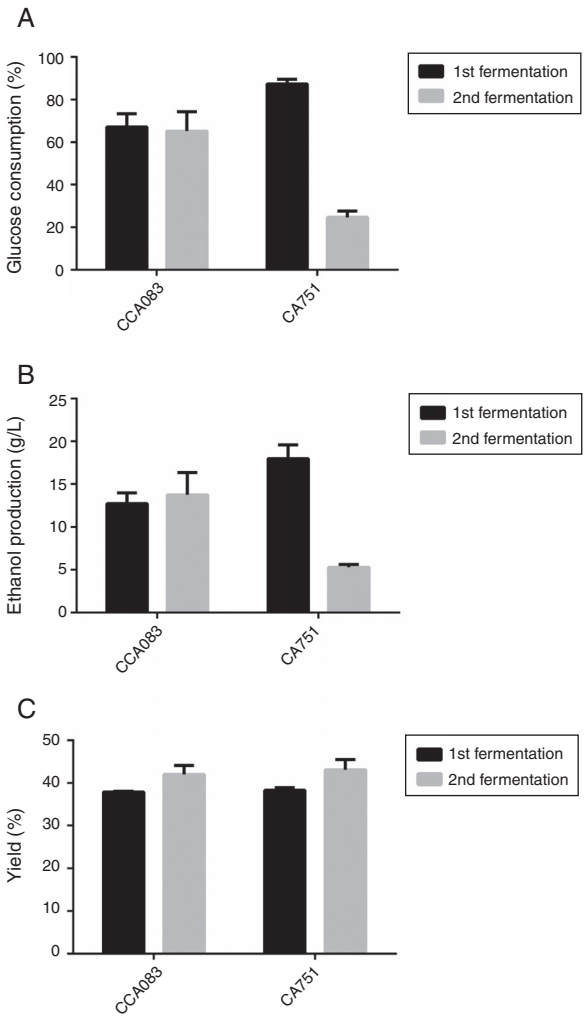

Figure 3 Fermentation performance of CCA083 and CA751 cachaça strains after a heat shock pretreatment prior to cell recycle. Glucose consumption (A), ethanol production (B) and fermentation yield (C) of two distillery strains after a heat 24 h of fermentation process. Black bars – first fermentation; white bars – second fermentation (cells recycle). *p*-Value <0.001.

shock pretreatment followed by a second fermentation process, the CA751 strain consumed less glucose but had the same yield (Fig. 3B), with a productivity of 0.15 g L^{-1} h^{-1}. The CCA083 strain showed a similar fermentative behavior of its first fermentation in response to a stress condition after heat shock treatment, achieving 0.38 g L^{-1} h^{-1} of productivity. Even though the fermentation yield has increased for both strains, the glucose uptake followed by ethanol production by the CCA083 strain was more relevant than the CA751 strain (Fig. 3C). This result may indicate that among the cachaça distillery strains used in this work, the one that stands out is CCA083 strain.

Stress accumulation was evaluated by assessing mitochondrion mutagenesis. Both CCA083 and CA751 strains showed high viability profiles during the first fermentation, reaching 86% and 75% respectively, with no petite formation (Fig. 4A). That leads us to believe that these strains accumulated a very low level of damage, which was not enough to stop cell division under growing conditions.

