PERFORMANCE OF A YEAST STRAIN SELECTED FROM AN INDUSTRIAL PROCESS AND USED AS INOCULUM

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ABSTRACT

This study monitored the population dynamics of yeast strains in industrial ethanol fermentation tanks throughout the 2019 crop season. The aim was to select a native strain with good fermentative performance for use as inoculum in the 2020 season. The custom strain RAA5 showed favorable characteristics for fermentation (ethanol yield > 0.465, conversion > 90%, biomass yield > 0.045%) as well as the ability to persist throughout the entire process. Monitoring of fermentation in the 2020 season confirmed that the custom yeast strain is robust, being detected in virtually all collections and dominating the tank in some months. It was concluded that the custom yeast selection strategy enhances the stability of alcohol fermentation by ensuring the permanence of the yeast strain or its reintroduction by raw materials.

Keywords: 1. yeast 2. bioethanol 3. fermentative 4. capacity, alcoholic fermentation

1 INTRODUCTION

Brazil was the world's leading producer of bioethanol until 2005, when it lost the top position to the United States of America. Currently, Brazil is the second largest ethanol producer, owing to advances in production technology, extensive arable lands, and favorable climatic conditions. Brazil is also the world's largest producer of sugarcane, which is the most efficient raw material for ethanol fermentation. Ethanol production from sugarcane juice is well-established in the country. These factors, together with the growing global demand for ethanol, make Brazil highly competitive in the international market. Furthermore, the Brazilian energy matrix is a prominent example of sustainability: 42.9% of the primary energy produced in Brazil comes from renewable sources [1].

Global biofuel production has grown steadily over the past decade, from 16 billion liters in 2000 to 27 billion liters in 2021. Fossil fuels are predicted to be increasingly replaced by biofuels. By 2050, it is estimated that 25% of the world's transportation energy will come from renewable sources [2], [3].

A diversity of species from the genus *Saccharomyces* are used as fermentation agents in Brazilian distilleries. Of these, the most commonly used strains belong to the species *Saccharomyces cerevisiae*. According to Del Rio [4], yeasts must exhibit certain characteristics to be efficient ethanol fermenters, such as a high rate of sugar fermentation, determined from the amount of sugar converted to ethanol in a given time. The higher the rate of fermentation, the higher the productivity, which leads to increased daily production, reduced costs, and reduced risks of process contamination by undesirable microorganisms. Another interesting characteristic of fermentative yeasts is tolerance to alcohol concentrations above 10% (w/v), given that low tolerance results in low ethanol yields and productivity during industrial fermentation. In addition to these factors, resistance to contaminants, population dominance, and physiological stability to withstand variations in process conditions are fundamental [5].

Yeast strains isolated from Brazilian industrial plants have been widely applied in sugar and alcohol production, such as the *S. cerevisiae* strains BG-1, SA-1, CAT-1, PE-2, and Y-904 [6], [7]. Combinations of two or more of these strains are used as starter cultures at the beginning of the crop cycle. At the end of the season, the prevailing population consists of the best adapted yeast cells, which might be either wild or commercial yeasts.

Monitoring of yeast population dynamics during alcohol fermentation can provide valuable information for process optimization. Collected data can be used for the selection of native yeasts with high fermentative potential as well as to adapt process conditions to the requirements of fermentative microorganisms through process design, improvement projects, and correction of operational procedures [8].

This study aimed to monitor and select custom yeasts from an ethanol fermentation process through analysis of population dynamics and fermentative performance in the 2019 season at an industrial unit located in the Midwest region and apply the selected strain as inoculum in this same unit in 2020 to assess its ability to survive throughout the crop season.

2 MATERIAL & METHODS

A. Samples

The samples were collected from an ethanol production process from sugarcane and byproducts in a unit operating continuously with cell recycling in the State of São Paulo, Brazil.

B. Yeast Identification

Yeasts were identified molecularly through the karyotyping technique. Chromosome isolation was made by modifying a protocol proposed by Blond and Vezinhét [9].

C. Fermentative capacity

The parameters were determined by mass balance calculations.

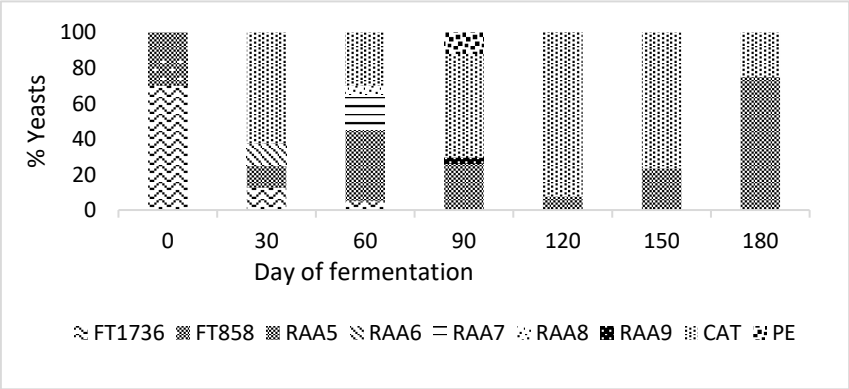
3 RESULTS & DISCUSSION

Fermentation was started using a mixture of CAT1, PE2, FT1736, and FT858 strains. In total, nine different yeasts inhabited the tank during fermentation, four of which were inoculum yeasts and five of which were native yeasts. The incidence of each strain, including inoculum yeasts, and the dynamics of yeast populations during the 2019 season are presented in Table 1 and Fig. 1, respectively.

