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Dominance and Persistence of PE strains (*Saccharomyces sensu stricto*) in Brazilian bioethanol fermentation tanks (three units, one season)

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***Abstract*-The purpose of this work was to follow up the dynamics of yeast population in fermentation tanks for ethanol production starting their processes with PE strain (Saccharomycessensustricto) as inoculum. This follow-up was carried out by using the pulsed-field technique. The results suggest that even though the replacement for indigenous strains took place at different times, the PE strain was unable to remain until the end of the season in the units assessed. In spite of the complete replacement of the PE strain, this fact cannot be associated with the entry of a certain strain, since those that eliminated the PE were also replaced by others at some point at the three units. Each process presents a different dynamics with regard to the yeast population in process, which changes depending on a number of biotic and abiotic factors favoring the installation of certain strains at different periods of the season.**

***Index Terms* -, bioethanol, PE strain (Saccharomycessensu stricto), yeast population dynamics, pulsed field**

I. INTRODUCTION

Contrary to what was initially believed, molecular biology techniques [1], [2], [3], [4] allowed the understanding that bioethanol produced in fermentation tanks are the product of metabolism of yeast naturally inhabiting feedstock and not of those introduced as inoculum at the start of the season. Since they are adapted to the particular conditions of fermentation tanks, these yeasts quietly replace those added as inoculum at the beginning of the season. As reported by UNICA [5], Brazil's ethanol industry production should reach approximately 24.7 million liters of ethanol in the 2017 season. As a result of intense work carried out at research centers to select highly efficient yeasts (select yeasts) from industrial processes, currently a great part of Brazilian units use some of these yeasts to start their processes [4].

Andrietta et al. [6] describe some process-isolated yeasts commercialized as inoculum to start fermentation processes.They are: BG 1 (Usina Barra Grande), CR1 (Usina Cresciumal), SA -1 (Usina Santa Adélia), CAT -1 (Usina Catanduva), PE-2 (Usina da Pedra).

Among the commercialized strains, PE has stood out as the most persistent in fermentation tanks when used as inoculum to start the season. Basso et al. (2) studied the permanence of indigenous strains in industrial processes in the period of 12 seasons. The results presented by the authors show that PE2 strain was capable of remaining in 58% of the distilleries where it was used as inoculum at the beginning of the season. Argueso & Pereira [7] accredit this permanence to the genomic complexity of this strain, which allows it to adapt to the industrial environment. Even though these results seem promising and point to the possibility of selecting yeast that can be used in Brazilian industries, the dynamics of yeast population in fermentation tanks needs to be known. This need originates from the fact that each unit has its particularities regarding biotic and abiotic factors, which are decisive in the selection of yeasts inhabiting the tanks. In this context, the purpose of this work is to follow up the dynamics of yeasts from three industrial processes using PE strain to start their seasons.

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II - MATERIAL AND METHODS

Industrial units: Three different industrial units were assessed, all of them based in the State of São Paulo, and having used PE strain to start their 2010/11 season. Collections were made monthly until the end of the season at each unit. The number of samples varied with regard to the number of crush months at each unit. The names of the units were kept confidential, with reference in this work as: Unit A, Unit B and Unit C.

Samples : The samples were previously diluted in 0.9% saline solution and cultivated in WLN differential medium (DIFCO # 0424) supplemented with 100 ppm of monensin for inhibition of bacteria found in the samples. The surface-spreading technique was used. Plates were incubated at 32°C for seven days for selection of different colony morphologies. The distinction of biotypes was made based on the morphological differentiation of the colony. The parameters used were size, color and texture. Different biotypes were, in duplicate, purified and maintained in PDA slant (Potato dextrose agar).

Yeast Identification: Yeasts were identified molecularly through the karyotyping technique. Chromosome isolation was made by modifying a protocol proposed by Blond and Vezinhét [8]. Chromosomes were spread using agarose gel in pulsed-field electrophoresis in CHEF III (Bio-Rad) equipment. The gel was colored with ethidium bromide prepared in a TAFE solution (0.5 l/ml) and analyzed under ultraviolet light (UVP BioImagem System). The chromosomal profile, made in duplicate for one of the different biotypes (colony morphology) isolated in each collection was compared with the PE strain profile, as well as between themselves.