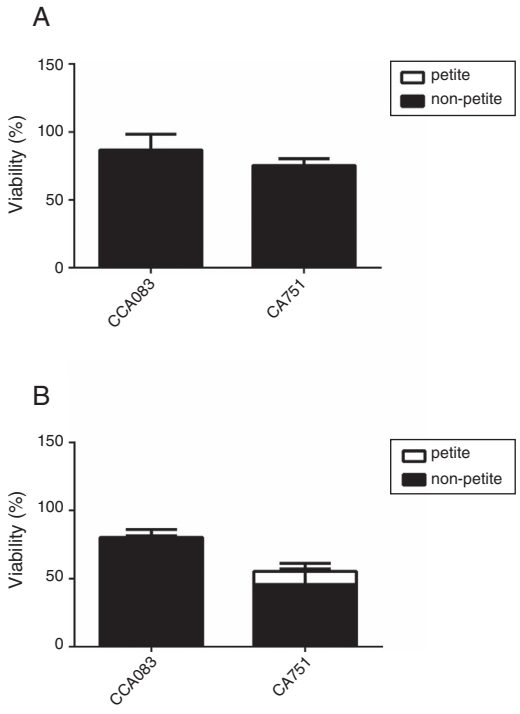

Figure 4 CCA083 and CA751 cells viability after 24 h of fermentation process. (A) Ratio of petite and non-petite formation after 24 h of fermentation. (B) Ratio of petite and non-petite formation after cells recycle. Black bars – non-petite; white bars – petite. *p*-Value <0.0001.

Nevertheless, on the second fermentation, both strains showed a decrease on viability (Fig. 4B). Nonetheless, CA751 has shown to be more sensitive, presenting 55% viable cells being 10% petite cells (Table 5).

Discussion

We demonstrate that ethanol productivity and yield are not the only aspects to be analyzed when it comes to improving fermentative *S. cerevisiae* strains. There is a substantial difference between laboratorial and industrial strains when it comes to the process robustness. This is tightly related to their ability to handle oxidative stress throughout the fermentation cycles. Under industrial conditions, the yeast cells are often reused and submitted to serial fermentations, and this could contribute to cell aging and mitochondrial mutation, which reduces fermentative capacity (Gibson, Prescott, & Smart, 2008).

It is remarkable that CA751 and CCA083 strains were capable of keeping more than 50% viable cells and maintained a low level of mitochondrion mutagenesis over the second fermentation process. The mechanisms behind this phenomenon are not yet fully known. However, it is acknowledged that ethanol replaces surface water on membranes and proteins, causing protein denaturation and loss of membrane fluidity, which ultimately leads to membrane leakage (Barry and Gawrisch, 1995). Furthermore, ethanol also causes an oxidative stress increase due to an overproduction of reactive oxygen species (Landolfo, Politi, Angelozzi, & Mannazzu, 2008) or inactivation of antioxidant

Table 5 Summary table of results presented in Fig. 4. CCA083 and CA751 cells viability after 24 h of fermentation process.[a]

	Strains	Viability (%)	Non-petite (%)	Petite (%)
1st fermentation	CCA083	87 ± 12	87 ± 12	–
	CA751	75 ± 5	75 ± 5	–
2nd fermentation	CCA083	80 ± 6	80 ± 6	–
	CA751	55 ± 7	45 ± 0.23	10 ± 1.8

[a] Average values from duplicate experiments ± standard deviation.

enzymes (Santiard, Ribiere, Nordmann, & Houee-Levin, 1995). Based on this, it could be concluded that the first adaptation in presence of ethanol and the heat shock stress may cause a set of metabolic changes related to the acquisition of tolerance against many severe stress conditions.

Currently, the searches for alternative strategies to increase ethanol production have been strongly studied. However, environmental factors that affect stress tolerance and performance are often neglected. High ethanol concentration, high temperature, acidity and increased osmolarity (due to high concentrations of sugar and salts) are some stress conditions faced by yeasts during the industrial processes, some of them acting synergistically (Basso et al., 2008). Therefore, the severe treatment is used to activate the adaptive metabolism response to heat stress increasing the heat shock proteins (hsp) expression and the accumulation of protective molecules, such as trehalose. Previous studies have demonstrated that yeast enzymes, such as pyrophosphatases, are protected by the presence of trehalose (Kim, Moon, Yun, & Jin, 2006). The loss of cytoplasm water can occur in yeasts exposed to osmotic shock and several mechanisms need to be activated in order to protect the cell from dehydration (Estruch, 2000). Walker (1998) reported that under osmotic stress conditions, glycerol was the most effective regulator of osmosis present in *S. cerevisiae* whilst trehalose was the most efficient carbohydrate for plasmatic membrane stabilization. Thus, yeast cell survival during the alcoholic fermentation depends on its ability to quickly adapt to environmental changes.

