Article Review
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.80422

Microbial contamination in the ethanol and cachaça fermentation process: impacts and applications

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Abstract

Brazil is a major powerhouse in the production of sugarcane. Consequently, several supply chains use it as a raw material, such as the food sector, mainly in the production of sugar and beverages, such as cachaça, and the biofuels sector, with the production of ethanol, an important product for the Brazilian economy. The production of cachaça and ethanol share an important stage known as fermentation, a fundamental process that defines the quality and yield of the alcoholic fermentation product, which is achieved using *Saccharomyces cerevisiae*. Different industrial strains have been selected to promote alcoholic fermentation efficiently and with high productivity. However, it is possible that microorganisms from various stages of the production chain reach the fermentation phase, compromising it. Contaminants can vary from different genera of yeasts, including *Dekkera* and *Pichia*, to bacteria, mainly belonging to the *Lactobacillaceae* family, which produce lactic acid. Contaminating microorganisms affect the fermentation stage and, as a consequence, the quality of the produced cachaça or the production efficiency of ethanol. Recent studies have shown that these contaminants, in addition to resulting in negative aspects of sugarcane fermentation, can also present interesting physiological characteristics that can be applied in bioprocesses in other productive sectors or to improve fermentation strains.

Keywords: cachaça; ethanol; alcoholic fermentation; contaminants; lactic bacteria.

Practical Application: Several species of sugarcane-contaminating microorganisms interfere with the ethanolic fermentation of cachaça and ethanol. Research efforts have been dedicated to understanding this contamination process and seeking control strategies and biotechnological applications for these microorganisms.

1. Introduction

With one of the largest sugarcane output rates worldwide, Brazil produced more than 654.5 million tons in the 2020/2021 harvest, an increase of 1.8% over the previous production. Such high yields of sugarcane directly contribute to the productivity of products that use it as a raw material. Some examples are foodstuffs such as sugar and cachaça, biofuels, or the sugar-energy sector (CONAB, 2021).

This review focuses on two relevant products made from sugarcane, namely, cachaça and ethanol. Sugarcane has been produced in the country since the colonial period, and the production of cachaça has accompanied it since then (Carneiro, 2020). The first sugar mills in Brazil emerged in 1532. At that time, cachaça was a secondary product of sugar production; however, it is currently an exclusively Brazilian product, and its production is recognized around the world (Alcarde, 2017). This distillate is produced practically in all Brazilian states, although Minas Gerais and São Paulo are the ones with the highest production (MAPA, 2019).

Sugarcane ethanol is a product with a significant economic impact in Brazil. In the 2020/2021 harvest alone, the country was responsible for generating 29.7 billion liters of ethanol from sugarcane (CONAB, 2021). Based on these factors, ethanol production arouses interest from the government and the private sector, which makes ethanol and the activities involved in its production an important point in discussions in our country. The relevance of ethanol is primarily associated with its use as a biofuel, an alternative to the use of fossil fuels. It is believed that the use of these fuels at a global level can contribute to the reduction of greenhouse gas emissions (Rodrigues Filho & Juliani, 2013).

The production processes of cachaça and ethanol have an important stage in common: fermentation, which is one of the most crucial processes for production. Initially, the sugarcane is received at the industry, unloaded, stored, and then passed to the milling stage, generating the juice. The sugarcane juice undergoes some steps that are specific to each line of production, followed by the fermentation process, a stage that, for each product, is of great significance due to its influence on quality and yield (Alcarde, 2017).

However, this stage can be compromised due to contamination by microorganisms from the field or those that are present along the chain of production of cachaça and ethanol.

Received 25 Dec., 2022
Accepted 6 June, 2023
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The microbial load may vary during the process, but the number of contaminants tends to increase throughout the production stages (Costa et al., 2015). Since there is no sugarcane sterilization step before fermentation, the contaminating microorganisms reach this crucial stage, where they cause significant damage. Selected strains of *Saccharomyces cerevisiae* compete during fermentation with unselected yeasts and contaminating bacteria (Alcarde, 2017; Bassi et al., 2018).

These contaminating microorganisms are capable of directly interfering with the quality of the cachaça and the yield of the fermentation stage, becoming unwanted in either process. Therefore, the aim of the present review was to conduct a survey of the contaminating microorganisms in the alcoholic fermentation processes of cachaça and ethanol and identify the impacts that this microbiota may have on the production chains.