III.RESULTS AND DISCUSSIONS

Table 1 presents the results for yeast population dynamics at the three units assessed during the harvest season at each one of them.At Unit A, with season from April to October, four indigenous yeast strains were observed. PE strain was found as the only yeast in fermentation tanks during the first thirty days of the season. As of May, it is possible to note the presence of an indigenous yeast (1), which dominates the process (62.5%). PE is eliminated in June and Indigenous 1, although dominant in the process, starts to coexist with two other indigenous strains (2 and 3). In July, Indigenous 1 still dominates the process, but it cohabits with two other strains (Indigenous 2 and 4) and Indigenous 3 is eliminated. During the months of August and September, Indigenous 2 is the only yeast strain found in the process. The season ends with two yeasts in the process, the dominant Indigenous 2, and Indigenous 1, which had been eliminated from the process in August and returned at the end of the season. This yeast (Indigenous 1) was likely not completely eliminated from the process, being capable of remaining, however, at very low concentrations. Some process oscillation, whether due to feedstock or another factor, promoted the population increase of that yeast.At Unit B, with season from May to December, the presence of a large number of yeasts was observed during this period. We noted the presence of nine different indigenous strains at this unit. From March to May, PE was the only strain present in the fermentation process. In June, we noted the presence of an indigenous strain, which is capable of installing at concentration equals to 83.3%. PE strain represented the minority of the population in the month of June. In July, a drastic change is noted in the population inhabiting the fermentation tank. Three indigenous strains (2, 3 and 4), appearing for the first time in this month, cohabit the process. Strains 2 and 3 appear at the same proportion (40%), with indigenous 4 at a concentration corresponding to 20% of the population. In August, when the season completed ninety days, the population in fermentation tanks comprises four different indigenous yeast strains. They are known as: *Indigenous 5*, *Indigenous 6*, *Indigenous 7* and *Indigenous 8*, respectively representing 40, 20, 20 and 20% of yeast population. In the month of September, a new indigenous strain, referred to as Indigenous 9, installs itself in the process with an aggressive behavior. This yeast dominates the process, representing 66.7% of the population, whereas the other 33.3% are represented by Indigenous 8, which appeared for the first time in the previous month. In the following months until the end of the season, the tank will be inhabited by these two strains only (Indigenous 8 and 9), with fluctuating proportions each month. In October, Indigenous 9 still dominates the process (80%). In November, the concentrations of these two strains match: 50% for each one of them. The season ends with 210 days and, at this time Indigenous 8 starts to dominate the process. Indigenous 9 is still present, but representing only 28.6% of the population total. At Unit C, with season from May to November, four indigenous yeast strains were observed. At the first collections, PE strain remained as the only strain in the process. Only in August, ninety days into the season, does the first indigenous strain appear (Indigenous 1), which was not able to eliminate the PE strain, still representing 85.7% of yeast population in the process. One hundred and twenty days into the season, PE is fully eliminated from the process, probably as a result of the presence of two indigenous yeasts (2 and 3), which appeared for the first time in the process, with indigenous 2 dominating the process this month with 66.6% participation of total yeasts. Indigenous 2 and 3 remain in the month of October, with Indigenous 2 dominating the process. The season ends with Indigenous 2 and 3, but a fourth strain (4) appears. Despite the introduction of this new strain (4), Indigenous 2 still dominates the process, representing 50% of yeast population. Each of the other yeasts represent 35% of total yeast population. Figure 1 shows the distribution of PE yeast at the three units assessed during the 2010 season. It is possible to note that the PE strain was unable to remain until the end of the season in all units. It is also possible to observe that the elimination of this strain from the process did not happen in the same month for the three units assessed. Unit A was the first to experience the replacement of PE strain by indigenous ones. At this unit, PE disappears in the month of June. In contrast, Unit C succeeds in maintaining PE in the process until August. As for Unit B, the PE strain remained until July only.

IV. CONCLUSIONS

Even though PE strain has been used as inoculum to start up an ethanol production plant, it was unable to remain in the process until the end of the season of all units assessed in this work. This fact does not disqualify this yeast to be used as start-up inoculum, since this strain was isolated from a sugar and ethanol process, placing it at an advantage compared with baker's yeasts. These particularities of PE, associated with the fact that it is commercialized dry and at large amounts, ends up promoting fermentation start-up with no risks of yeast-related accidents. This work has elucidated that there is a great variation in yeast microbiota during different periods of the season. These variations are certainly associated with process oscillations both of biotic and abiotic nature. This drastic change and the dynamics in the composition of the yeast population in fermentation tanks is easily understood, since seasons are long (lasting up to 200 days), which leads to feedstock processing (sugarcane) with different contents at each period. Operational conditions may also undergo external changes, causing a quick replacement of process yeasts. It is important to highlight that there was no perceptible change in fermentation performance with the replacement of PE strain in none of the units assessed. Without the follow-up of yeast population through karyotyping, it would have been impossible to note the elimination of PE strain from the processes.

