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Potential evaluation of *Saccharomyces cerevisiae* strains from alcoholic fermentation of mango pulp

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The fermentation characteristics of different strains of *Saccharomyces cerevisiae* isolated from sugar cane musts for the production of cachaça (Brazilian sugar cane spirit) and from beer musts were analyzed based on the kinetic parameters of alcoholic fermentation in mango pulp. The commercial pressed baker's yeast was used as a standard inoculum. The results show that the yeast strains tested from sugar cane must and beer must had low fermentation performance when inoculated into mango pulp. They did not meet the selection criteria for fermentative cultures, such as productivity, efficiency, ethanol yield and ethanol production. It is observed that the commercial pressed baker's yeast showed greater adaptability in the mango must than the other yeasts.

Key words: Alcohol fermentation, industrial microbiology, mango, yeast.

INTRODUCTION

Fruit fermented beverages are promising products due to the tendency of their consumer acceptance as showed in researches, and to their contribution to the reduction in postharvest losses of perishable fruits (Sandhu and Joshi, 1995). The consumer market is becoming increasingly exigent about product quality, which places the food industry under pressure with regard to the adequacy and improvement of its products. The search for improvements becomes evident, for instance, in studies that aim to improve yeast strains in order to make the fermentation process more effective and productive. *Saccharomyces cerevisiae* used in the fermentation process to obtain alcoholic beverages must present some essential features, such as high yield, high ethanol tolerance, quick conduction of fermentation in order to prevent contamination by other micro-organisms, balanced production of secondary compounds and to be easily removed at the end of fermentation (Oliveira, 2001).

Oliveira et al. (2005) evaluated different yeast strains for the production of cachaça, a Brazilian sugar cane spirit. From their observations, it was found that there are significant differences between the fermentation potential among different strains of *S. cerevisiae* and that the ethanol yield was the most important factor in the differentiation of the strains. Brazil is the world's largest

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producer of fruits and the Northeast region is the country's biggest producer. The northern part of the state of Minas Gerais produced 41,951 tonnes of mangoes of the varieties Palmer and Tommy Atkins. However, several factors act negatively on the flow and efficient allocation of mangoes production and therefore postharvest losses represent a significant portion of this production. Various techniques are being developed and used in order to increase the postharvest life of the fruit as well as to allow its full use. Among these techniques is fermentation, which is a highly viable alternative for developing new products and adding value. It is an efficient and low-cost technology that has become one of the alternatives to the use of the fruits. It also represents a new branch for horticulture industry and a tool for the development of new beverages (Chitarra and Chitarra, 1990; Silva et al., 2007; Asquieri et al., 2008; Banco do Nordeste, 2008).

This research was carried out to evaluate and compare the potential in a laboratory-scale mango pulp fermentation of seven strains of *S. cerevisiae* (LC03, LC06, LC07, LC17, UFMG 1007, UFMG 1031 and UFMG 905) from sugar cane musts for the production of cachaça and from musts used in the production of beer, compared with *S. cerevisiae* cells from commercial pressed baker's yeast.

MATERIALS AND METHODS

Microrganisms and preservation

In the fermentation tests, eight strains of *S. cerevisiae* were used. Among these strains, one was isolated from commercial pressed baker's yeast. The other strains were isolated and identified by the Laboratory of Taxonomy, Biodiversity and Biotechnology of Fungi, Department of Microbiology, Institute of Biological Sciences, UFMG, and coded as LC03, LC06, LC07, LC017, UFMG 905, UFMG 1007 and UFMG 1031. Yeast cells of *S. cerevisiae* from commercial pressed baker's yeast were used as a standard, because they have already shown good fermentation results in fruit musts, such as banana (Lara, 2007; Alvarenga et al., 2011) and mango (Alvarenga et al., 2013) in previous studies conducted in the Laboratory of Industrial Microbiology and Biocatalysis (LAMIB), Department of Food, Faculty of Pharmacy, UFMG. All the yeast strains were maintained in GYMP Broth at -80°C covered with a thin layer of sterilized mineral oil in order to avoid air contact.