2. Fermentation

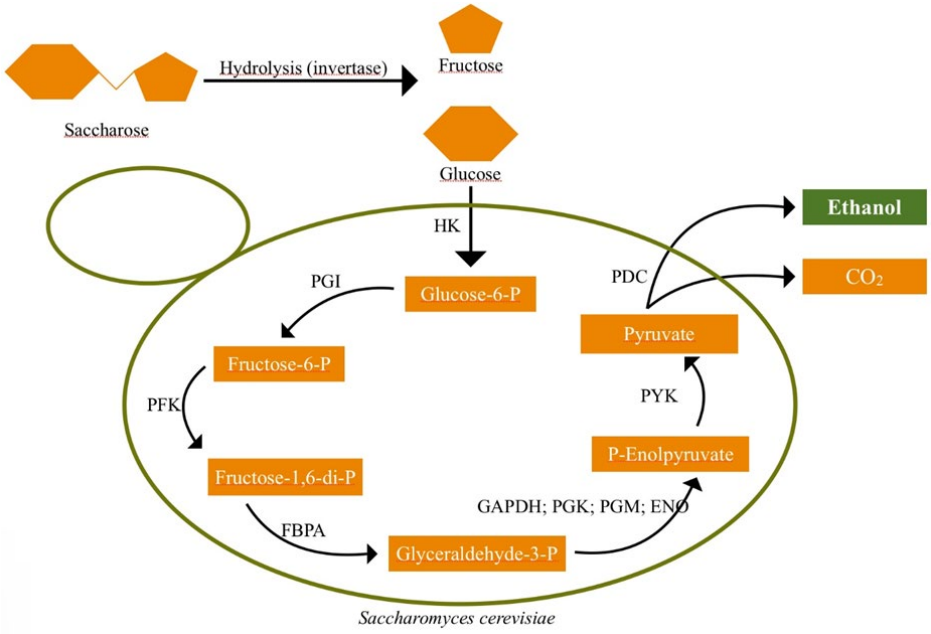
Alcoholic fermentation can be achieved mainly by yeasts, although some other fungi and bacteria are also able to convert sugars into ethyl alcohol and carbon dioxide (Malakar et al., 2020). In the alcoholic beverage industry, the most widely used microorganisms are yeast species belonging to the genus *Saccharomyces*, mainly *S. cerevisiae*. In addition, specific strains are used in the fermentation process according to the type of product, so that each one has its sensory characteristics, reaching the desired quality level and maximizing the alcohol yield during the fermentation period (Walker & Stewart, 2016). Figure 1 illustrates the metabolic pathway of ethanolic fermentation by *S. cerevisiae*. In the fermentation process to produce fuel ethanol, specific strains of *S. cerevisiae* are also used, such as PE-2 strain, which has high ethanol production to provide as much ethanol as possible during the industrial fermentation period (Bassi et al., 2018).

The yeast *S. cerevisiae* requires specific conditions for growth and to carry out the alcoholic fermentation more effectively. For example, the ideal conditions of the substrate for alcoholic fermentation by *S. cerevisiae* include a minimum water activity (a_w) of 0.65, pH of the environment between 4.5 and 6.5, and temperature between 20 and 30°C. Since *S. cerevisiae* does not work well under strictly anaerobic conditions, it requires some oxygen during fermentation. This yeast uses the sugar available in the environment to promote fermentation. It can consume different sugars, and in the case of fermentation of cachaça and ethanol from sugarcane, the yeast uses mainly the available sucrose. With the breakdown of sugar, a pyruvate molecule is produced, which undergoes decarboxylation, and, after a final reduction step, ethanol and CO_2 molecules are formed (Walker & Stewart, 2016).

The industry aims to provide these conditions so that the fermentation process is as efficient as possible and, thus, produces quality products. Therefore, for each process, both for the production of cachaça and ethanol, specific strains of *S. cerevisiae* are selected which present better performance under the conditions of ethanolic fermentation.

2.1. Cachaça fermentation

The microorganisms used in the fermentation of cachaça are the objects of several studies that aim to understand the effects they cause on the beverage as well as select strains resistant to the process that produces desirable secondary compounds for a quality cachaça. The fermentation stage of cachaça can be initiated in different ways, with the use of selected strains being the most common. However, some distilleries make use of natural yeast, pressed yeast, or mixed yeast, a mixture of the two forms of yeast (Alcarde, 2017).



HK: hexokinase; PGI: phosphoglycoisomerase; PFK: phosphofructokinase; FBPA: fructose bisphosphate aldolase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PGK: phosphoglycerate kinase; PGM: phosphoglyceromutase; ENO: enolase; PYK: pyruvate kinase; PDC: pyruvate decarboxylase.

Figure 1. Simple metabolic pathway of ethanolic fermentation by *S. cerevisiae*.

2.1.1. Dry yeast

In Brazil, it is possible to find premium cachaça aged for years with high quality. It is common to use dry yeast, which consists of laboratory-tested yeasts that have specific characteristics for the cachaça fermentation process. They demonstrate good yields and tolerance to the conditions of the fermentation process, such as temperature, acidity, and the high alcohol content of the process, in addition to the chemical quality of the product (Alcarde, 2017). Several strains of *S. cerevisiae* have been studied for this process. Different strains interfere with the chemical composition of cachaça. Among the strains used in the industry, CA-11 and CANAMAX produce cachaça with the highest chemical and sensory quality, both of which were developed by universities in Minas Gerais. Nevertheless, other strains, including BG-1 and CAT-1, also produce this distillate within the standards established by Brazilian legislation and are used in some distilleries (Alcarde et al., 2012; Alcarde, 2017).

2.1.2. Compressed yeast

Some other distilleries prefer to carry out the fermentation process using compressed yeast, also known as baking yeast. To this end, a solid mass containing *S. cerevisiae* cells is added to the sugarcane must, with a Brix between 8 and 10°Brix and temperatures between 30 and 32°C. When the Brix is reduced to half, successive additions of must are started until reaching a 16–18°Brix with a final volume of approximately 20–30% of vat occupancy. Only then is the vat completed and fermentation initiated (Alcarde, 2017).