Because of the potential offered by the highly adaptable metabolism of *S. cerevisiae*, the selection of wild yeasts as fermentation agents could enable the increase on efficiency of alcohol production. Accumulation of respiratory deficient cells, named petites, can have detrimental effects on fermentation performance, such as reduced fermentation rates and loss of flocculation (Gibson et al., 2008; Good, Dowhanick, Ernandes, Russell, & Steawart, 1993). Then, it is important to note that an acute stress (heat shock) prior to fermentation is capable of activating stress response mechanisms that enhances the yeasts' resistance and increases its viability after the second fermentation. The cachaça strains analyzed in this work have shown a striking performance in fermentation conditions. Although their isolation niche is quite different, the selective pressure exerted on these strains have led to similar phenotypes that are relevant to the ethanol production.

On the other hand, the isolated strains can keep a higher productivity compared to CAT-1 and PE-2 strains after 24 h of fermentation. Moreover, the cachaça strains have a lower accumulated cell damage compared to the controls. That, indicates that these cells are far less sensitive to the stresses during the fermentation cycles. With that in mind, the goal of this work was to demonstrate that production, productivity and yield should not be the only properties analyzed for improvements of current strains to be used in industries. It is noteworthy that Basso et al. (2008) has karyotyped the strains during the fermentation cycle and have concluded that inoculated strains are not capable of keeping its population throughout the subsequent fermentation cycles. That is due to its inability to survive the stresses and compete with other contaminant microorganism strains. By keeping its high density, a certain strain can consume more sugars and it is expected that this would increase the productivity by keeping the contaminant microorganisms on a low density throughout the fermentation cycles.

Despite the low sugar concentration compared to the concentrations in sugarcane juice and/or molasses, the industrial strains used in this work have reached high ethanol production levels. It is important to emphasize that even though not all glucose was consumed, CCA083 was still able to convert all sugars absorbed into ethanol. It is believed that the inability to metabolize more sugar was due to the high ethanol concentrations. Considering that recycle is a usual practice in the industries, the residual glucose could be used for a next round of fermentation given more substrate. Besides, further improvement of these strains (e.g. adaptive metabolic engineering) can be done in order to increase their resistance to inhibitors from lignocellulosic biomass pretreatment and ethanol (Jönsson & Martín, 2016).

In this context, the cachaça strains used in this work, CCA083 and CA751, could be an experimental platform for future genetic modifications for application in the ethanol fermentation process and potentially to the lignocellulosic biomass fermentation. This could dramatically decrease the cost of inoculums preparation for fermentation at industries and consequently enabling a faster application of the technology. Among the three cachaça strains evaluated, the strain CCA083 has shown the best performance in terms of yield and productivity. These results could bring up to light a new framework for industries and create new possibilities for researchers to increase the stress tolerance on first and second-generation ethanol production strains.

Funding

The Innovation and Research Funding (FINEP) of the Brazilian Ministry of Science and Technology and the Brazilian Research Council (CNPq) funded this study.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are thankful to Vinícius Mattos de Mello and Igor Patrick Vasconcelos Vieira for conducting the experiments of this paper.

References

Adamis, P. D., Panek, A. D., & Eleutherio, E. C. A. (2007). *Toxicology Letters, 173*, 1–7.

Amorim, H. V., Lopes, M. L., Oliveira, J. V. C., Buckeridge, M. S., & Goldman, G. H. (2011). *Applied Microbiology and Biotechnology, 91*, 1267–1275.

Baron, J. A., Laws, K. M., Chen, J. S., & Culotta, V. C. (2013). *Journal of Biological Chemistry, 288*, 4557–4566.

Barry, J. A., & Gawrisch, K. (1995). *Biochemical Journal, 34*(27), 8852–8860.