Inocula preparation

For the preparation and standardization of inocula, all of the strains were streaked on the surface of the culture medium yeast extract–malt extract - YM agar (malt extract 0.3%, yeast extract 0.3%, peptone 0.5%, 1.0% glucose, 2.0% agar) containing 0.02% of chloramphenicol in a Petri dish. After 48 h incubation at 30°C, the same cultures were resuspended in saline solution (0.85). To standardize the initial number of cells, the parameter used was inocula turbidity determined by reading absorbance at 600 nm with a spectrophotometer (Femto). The suspensions were prepared aseptically with colonies progressively diluted in saline solution until absorbance of 0.7, which corresponds to an inoculum of 10^8 cells per ml.

Must preparation

The mango pulp was enzimatically hidrolized using Pectinex Ultra SP (0,025%) with enzimatic activity of 4000 PG. The must was composed of a 1:1 mixture of sterile distilled water and pulp, adjusted to 18 ° Brix with a solution of commercial sucrose and initial pH value of 4.5. The must was pasteurized (65°C for 30 min).

Laboratory-scale fermentation

The tests were performed in triplicate in 250 ml Erlenmeyer flasks, containing 100 ml of must inoculated with 10 ml of the suspension with the concentration of yeast cells previously standardized. The flasks were incubated in orbital shaker at 30 ± 2°C for 24 h and 150 revolutions for minute. For the analyses, samples of the musts were taken immediately after inoculation and after fermentation. The samples were centrifuged at 1006 *g* for 15 min.

Analytical methods

The final product obtained from fermentation were analyzed for the determination of total reducing sugars (TRS) by the method described by Miller (1959); alcohol content (°GL) with the methodology described by Salik and Povoh (1993); total titratable acidity (TTA), density and pH according to the methodologies of Adolfo Lutz Institute (IAL, 2008).

Calculation of the kinetic parameters

In order to determine the kinetic parameters of fermentation with the use of different strains of the yeast *S. cerevisiae*, the following values were calculated: ethanol yield (%), yeast efficiency, productivity (g. L^{-1}. h^{-1}), the conversion factor of substrate into product ($Y_{p/S}$), rate of substrate consumption (g. h^{-1}) and rate of TRS conversion (%).

Ethanol yield

The ethanol yield was determined by the amount of ethanol formed in relation to the theoretical quantity, through the conversion of sugars present in the must. It obeys the stoichiometry in which the expected amount of ethanol is calculated considering that 1 g of TRS produces 0.511 g of ethanol, expressed in percentage.

Efficiency

The efficiency of the fermentation expresses the ethanol production in relation to the theoretical production according to the content of sugar determined in the must. It determines the efficiency of the conversion of fermentable sugars (TRS) into ethanol, expressed in percentage.

Ethanol productivity

The ethanol productivity expresses the mass of ethanol produced (g) volume (l) in the fermentation medium per unit of

time (h). It allows the determination of the rate of transformation of fermentable sugars (TRS) into ethanol.

Conversion factor of substrate into product ($Y_{p/S}$)

The conversion factor of substrate into product ($Y_{p/S}$) is the relationship between product formation and substrate consumption. It is based on the stoichiometry of the reaction in which 1 g of sugar will produce 0.511 g of ethanol.

Rate of substrate consumption

The rate of substrate consumption determines the rate at which the sugar is consumed by the yeast for the production of biomass and by-products such as ethanol. It is expressed in grams of sugar consumed per unit of time (g. h^{-1}).

Rate of substrate conversion

The rate of TRS conversion determines the amount of reducing sugars consumed in relation to the total of available sugars in the fermentation medium. It is expressed in percentage (%).

Data analysis

The data were statistically analyzed using the Analysis of Variance (ANOVA) followed by the Tukey Multiple Comparison test ($p < 0.05$) using the software SPSS Statistic 19®.

