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ARTIGO ORIGINAL



### Technological Advances in Ethanol Production: The Role of Sugarcane, Corn and Yeast Lineages

Avanços Tecnológicos na Produção de Etanol: O Papel da Cana-de-Açúcar, Milho e Linhagens de Leveduras

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#### RESUMO

As culturas energéticas, apresentam um alto potencial como matéria-prima para suprir a demanda por energia, as mais promissoras são a cana-de-açúcar e o milho que possuem potencial de biotransformação. Neste contexto, o estudo visa avaliar o panorama de produção de cana-de-açúcar e milho no Brasil, averiguar os estados que se destacam na produção de etanol a partir destas culturas, bem como avaliar as características das principais linhagens de Saccharomyces cerevisiae utilizadas na fermentação para a produção de etanol. A metodologia empregada foi a pesquisa bibliográfica no modelo da cienciometria. Os resultados mostram que o Brasil é um importante produtor de cana-de-açúcar e milho. A produção de etanol é impulsionada pelos estados de São Paulo e Goiás com etanol de cana-de-açúcar, enquanto Mato Grosso é o maior produtor de etanol de milho. As leveduras utilizadas nestes processos possuem diferenças principalmente em relação a tolerância dos fatores de estresse como a temperatura e tempo prolongado de fermentação, além de altas concentrações de etanol presentes no meio fermentativo. A busca e o desenvolvimento de linhagens de Saccharomyces cerevisiae selecionadas e geneticamente modificadas relacionadas aos fatores intrínsecos do processo tanto da cana-de-açúcar quanto de milho podem assegurar a eficiência e a sustentabilidade do processo.

**Palavras-chave**: Culturas energéticas. Fermentação. *Saccharomyces cerevisiae*. Biocombustíveis.

#### ABSTRACT

Energy crops have high potential as raw materials to meet the demand for energy, the most promising are sugar cane and corn, which have biotransformation potential. In this context, the study aims to evaluate the panorama of sugarcane and corn production in Brazil, investigate the states that stand out in the production of ethanol from these crops, as well as evaluate the characteristics of the main strains of *Saccharomyces cerevisiae* used in fermentation for the production of ethanol. The methodology used was bibliographical research in the scientometrics model. The results show that Brazil is an important producer of sugar cane and corn. Ethanol production is driven by the states of São Paulo and Goiás with ethanol from sugar cane, while Mato Grosso is the largest producer of corn ethanol. The yeasts used in these processes differ mainly in relation to tolerance of stress factors such as temperature and prolonged fermentation time, in addition to high concentrations of ethanol present in the fermentation medium. The search and development of selected and genetically modified *Saccharomyces cerevisiae* strains related to the intrinsic factors of the process.

Keywords: Energy cultures. Fermentation. Saccharomyces cerevisiae. Biofuels

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#### 1. INTRODUCTIO

The growing need for energy sources, driven by the advancement in people's quality of life, has led to a global search for energy and sustainable solutions. Within this context, biofuels originating from biomass, such as ethanol, emerge as an environmentally and economically viable alternative. This is due to its advantages, such as the ability to reduce greenhouse gas emissions (BARBOSA et al., 2022). In this perspective, energy crops have a high potential as a raw material to meet this demand.

Energy crops are plants that have a high capacity for converting solar energy into biomass through photosynthesis, thus accumulating energy in the form of organic matter, which can be further processed and generate bioenergy or biofuels, such as ethanol. The choice of these crops takes into account their high productivity and lower environmental impact (JESWANI; CHILVERS; AZAPAGIC, 2020). The widely used energy crops are sugar cane and maize. These two crops have intrinsic characteristic that make them ideal for the production of biofuels.

Sugarcane, a crop considered as a commodity, has high productivity and high levels of fermentable sugars, which have great potential for transformation, being able to replace energy from fossil fuels efficiently (MANOCHIO et al., 2017). In Brazil, sugarcane is used both in the production of anhydrous and hydrous ethanol. This country is considered the world's largest producer of sugarcane and the second largest producer of ethanol. According to the Companhia Nacional de Abastecimento (CONAB, 2020), in the 2020/21 harvest this country had a productivity of 650 million tons of sugarcane and 30.6 billion liters of ethanol that were destined for domestic consumption and exportation.

