INCIDENCE OF DIFFERENT PHENOTYPES OF FLOCCULENT YEAST STRAINS IN BRAZILIAN STATES DURING 2021/2022 SEASON

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ABSTRACT

This work aimed to provide an overview of the incidence of different flocculent yeast strains inhabiting fermentation processes in different states of Brazil during the 2021/2022 season, specifically in the Northeast, Central-West, and Southeast Brazilian regions. In this context, we assessed 259 isolates from 99 samples collected from 17 industrial units located in the following states: Bahia, Goiás, Mato Grosso do Sul, Minas Gerais, and São Paulo during 2021/2022 season. The yeast isolates were molecularly identified using karyotiping and characterized regarding their flocculation capacity. The evaluation resulted in a classification of the strains as follows: NF (non-flocculants); FL (light flocculants) and FP (heavy flocculants).
General aspects of flocculation process are discussed, and some implications of flocculent yeast strains in the fermentation processes to bioethanol production are lightly addressed, considering months along the season and different Brazilian states.

Keywords: Yeast. Fermentation processes. Bioethanol. Flocculation. *Saccharomyces cerevisiae*.

1 INTRODUCTION

Yeast flocculation in cultivation tanks is a recurring problem in ethanol production, which can harm bioethanol yield. A more notable consequence is the lack of uniformity of the fermented must, which needs to be centrifuged so that the yeast cells separate from the wine and return to the process while the wine is distilled. The success of centrifugation results from the perfect homogeneity of the mixture, which is not possible when the mixture is flocculated. The main causes of flocculation are the presence of flocculation-promoting bacteria and/or the installation of a strain that flocculates under certain conditions. On the other hand, not all native strains present the characteristic of heavy flocculation, which is undesirable. Some of the native strains, although they have a flocculation profile, are light flocculants and do not impact processes in the same way. In this sense, it is common to identify the presence of flocculent yeasts, which are introduced into the process by the must and end up cohabiting the process with yeasts that essentially do not have this property. Thus, in this context, this work aimed to identify different phenotypes of yeast strains, considering flocculation criteria: non-flocculants (NF); light flocculants(LF) and heavy flocculants(HF) along the 2021/2022 season and evaluate the incidence of the of yeast strains according to different phenotypes of flocculation profiles in fermentative processes in different Brazilian states of 2021/ 2022 season. A total of 99 samples from 17 Brazilian industrial units distributed in 5 states (Bahia, Goiás, Mato Grosso do Sul, Minas Gerais and São Paulo) belonging to 3 different regions (Northeast, Central-West and Southeast) were evaluated.

2 MATERIAL & METHODS

Samples: Throughout the 2021/2022 season (April to December), 99 samples were collected from 17 industrial units producing ethanol from sugarcane that operate continuously with cell recycling in the following Brazilian states: Bahia, Goiás, Minas Gerais, Mato Grosso do Sul and São Paulo. The industrial units producing bioethanol used as initial inoculum different compositions containing strains of Saccharomyces sensu stricto, which included commercially selected yeast strains (PE-2, CAT-1, BG-1, FT858) and/or native strains, previously characterized, and/or baker's yeast strains.

All samples were previously diluted in 0.9% saline solution and cultured in WLN differential medium (DIFCO #0424) supplemented with 100 ppm monensin to inhibit bacteria found in the samples. The superficial spreading technique was used. The plates were incubated at 32°C for seven days to screen for different colony morphologies. The distinction between biotypes was made based on the morphological differentiation of the colony. The parameters used were size, color and texture. Different biotypes were purified and maintained on PDA (Potato Dextrose Agar) slant. The collections resulted in 259 isolates that were later analyzed for the molecular identity using Kariotyping and flocculation profile of the isolates.

Yeast Identification: Yeasts were molecularly identified using the karyotyping technique. Chromosomal isolation was performed by modifying a protocol proposed by Blond and Vezinhét[1] .The chromosomes were spread using agarose gel in pulsed field electrophoresis in CHEF III equipment (Bio-Rad). The gel was colored with ethidium bromide prepared in TBE solution (1X) and analyzed under ultraviolet light (UVP BioImagem System).