Table 1: Yeast population (%) present at the three units studied during the harvest season months.

Months	*March (%)*	*April (%)*	*May (%)*	*June (%)*	*July (%)*	*August (%)*	*September (%)*	*October (%)*	*November (%)*	*December (%)*
UNIT A										
PE	100	100	37.5	0.0	0.0	0.0	0.0	0.0	SEASON END	
Indigenous 1	0.0	0.0	62.5	59.3	46,4	0.0	0.0	20		
Indigenous 2	0.0	0.0	0.0	34.4	32.1	100	100	80		
Indigenous 3	0.0	0.0	0.0	6.3	0.0	0.0	0.0	0.0		
Indigenous 4	0.0	0.0	0.0	0.0	21.5	0.0	0.0	0.0		
UNIT B										
PE	100	100	100	83.3	0	0.0	0.0	0.0	0.0	0.0
Indigenous 1	0.0	0.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0
Indigenous 2	0.0	0.0	0.0	0.0	40.0	0.0	0.0	0.0	0.0	0.0
Indigenous 3	0.0	0.0	0.0	0.0	40.0	0.0	0.0	0.0	0.0	0.0
Indigenous 4	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0
Indigenous 5	0.0	0.0	0.0	0.0	0.0	40.0	0.0	0.0	0.0	0.0
Indigenous 6	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0
Indigenous 7	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0
Indigenous 8	0.0	0.0	0.0	0.0	0.0	20.0	33.3	20.0	50.0	71.4
Indigenous 9	0.0	0.0	0.0	0.0	0.0	0.0	66.7	80.0	50.0	28.6
UNIT C										
PE	100	100	100	100	100	85.7	0.0	0.0	0.0	SEASON END
Indigenous 1	0.0	0.0	0.0	0.0	0.0	14.3	0.0	0.0	0.0	
Indigenous 2	0.0	0.0	0.0	0.0	0.0	0.0	66.6	66.3	50.0	
Indigenous 3	0.0	0.0	0.0	0.0	0.0	0.0	33.4	36.4	25.0	
Indigenous 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	

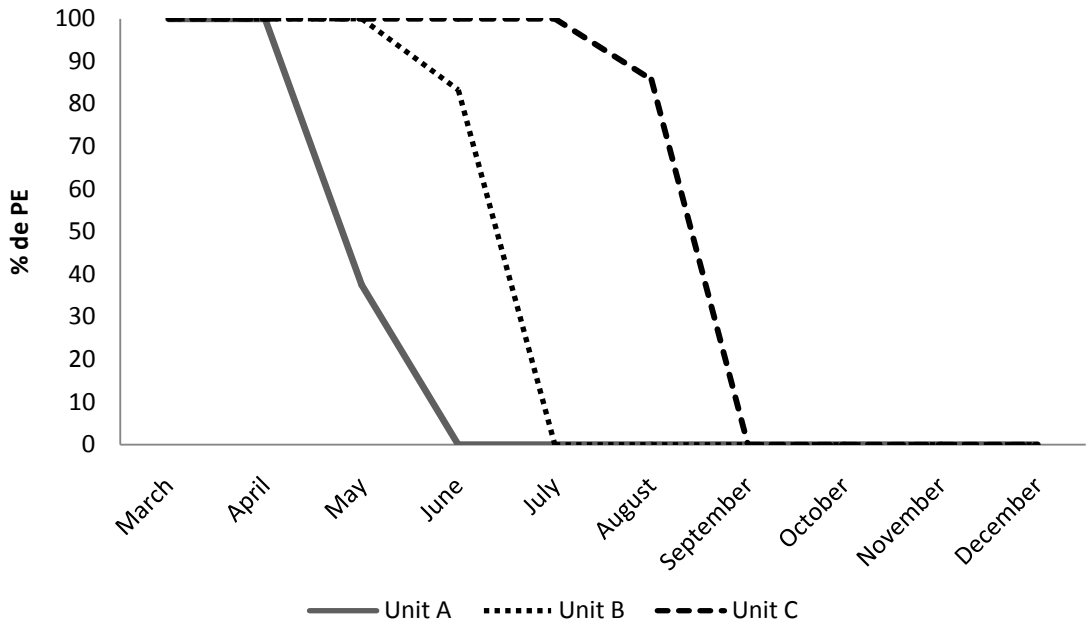

Figure 1: PE strain distribution during the season months at the three units assessed.

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