RESULTS AND DISCUSSION

The Table 1 presents the mean of the kinetic and physic-chemical parameters obtained for the fermentation tests. Table 2 presents data comparing three yeasts used in this study with data from the fermentation of banana pulp. The fermentation tests with different strains of *S. cerevisiae* showed low values for the parameters analyzed, except the one from the commercial pressed baker's yeast. The parameter efficiency was not statistically different from the commercial yeast for the strains LC03, LC06, LC07 and LC17. Once the yeasts were not able to fully consume the fermentable sugars by converting them to ethanol, all other parameters that are based on the conversion of substrate (sugar) contained in the must into product (ethanol) were compromised (Table 1). Fermentation carried out with the commercial yeast differed from the fermentation with the other strains of *S. cerevisiae* analyzed, presenting higher rates for ethanol content (5.81 g. L^{-1}), ethanol yield (70.20%), efficiency (92.90%), ethanol productivity (0.24 g. L^{-1}. h^{-1}) , rate of TRS conversion (78.60%) and rate of substrate consumption (0.510 g. h^{-1}). The values obtained were close to those reported by Alvarenga et al. (2011) that examined the potential of different strains of *S. cerevisiae* for the fermentation of banana pulp. The fermentation of mango pulp using the commercial pressed baker's yeast did not differ statistically from the strains LC03, LC06, LC07 and LC17 regarding the conversion factor of substrate into product. The rate of substrate consumption (0.510 g. h^{-1}) differed statistically from other strains, except for the strain UFMG1007. When comparing the rate of TRS conversion, there is no statistical difference between the strains UFMG 1007, UFMG 905 and the commercial yeast.

Oliveira et al. (2001) analyzed the fermentative capacity of different yeast strains on synthetic medium with a glucose concentration of 150 g. L^{-1}. In that study, yeasts were classified into distinct groups based on the analyzed parameters such as ethanol yield (%) rate of TRS conversion (%), efficiency (%) the conversion factor of substrate into product ($Y_{p/S}$), among others. Regarding the $Y_{p/S}$ parameter, the authors classified the yeasts into three groups: very high level when the yeasts presented $Y_{p/S}$ values from 0.491 to 0.510 g. g^{-1}; high level with values from 0.451 to 0.490 g. g^{-1}; and mid-level with $Y_{p/S}$ ranging from 0.420 to 0.450 g. g^{-1}. According to this classification, the $Y_{p/S}$ of the yeasts from the commercial pressed baker's yeast (0.470) used in the present study (0.470) is considered high.

The strains UFMG 905 and UFMG 1007 were also analyzed in other studies. Alvarenga et al. (2011) used these strains for the fermentation of banana pulp (Table 2). Oliveira et al. (2001) used the same strains while analyzing the fermentation characteristics of different yeast strains on synthetic medium containing glucose. Silva et al. (2006) studied the kinetic parameters of these strains of *S. cerevisiae*, including flocculation capacity. studied the production of volatile compounds by these yeasts isolated from cachaça distilleries. Furthermore, Marini et al. (2009) did a comparative study including these strains among others of *S. cerevisiae* as starter cultures for the traditional and industrial production of cachaça. The values for the kinetic parameters found in the present study were lower than those reported by Alvarenga et al. (2011). In general, in both the current study and the one conducted by Alvarenga et al. (2011), the parameters evaluated in the fermentation of banana pulp with yeast strains UFMG 905 and UFMG 1007 were lower when compared to commercial yeast.

The results obtained for $Y_{p/S}$ and ethanol productivity were lower than those reported by Marini et al. (2009) and Silva et al. (2006) for the strains UFMG 1007 and UFMG 905, respectively. For the strain UFMG 1007, the authors reported values of $Y_{p/S}$ of 0.391 ± 0.007 and ethanol productivity of 6.19 ± 0.00 g. $L^{-1}.h^{-1}$ and for the strain UFMG 905, $Y_{p/S}$ of 0.439 and ethanol productivity of 6.85 g. $L^{-1}.h^{-1}$. It is important to emphasize that efficiency takes into account the content of ethanol produced in relation to the amount of TRS consumed, while the yield is calculated by the ratio between the

Table 1. Mean values of physico-chemical and kinetic parameters obtained from fermentation tests.