Table 1. Incidence of yeast strains during fermentation in the 2019 season.

Day	No. of strains	Strains
0	3	FT1736//FT858/RAA5
30	4	CAT/FT1736/RAA5/RAA6
60	5	RAA5/CAT/RAA7/RAA8/FT1736
90	4	CAT/RAA5/PE/RAA9/
120	2	CAT/RAA5
150	2	CAT/RAA5
180	2	RAA5/CAT

Fig. 1. Yeast population dynamics in the 2019 season



At the end of 2019, all yeasts inhabiting the tanks were subjected to fermentation for analysis of fermentative capacity. The results are described in Table 2.

Table 2. Fermentative capacity of native yeasts.

Strain	$Y_{x/s}$	$Y_{p/s}$	P	k	C	P_{sp}	k_{sp}	Best parameters
RAA5	0.0480	0.4615	2.5442	5.8833	91.26	0.4008	0.8739	$Y_{x/s}$ and C
RAA6	0.0499	0.4522	2.3536	5.5548	88.62	0.3781	0.8415	$Y_{x/s}$
RAA7	0.0548	0.4486	2.3299	5.5421	86.31	0.3416	0.7660	$Y_{x/s}$
RAA8	0.0525	0.4508	2.3122	5.4732	85.31	0.3584	0.7999	$Y_{x/s}$
RAA9	0.0465	0.4592	2.4806	5.7648	89.55	0.4127	0.9041	$Y_{x/s}$

$Y_{x/s}$, cell yield (g dry weight g^{-1} fermentable sugar consumed); $Y_{p/s}$, ethanol yield (g dry weight g^{-1} fermentable sugar consumed); P, productivity (g ethanol L^{-1} h^{-1}); k, rate of substrate consumption (g fermentable sugar consumed L^{-1} h^{-1}); C, conversion (%); P_{sp}, specific productivity in relation to cell biomass production; and k_{sp}, rate of substrate consumption per unit weight of cell biomass production.

The results of the fermentation capacity assay suggest that both RAA5 and RAA9 may be used as inoculum, as they were classified as superior. However, it is necessary to investigate the persistence of yeasts during fermentation. RAA5 was found to have good fermentative performance as well as the ability to persist in tanks. The strain was identified throughout the season in all collections, even if not as the dominant yeast.

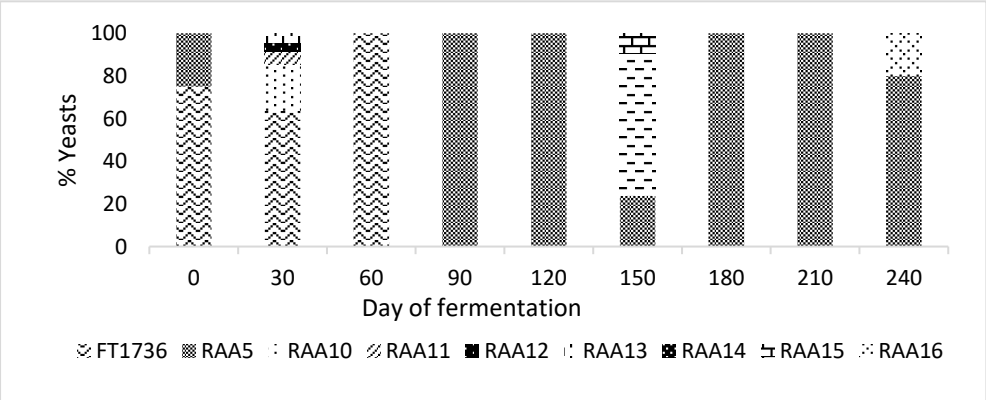
The 2020 fermentation season was started using a mixture of FT1736 and the custom yeast RAA5. Throughout the season, a total of nine yeasts inhabited the tanks, two of which had been used as inoculum (FT1736 and RAA5) (Table 3).

Figure 2 depicts the dynamics of yeast populations in the 2020 season.

Table 3. Incidence of yeasts during fermentation in the 2020 season.

Day	No. of strains	Strains
0	2	FT1736/RAA5
30	5	FT1736/RAA10/RAA11/RAA12/RAA13
60	1	FT1736
90	1	RAA5
120	1	RAA5
150	3	RAA5/RAA14/RAA15
180	1	RAA5
210	1	RAA5
240	2	RAA5/RAA16

Fig. 2. Yeast population dynamics in the 2020 season



From the results of Table 3, we can conclude that the custom yeast RAA5, selected in the 2019 season and used as inoculum in the 2020 season, is robust.
Steckelberg [10] observed that using custom yeasts as inoculum is more promising than using selected strains available in the market, such as PE, CAT, and FT.

4 CONCLUSION

In view of the results, we conclude that selection of custom strains through monitoring and characterization of industrial units and subsequent use of these strains as inoculum in the same units contributes to high fermentation stability, given that yeasts persist throughout the season or are reintroduced from raw materials.

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