Another promising crop is corn, which has also been highlighted as an energy crop, being used for different processes including the production of biofuels. From corn, it is possible to generate a variety of products and by-products, such as beverages, polymers, biofuels and oil, in addition to being used in human food, making ir the main agricultural crop in the world (MIRANDA, 2018). The United States and China are the largest corn producers, corresponding to 54% of world production, while Brazil and European Union together represent 15%, these countries together are responsible for about 69% of world production of this grain (EICHOLZ et al., 2020).

Brazil is considered the third largest producer of corn, showing growth at each harvest, reaching 4.67% per year in production and 2.95% per year in productivity, higher than those observed in the world (CONAB, 2018). The Centro Oeste region, with emphasis on the

states of Mato Grosso and Mato Grosso do Sul, is the largest producer of corn, with Mato Grosso being the highest in crop productivity due to the technologies adopted for planting, such as no-tillage, crop rotation and nutrient cycling in the soil (LOPES; SANTOS; BATISTOTE, 2022).

In this region there are corn harvests, which triggered several problems, the main one being the lack of structure for storage, among others. This situation spurred the installation of new distilleries in the state of Mato Grosso, aiming at the production of ethanol from this grain. This state stands out as a pioneer in the production of corn ethanol in Brazil (DA SILVA et al., 2020). Corn ethanol production is expanding and the Mato Grosso region is an important hub for this activity (LOPES; SANTOS; BATISTOTE, 2022), with five distilleries in operation and another fifteen implementation projects in the licensing phase, which indicates a trend towards increased production of corn ethanol in the country, according to the Associação de Produtores de Bioenergia de Mato Grosso do Sul (BIOSUL, 2021).

World production of bioethanol was approximately 118.2 billion liters in 2020 according to RFA Renewable Fuels Association (RFA, 2023). Brazil presents consolidated Technologies in relation to the production of ethanol from sugarcane, is the second largest producer with 31% of world production, behind only the United States with 53% in 2020 (RFA, 2023). Regarding the fermentation process, the selected yeasts have numerous characteristics, such as high viability rate, fermentability and resistance to stress factors.

Therefore, the choice of yeasts based on their characteristics is an essential factor to ensure yield and efficiency in ethanol production (AZHAR et al., 2017). Brazil and United States are the largest producers of ethanol, however they differ in production methods, in Brazil, sugarcane juice and selected yeasts are used, while in the United States corn hydrolyzed is fermented using conventional and genetically modified yeasts. The choice of yeast strain is fundamental in the process, in order that high ethanol yields can be obtained depending on the raw material and industrial conditions used (SECCHES et al., 2022).

These microorganisms are essential for the conversion of sugar into bioethanol. The yeasts used are Saccharomyces cerevisiae, which have high industrial performance, as they have ideal characteristics for fermentation, such as high viability rate, high ethanol tolerance, fast and efficient substrate conversion, making it a suitable option for large-scale industrial processes (FAVARO; JANSEN; VAN ZYL, 2019). Technological advances and the choice of yeasts have enabled the largest ethanol producers to be at the forefront of world bioethanol production. In this context, the study aims to evaluate the panorama of sugarcane and corn production in Brazil, as well as to ascertain the states that produce the most ethanol

from these crops and to evaluate the characteristics of the main lineages of *Saccharomyces cerevisiae* used in the fermentation of these cultures for ethanol production.

### 2. MATERIALS AND METHODS

The study was carried out at the Biotechnology, Biochemistry and Biotransformation Laboratory of the Center for Natural Resources Studies – CERNA of the State University of Mato Grosso do Sul, Dourados/MS.

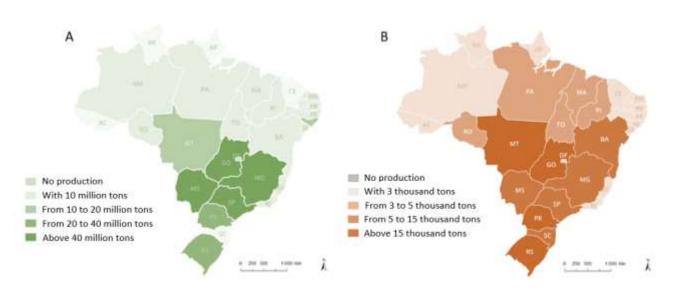
The methodology used in this study was bibliographical research, which involved a preliminary survey of the panorama of publications on the topic. It was based on exploratory quantitative research associated with descriptive research that was used to analyze scientific production, through scientometrics. The search terms were "Ethanol production, sugar cane, corn", in English. The articles were read and the data selected. Subsequently, the data was compiled in Excel 2019 software according to the structure of this research.

