PROSPECTING WILD FERMENTING YEASTS FROM BRAZILIAN BIOMES FOR APPLICATION IN BREWERIES

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ABSTRACT

Brazil is a continental country with a wide and important variety of biomes. Containing, among others, an abundance of organisms yet to be explored and enormous potential for applications in the biotechnology industry. The craft beer market has shown constant growth in recent years. As a result of this fact, several needs for this sector began to emerge, such as the search for new strains of yeast capable of carrying out ethanolic fermentation. Currently, most of the products found on the market use similar varieties of *Saccharomyces cerevisiae*. Therefore, the search for wild yeasts that can provide organoleptic characteristics different from the strains frequently used, can expand the range of product varieties. Over the last 5 years, samples were collected from Acre, Santa Catarina and São Paulo, from plants and bee nests. Different species of yeasts were selected for the fermentation potential and genetically identified. To evaluate yeast potential to produce beer, fermentation was performed at a high-scale level in different breweries. Those findings show the possibility to use wild yeasts in the brewery fermentation and also amplify our understanding of the ecological niches of wild yeasts and their potential for biotechnological industry.

Keywords: Saccharomyces, Biome, Yeast, Beer, Fermentation

1 INTRODUCTION

Brazil possesses unique and plentiful habitats with one of the most abundant biodiversity on the planet, having a symbiotic interaction for their maintenance and adaptation to environmental changes. Brazilian micro biodiversity could provide inputs to improve the food industry, in addition to its possible application in biotechnology. Among those, novel yeast strains could be found not only those from *Saccharomyces* genus, but also from non-*Saccharomyces*.

With the development of new techniques for isolating and identifying microorganisms, the use of wild yeasts in applications within the biotechnology industry has expanded. These organisms have been used by humanity for thousands of years in the manufacture of food and beverages, which led to a process of domestication of yeasts, mainly those belonging to the genus Saccharomyces, generating standardized strains for each type of process.

The growth of the craft beer industry has driven the sector in the search for new inputs, especially national inputs that bring Brazilianness to the product. Most of the supplies used are imported, which generates a high cost for the final product and does not yet bring unique characteristics of our terroir. Showing the need to search for new yeast strains in Brazilian biomes, capable of carrying out ethanolic fermentation in beer wort and providing organoleptic characteristics (such as aroma and flavor) different from the frequently commercial strains[1].

Brazil has an advantage in relation to the search for new yeasts with biotechnological potential for beer production, as it is a megadiverse country, with a biota estimated at 15 and 20% of the planet's living beings. In relation to fungi alone, more than 13 thousand species have been cataloged in Brazil, corresponding to 14% of the world's diversity. All this biodiversity is due to some specific and unique biomes within this continental country[2,3].

Therefore, the present project aims to capture and prospect wild yeasts from national biomes, not only to bring unique characteristics to the brewing industry, but also to explore the richness and potential of the Brazilian microbiome.

2 MATERIAL & METHODS

Environmental samples were collected over the last five years from different regions; from Samphire plant, a halophyte perennial plant from Palhoça beach, SC, from native bee nests from the region of Kaxinawá tribe near Jordan River at the Amazon Forest, AC, from an artificial bee colony located at CEPE - USP, east metropolitan area of São Paulo, SP, and from *Apis mellifera* artificial colony at Itu, SP [4, 5, 6].

Collected samples were diluted in NaCl 0,9% in a sterile falcon tube and 50uL of the solution were plated on YPD 6% RBC medium, after 3 days at 30°C, white creamy colonies were selected and transferred to the YPD medium and fermentation tests were performed in DME 10°.

Isolated yeast were selected for their fermentation potential and their genomic DNA were extracted, amplified, purified and quantified before being sequenced. Assembled contigs were subjected to a similarity search in the GenBank/EMBL/DDBJ database using Blast 2.10.1 software. For phylogenetic analysis, an alignment was created with Mega Version 3.1 using the default settings for multiple sequence alignments with reference to the sequences of each fungal species available in GenBank. Samples were identified with more than 99% of similarity.

To evaluate yeast potential to produce beer, fermentation was performed at a high-scale level at different breweries, and different beer styles.

3 RESULTS & DISCUSSION

The process of isolating yeast showed that even *Saccharomyces* and non-*Saccharomyces* can be found in the wilderness, from different Brazilian biomes. As seen in Table 1, listing the collecting site, sample and identified yeast.

Table 1: List of the collection site with the samples, identified yeasts and code used in this project

Collecting site	Sample	Yeast identification	Code
Palhoça beach, SC	Samphire plant	*Saccharomyces cerevisiae*	YSC01
Jordan river, AC	Native bee nest (*Nannotrigona spp*)	*Saccharomyces cerevisiae*	YSC02
Itu, SP	Artificial bee colony (*Apis mellifera*)	*Wickerhamomyces anomalus*	YWA01
São Paulo, SP	Native queen bee (*Tetragonisca angustula*)	*Debaryomyces hanseni*	YDH01

After the genetic identification, yeasts were analyzed in a high-scale batch to produce beer. Different beer recipes and styles were determined according to the organoleptic characteristic produced by each yeast, as well as the style the brewer wished to produce. Yeasts were propagated at each brewery until they reached the necessary pitch for each beer wort volume. On Table 2 is listed the breweries, yeast used, beer style, volume produced and beer name.

Table 2: List of breweries, yeast used, beer style, volume produced and beer name

Brewery	Yeast	Beer style	Volume	Beer name
Lohn	YSC01	Brazilian Pale Ale	2000 L	Toda Nossa
Nacional	YSC02	Amber Ale	499 L	Garafada Jurema - Musas 2024
Nacional	YSC02	Brazilian IPA	500 L	Simbiótica - Pint of Science 2024
Cybeer	YSC02	Brazilian Pale Ale	250 L	Amazon IA
Zev collab with Nacional	YDH01	Imperial IPA	2500 L	Queen BEEa
Nacional	YWA01	Wild Ale	30 L	Origem das espécies - PInt of Science 2020
Goose Island	YWA01	Table Saison	800 L	Sonhos tropicais - Pint of Science 2022

All beers produced had unique flavors, bringing up the possibility of prospecting wild yeast into the brewery process. Two of those beers are now routinely produced by Lohn and Cybeer. Besides that, Toda Nossa has received a silver medal at the World Beer Cup. The AmazonIA already had its recognition for excellence, receiving a gold medal at regional and national level at Copa Brasil de Cerveja (Abracerva). All beer labels are shown at Figure 1.

Figure 1: Images of those beer labels fermented with wild yeast isolated from native biomes.

4 CONCLUSION

Those finds show the possibility to use wild *Saccharomyces* and non conventional yeasts in the brewery fermentation, bringing new flavors to the industry. And also amplifying our understanding of the ecological niches of wild yeasts, their symbiotic relationship with native bees and their potential for biotechnological industry.

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