Flocculation Profile of Yeast Strains: The 259 yeast isolates were characterized regarding their flocculation capacity by visual assessment after being subjected to growth in liquid culture medium under agitation, in ideal conditions for the fermentation process (32° C, 24h). The evaluation resulted in a classification of the strains as follows: NF (non-flocculants); FL (light flocculants) and FP (heavy flocculants). NF (non-flocculating) strains correspond to the homogeneous growth phenotype in liquid culture medium under ideal growth conditions. FL lines (light flocculants) correspond to the heterogeneous growth phenotype, with cells arranged in microflocs in suspension, when subjected to growth in liquid culture medium under ideal conditions. Strains classified as FP (heavy flocculants) correspond to the heterogeneous growth phenotype, with cells that grow in aggregate forming pellets or micropellets, separated from the liquid culture, when they are submit under ideal growth conditions.

3 RESULTS & DISCUSSION

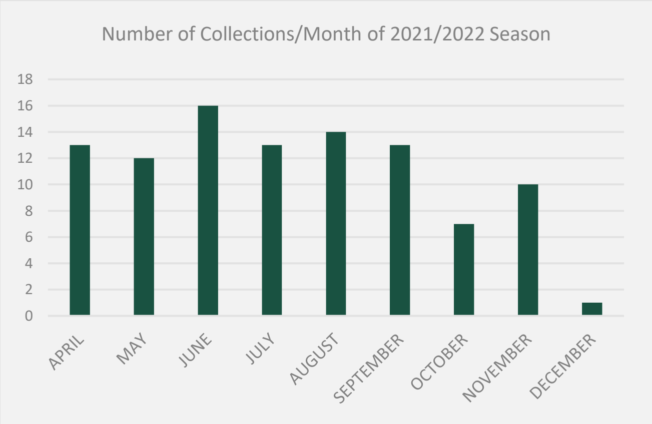

Figure 1 – Number of collections of samples during 2021/2022 season. (N=99)

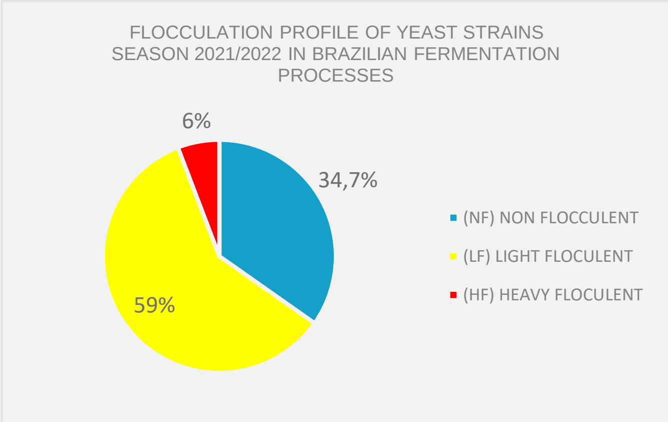

Figure 2 – Flocculation profile of all yeast strains evaluated during 2021/2022 season. (N=259)

As mentioned above, a total of 99 samples from 17 Brazilian industrial units distributed in 5 states: Bahia, Goiás, Mato Grosso do Sul, Minas Gerais and São Paulo) belonging to 3 different regions (Northeast, Central-West and Southeast) were evaluated .The number of sample collections were distributed during the season 2021/2022 according to Figure 1. Although the season generally occurs between April to December, in the most of Brazilian states, it is possible to notice a decrease number of collection in the third trimester because the end season usually varies from one industrial unit to another. So, some industrial units finished the season before the others.

As result of molecular identification using the karyotype technique in the 259 yeast isolates, were found 116 different yeast strains These results indicate the re-incidence of some strains, as described by several authors.[2,3,4,5]

The yeast strains' flocculation capacity was visually assessed after cultivating the strains in liquid cultivation medium under ideal fermentation conditions.

The results obtained from flocculation assessment (Figure 2) shown the prevalence of flocculent yeast strains (64%), in which 59% correspond to LF (light flocculent) yeast strains and 6% to HF (heavy flocculent) yeast strains. Conversely, 34,7% represent NF (non-flocculent) yeasts strains.

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In order to ascertain whether there exist significant variations in the fermentative processes across various Brazilian states, taking into account the prevalence of flocculent yeast strains, we assessed the composition of each group of samples from diverse Brazilian states, (Figure3).