Scientometrics is a tool that allows the exploration and compilation of quantitative and statistical data, which makes it possible to observe gaps that can be discussed to build knowledge (PARRA; COUTINHO; PESSANO, 2019).

# 3. RESULTS AND DISCUSSION

Brazil has its economy based on agribusiness, its territorial extension and climatic diversity collaborate with the productivity of crops such as sugarcane and corn (Figure 1). This country occupies a prominent position in the world in the production and exportation of products derived from these raw materials. The data show that the states of Mato Grosso do Sul, Minas Gerais, São Paulo and Goiás have national relevance in the production of sugarcane (Figure 1A), while in the case of maize, the states Mato Grosso, Goiás, Paraná and Rio Grande do Sul (Figure 1B).

Brazil stands out not only in the production of sugarcane and corn, but also in the transformation of these two crops into ethanol. This is the results of technological advances and investments in the modernization of industrial plants, which allow for more efficient and sustainable processes. In this scenario, the Centro Oeste region of Brazil has stood out, as in addition to being responsible for a large portion of the production of sugarcane and corn, it is also recognized for its strong and dynamic agribusiness (LOPES; SANTOS; BATISTOTE, 2022).



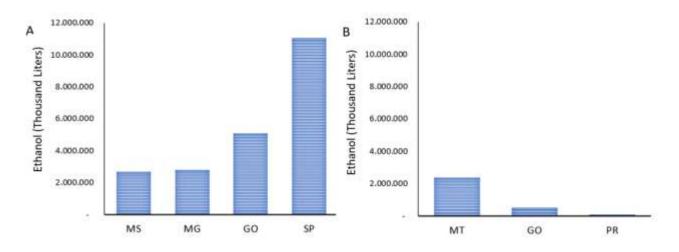
**Figure 1.** Brazilian sugarcane producers' states (A) corn (B). Source: Adapted from Monitoring the Brazilian crop - 2019/20 Crop (CONAB, 2020).

The state of Mato Grosso is considered the pioneer and largest producer of ethanol from corn in Brazil (DA SILVA et al., 2020). However, it is important to point out that the entire production chain must be attentive to the quality of its process, from the raw materials used, the transformation process, the processing efficiency, the utilization of co-products generated and the environmental sustainability (MIZIK; GYARMATI, 2021).

Ethanol plays in important role in the Brazilian energy matrix, being highly productive and sustainable, in addition to generating socioeconomic development in the regions where the industrial units are installed. In the specific case of sugarcane ethanol, the states of São Paulo and Goiás are the largest producers, while Mato Grosso and Goiás stand out as the main producers of corn ethanol (Figures 2A e B). These advances in the production of biofuels have been possible thanks to the development of technologies that improve the performance of the crop, in addition to the favorable edaphoclimatic conditions in some regions of the country.

This highlight in ethanol productivity is the result of several factors. Among these, the territorial extension and quality of arable land available in the country. In addition, they contribute significantly to genetic advances in sugarcane and corn varieties, jointly with improved agricultural practices and the adoption of mechanized harvesting methods, resulting from the modernization of industrial facilities (MOURAO et al., 2020). Ethanol plays a crucial role in the Brazilian energy matrix, being highly productive and sustainable, in addition to generating socioeconomic development in the regions where the industrial units are installed. Corn as well as sugarcane are important energy crops for ethanol production,

due to their high productivity and profitability (SILVA; DO SOCORRO MASCARENHAS; BATISTOTE, 2022).



**Figure 2.** Ethanol production from sugarcane (A) corn (B) in the mai Brazilian states. Source: Adapted from the Brazilian crop monitoring - 2020/21 Crop (CONAB, 2022).