Basso, L. C., Amorim, H. V., de Oliveira, A. J., & Lopes, M. L. (2008). *FEMS Yeast Research, 8*, 1155–1163.

Basso, L. C., Basso, T. O., & Rocha, S. N. (2011). In M. A. S. Bernardes (Ed.), *Biofuel production* (pp. 85–100). Rijeka: InTech.

Da Silva, R. O., Batistote, M., & Cereda, M. P. (2013). *Brazilian Archives of Biology and Technology, 56*(2), 161–169.

Della-Bianca, B. E., & Gombert, A. K. (2013). Stress tolerance and growth physiology of yeast strains from the Brazilian fuel ethanol industry. *Antonie Van Leeuwenhoek, 104*(6), 1083–1095.

Dorta, C., de Oliva-Neto, P., de Abreu-Neto, M. S., Nicolau-Junior, N., & Nagashima, A. I. (2006). *World Journal of Microbiology and Biotechnology, 22*(2), 177–182.

Estruch, F. (2000). *FEMS Microbiology Review, 24*(4), 469–486.

Fischer, C. R., Klein-Marcuschamer, D., & Stephanopoulos, G. (2008). *Metabolic Engineering, 10*, 295–304.

Gibson, B. R., Prescott, K. A., & Smart, K. A. (2008). *Letters in Applied Microbiology, 46*, 636–642.

Good, L., Dowhanick, T. M., Ernandes, J. E., Russell, I., & Steawart, G. G. (1993). *Journal of the American Society of Brewing Chemists, 51*, 35–39.

Hahn-Hägerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., & Gorwa-Grauslund, M. F. (2007). *Applied Biochemistry and Biotechnology, 74*, 937–953.

Ivorra, C., Perrez-ortin, J. E., & Olmo, M. (1999). *Biotechnology and Bioengineering, 64*, 698–708.

Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, *199*, 103–112. http://dx.doi.org/10.1016/j.biortech.2015.10.009

Kim, I. S., Moon, H. Y., Yun, H. S., & Jin, I. (2006). *Journal of Microbiology, 44*(5), 492–501.

Landolfo, S., Politi, H., Angelozzi, D., & Mannazzu, I. (2008). *Biochimica et Biophysica Acta, 1780*, 892–898.

Lopes, M. L., Paulillo, S. C., Godoy, A., Cherubin, R. A., Lorenzi, M. S., Giometti, F. H., et al. (2016). *Brazilian Journal of Microbiology, 47*(Suppl. 1), 64–76.

Lynd, L. R., Ahn, H. J., Hill, A. G. P., & Klapatch, T. (1991). *Biotechnology and Applied Biochemistry, 28*, 549–569.

Mannarino, S. C., Amorim, M. A., Pereira, M. D., Moradas-Ferreira, P., Panek, A. D., Costa, V., et al. (2008). *Mechanisms of Ageing and Development, 129*, 700–705.

Mussatto, S. I., Dragone, G., Guimarães, P. M. R., Silva, J. P. A., Cameiro, L. M., Roberto, I. C., et al. (2010). *Biotechnology Advances, 28*, 817–830.

Pereira, F. B., Gomes, D. G., Guimarães, P. M. R., Teixeira, J. A., & Domingues, L. (2012). *Biotechnology Letters, 34*, 45–53.

Santiard, D., Ribiere, C., Nordmann, R., & Houee-Levin, C. (1995). *Free Radical Biology and Medicine, 19*, 121–127.

Seo, H. B., Kim, H. J., Lee, O. K., Ha, J. H., Lee, H. Y., & Jung, K. H. (2009). *Journal of Industrial Microbiology and Biotechnology, 36*(2), 285–292.

Virmond, E., Rocha, J. D., Moreira, R. F. P. M., & José, H. J. (2013). *Journal of the Brazilian Chemical Society, 30*(2), 197–229.

Walker, G. M. (1998). *Yeast physiology and biotechnology*. pp. 203–264. Wiley.