The flocculent yeast strains were prevalent in the majority of Brazilian states, with a prevalence of 100% in BA, 76,4,5% in GO, 78,5% in MG, and 61,9% in MS, with the exception of SP, which exhibits the prevalence of NF (non-flocculent) yeast strains. Nonetheless, despite the aforementioned states, among the phenotypes of flocculent yeast strains, the most prevalent strains exhibited a light flocculation profile. On the contrary, São Paulo (SP) exhibits a higher prevalence of non-flocculent yeast strains, however, it also exhibits the highest prevalence of HF (heavy flocculent) strains. Additionally, Minas Gerais(MG) and Bahia(BA) should be highlighted. Minas Gerais exhibited HF yeast strains profile in a similar manner to that of So Paulo, albeit with a lower incidence. Bahia state should be emphasized because it presented only strains with LF profile exclusively.

The convergence data among the profile of yeast strains phenotype of flocculation and distribution of the incidence of them along all the season months is shown in Figure 4, in which it is shown the NF and LF yeast strains' profiles are present during all the months of the season. On the other hand, the profile of HF yeast strains appears to be low in the first and second trimesters, increasing significantly in the third trimester (October, November, and December).

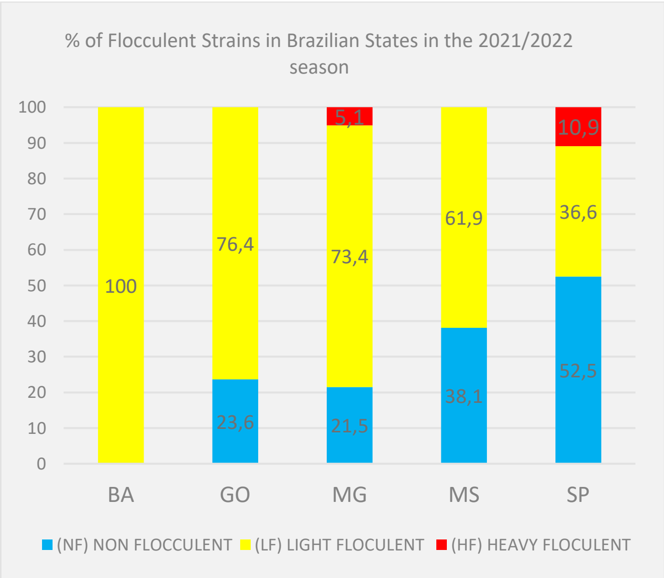

Figure 3 – Incidence of different phenotypes of flocculent yeast strains in the Brazilian States in the 2021/2022 season. (N=259)

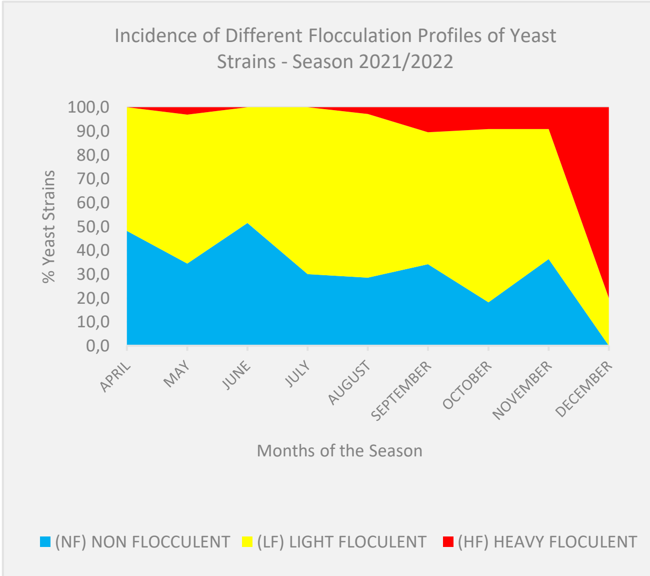

Figure 4 – Incidence of different phenotypes of flocculent yeast strains along the season 2021/2022 (N=259)

4 CONCLUSION

In conclusion, flocculent yeast strains are present along all the season and in different fermentation processes in the Brazilian regions/states. However there are differences from light flocculent yeast strains (LF) from heavy flocculent ones (HF), not only for the biological characteristics and population dynamics with yeast strains cohabiting the process with yeasts that essentially do not have this flocculent profile property, but also in implications to the industrial processes.

Finally, these findings open new perspectives to find innovative strategies to deal with undesirable yeast strains profiles in different regions and places according to the placement of industrial units and different moments along the season.

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