The choice of industrial yeast lineage must consider the characteristics of the process, since the production of sugarcane ethanol differs from production using corn as raw material. The data show that in the sugarcane juice-based ethanol production process selected yeast strains are used, such as Barra Grande 1 (BG-1), Santa Adélia 1 (SA-1), Catanduva 1 (CAT-1) and Pedra 2 (PE-2). However, corn due to its peculiarities, genetically modified yeast strains are used such as Ethanol Red Innova® Lift and Innova® Force (Table 1).

The processes that use the fermentative Route are considered to be more efficient, safer, with high profitability and environmental advantages (AWASTHI et al., 2022). In this process, yeasts are used which are generally selected in specific niches, since for to obtain an efficient conversion of substrate to ethanol, more robust yeasts are required and destined to industrial processes. These microorganisms have a high ability to convert sugar into ethanol (CARRIGLIO; BUDNER; THOMPSON-WITRICK, 2022).

To guarantee expressive numbers in the production of ethanol, some strategies are necessary, such as increasing production efficiency. In this context, fermentative yeast strains play a key role. Yeast selection and genetic engineering have been employed to optimize physiological and genetic characteristics, aiming to meet the specific demands of ethanol production. **Table 1.** Lineages of *Saccharomyces cerevisiae* selected, conventional and genetically modified, which are used in the fermentation process from sugarcane and corn to produce ethanol

Yeast strains	Characteristics	Substrate type	Reference
Barra Grande 1 (BG-1), Santa Adélia 1 (SA-1), Catanduva 1 (CAT- 1), Pedra 2 (PE-2)	These strains have survival capacity and process dominance; low glycerol and foaming; no flocculation; high yield in ethane production;	Sugarcane	Nagamatsu et al. (2021)
Ethanol Red ®	Has high tolerance to ethanol; high alcoholic yields and maintains greater cell viability; tolerates high concentrations of sugar;	Corn	Lesaffre Advanced Fermentations (2023)
Innova® Lift	Strain with expression of glucoamylase; Exhibits tolerance to temperature and organic acids; Reduced needly of nutrients; Suitable for longer fermentation time (57h or more)	Corn	Novozymes (2023)
Innova® Force	Expression of multiple starch degrading enzymes; Robust to high concentrations of ethanol, high drying solids (up to 38%), temperature and organic acids. Less need for nutrients	Corn	Novozymes (2023)

The *S. cerevisiae* yeasts play a crucial role in various industrial processes, such as the production of alcoholic beverages, baking, and bioethanol manufacturing. However, when exposed to industrial environments, they face significant challenges that can lead to abrupt changes in their metabolism (SILVA et al., 2023).

According to Favaro, Jansen and Van Zyl (2019), in the fermentation process with corn, the yeasts are exposed to more extreme conditions, they ferment for more extreme conditions, lasting more than 50 hours at high concentrations of ethanol in the medium. Thus, strains with better performance and with the ability to efficiently metabolize substrates are required. Regarding fermentation in sugarcane juice, the process takes around 8 to 10 hours with yeast recycling and high viability rates (MANOCHIO et al., 2017).

The selected Brazilian industrial yeast strains emerged from na adaptation process in the sugarcane juice-based medium, originating a portfolio of highly robust strains, which ferment in non-aseptic conditions by cell cycle, high temperatures and permanence in fermentation vats with high cell density, with an production of 10 to 17% (v.v<sup>-1</sup>) according to (JACOBUS et al., 2021).

In the United States, studies focused on genetic engineering have developed modified yeasts for fermenting corn hydrolysate. However, the lineage Ethanol Red is a conventional

yeast for the production of corn ethanol, capable of producing high concentrations of ethanol (GRONCHI et al., 2019). The use of selected, conventional and genetically modified yeast strains plays a key role in productive success.

# 4. FINAL CONSIDERATIONS

Brazil has a high role in the production of biofuels, and has important crops such as sugarcane and corn, such crops have high productivity and are present in several regions of the country.

The Sudeste region has the highest production of sugarcane ethanol, however the Center-West region has stood out in the production of biofuels from energy crops, such as corn and sugarcane, with optimistic prospects for increased production in the coming years.

The choice of yeasts is essential for the efficiency of the fermentation process depending on the raw. Thus, selected and genetically modified yeasts, with characteristics of resistance to high concentrations of ethanol, the stress conditions and efficient fermentative capacity play an extraordinary role, to meet the growing demand for ethanol and boost the biofuel sector in a sustainable way.

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