Strains	Ethanol[1]	TRS f[2]	TA_f[3]	pH_f[4]	Yield[5]	Effic.[6]	Produc.[7]	Y p/s[8]	Conv. s/p.[9]	Conv. TRS
LC03	2.16^{bc}	10.71^{a}	0.28	3.93^{a}	25.70^{c}	74.80^{abc}	0.09^{bc}	0.380^{abc}	0.24^{c}	34.85^{c}
LC06	2.00^{c}	10.62^{a}	0.26^{d}	3.86^{b}	24.12^{c}	71.90^{abc}	0.08^{c}	0.390^{abc}	0.23^{c}	34.72^{c}
LC07	2.33^{bc}	10.81^{a}	0.27^{d}	3.71^{d}	27.27^{bc}	77.90^{abc}	0.10^{bc}	0.400^{abc}	0.25^{c}	35.50^{c}
LC17	2.20^{bc}	11.63^{a}	0.17^{f}	3.69^{d}	25.60^{c}	83.84^{ab}	0.09^{bc}	0.430^{ab}	0.22^{c}	30.83^{c}
UFMG 1007	2.93^{b}	6.11^{bc}	0.23^{e}	3.60^{e}	35.23^{b}	56.50^{bc}	0.12^{b}	0.290^{bc}	0.42^{ab}	62.43^{ab}
UFMG 905	2.21^{bc}	7.34^{b}	0.34^{a}	3.77^{c}	26.50^{c}	48.95^{c}	0.09^{bc}	0.250^{c}	0.38^{b}	55.05^{ab}
UFMG1031	2.60^{bc}	7.00^{b}	0.29^{c}	3.87^{b}	31.12^{bc}	55.05^{bc}	0.10^{bc}	0.280^{bc}	0.38^{b}	56.90^{b}
Commercial yeast	5.81^{a}	3.94^{c}	0.31^{b}	3.69^{d}	70.20^{a}	92.90^{a}	0.24^{a}	0.470^{a}	0.51^{a}	78.60^{a}

Means followed by the same letters in the same column do not differ by Tukey multiple comparison test ($p < 0.05$); 1, Ethanol (g; L^{-1}); 2, final content of total reducing sugars (g; L^{-1}); 3, titratable acidity (g; L^{-1}); 4, hydrogenic potential (pH); 5, yield in ethanol (%); 6, efficiency (%); 7, productivity in ethanol (g; L^{-1} h^{-1};); 8, conversion factor of substrate into product; 9, rate of substrate conversion (g; h^{-1}); 10, rate of TRS conversion (%).

Table 2. Comparison of parameters: ethanol production, efficiency and ethanol yield of fermentations conducted with the strains UFMG 905, UFMG 1007 and commercial pressed baker's yeasts compared with existing data on the fermentation of banana pulp.

Strains	Parameters					
	Ethanol (g. L^{-1})		Efficiency (%)		Yield in ethanol (%)	
	Banana pulp	Mango pulp	Banana pulp	Mango pulp	Banana pulp	Mango pulp
UFMG 905	5.64	2.21	76.82	48.95	73.69	26.46
UFMG 1007	5.68	2.93	76.53	56.74	73.90	35.23
Commercial yeast	7.84	5.81	96.41	92.89	94.06	70.17

ethanol obtained and the ethanol expected considering the initial TRS content in the must. This explains why the efficiency values are higher than yield values. The parameters ethanol production, efficiency and ethanol yield in fermentations conducted with the strains UFMG 905 and UFMG 1007 in this study were lower than the ones reported by Alvarenga et al. (2011). These results may be due to differences between the musts since the author used banana musts in his tests, while in this study the mango must was used. However, the fermentation conducted with commercial pressed baker's yeast showed similar values to those reported by the author.

The yield is based on the hourly quantity of ethanol produced, reflecting directly on the efficiency of the yeast in transporting the carbohydrates found in the must (glucose, fructose, sucrose and others) to the interior of its cell. Alvarenga et al. (2011) points out that productivity is excellent aspect for selection of yeasts, the higher the values, the more suitable the yeast is for the fermentation process. The strains from sugar cane must and beer must used in the present study had low fermentation performance when inoculated into mango pulp and did not meet the selection criteria for fermentative cultures, such as productivity, efficiency, ethanol yield and ethanol production. The results showed that the commercial pressed baker's yeast had greater adaptability in mango must than the other strains. Due to this fact, the commercial pressed baker's yeast was selected for obtaining mango wine.

CONFLICT OF INTERESTS

The authors declared that there have no conflict of interests.

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