2.1.3. Natural yeast

Natural yeast, also known as "caipira" yeast, consists of yeasts found naturally in sugarcane cultivation and the production of cachaça. For their use in fermentation, the yeasts are stimulated to multiply before starting the fermentation process. For this growth to occur, the sugarcane juice with 6–7°Brix, containing the yeasts, is mixed with corn flour, rice, and bread bran and heated to 28°C, followed by the addition of more juice over the course of 10 days, after which the preparation is ready for fermentation (Alcarde, 2017; Gabriel et al., 2012). This approach is known as "country starter," which includes microbial cultures from different sources in the fermentation process for a period between 18 and 30 h (Portugal et al., 2016).

2.2. Ethanol fermentation

In ethanol fermentation, it is common to use the industrial yeasts *S. cerevisiae* PE-2 or CAT-1 because they exhibit the best performance in stressful conditions of fermentation and better yields when compared to other fermentation yeasts (Amorim et al., 2011; Bassi et al., 2018; Reis et al., 2013). The strain PE-2 presents specific genomic characteristics for this process, mainly regarding the telomeric and subtelomeric regions of the chromosomes, which explains the adaptation of this yeast to different processes. CAT-1 presents genomic regions with loss of heterozygosity, with 58% of the protein-coding gene alleles that are different from the reference strain S288c. Compared with baker's yeast, both PE-2 and CAT-1 strains have a higher number of gene copies for pyridoxine (vitamin B6) and thiamine (vitamin B1) biosynthesis. This characteristic may confer better fitness in fermentation media with a high concentration of sugars and a low content of vitamins, such as sugarcane juice must. In addition, the CAT-1 strain demonstrates a greater lag phase than S288c in this type of fermentative environment (Lopes et al., 2016).

The production of bioethanol subjects the yeast to stressful conditions during the fermentation stage, including high temperature and alcohol concentration, increased osmotic pressure, and low pH, among other factors (Bassi et al., 2018). Bioethanol fermentation in Brazil is also characterized by yeast recycling, in which the yeasts are collected after fermentation and treated for a new fermentation cycle. Yeast recovery consists of separating the cells from the fermented must by centrifugation and subjecting them to sulfuric acid. The conditions for recovery also generate a stressful environment, thus requiring the need of selected strains that can resist to such conditions (Bassi et al., 2018; Santos et al., 2021). In summary, the characteristics of the fermentation process directly influence the biological features of the yeasts that can persist in industrial fermentation for bioethanol production. This is coherent with the general observation that industrial strains are more robust and tolerant than laboratory and bakery strains, which reinforces the importance of selecting high-performance strains according to the fermentation process of each distillery (Lopes et al., 2016).

3. Contamination and impacts

In the industry, for both cachaça and ethanol production, there is no sterilization step, thus preventing the must from reaching the fermentation stage free of microorganisms (Alcarde, 2017; Lopes et al., 2016; Paraluppi & Ceccato-Antonini, 2019). In the reality of sugarcane industries, there is a range of yeasts and bacteria that accompany the raw material from its collection in the field and perpetuate throughout the subsequent production stages. The types of microorganisms may vary throughout the process, and the main genera that have been identified directly in the raw material include *Arxiomyces*, *Alternaria*, *Bacillus*, *Bipolaris*, *Chaetomium*, *Coniochaeta*, *Curvularia*, *Cryptococcus*, *Debaryomyces*, *Drechslera*, *Exserohilum*, *Kerinia*, *Lactiplantibacillus*, *Limosilactobacillus*, *Leuconostoc*, *Leptosphaeria*, *Pichia*, *Torulaspora*, *Tremella*, and *Saccharomyces* (Costa et al., 2015).

3.1. Yeast contamination

In some studies, researchers divide contaminating yeasts into two groups: non-*Saccharomyces* yeasts, which include all yeasts that do not belong to this genus, and wild strains of *Saccharomyces*, which were not developed especially for industrial fermentation processes. The wild strains of the genus *Saccharomyces* are the most abundant in the environment and are capable of dominating the fermentation process (Costa et al., 2015; Pandey et al., 2019). Non-*Saccharomyces* yeasts have poor fermentative performance, and their uncontrolled growth can lead to high production of acetic acid, glycerol, acetaldehyde, hydrogen sulfide, and ethyl carbamate (Portugal et al., 2016).

The contaminating yeast species of cachaça and ethanol fermentation, according to the consulted literature, pertain to the genera *Dekkera*, *Torulaspora*, *Saccharomyces*, *Candida*, *Pichia*, *Wickerhamomyces*, *Meyerozyma*, *Hanseniaspora*, and *Schizosaccharomyces* (Alcarde, 2017; Bassi et al., 2018; Brexó et al., 2018; Chamnipa et al., 2018; Conceição et al., 2015; Lopes et al., 2016; Pandey et al., 2019; Pongcharoen et al., 2018; Portugal et al., 2016).

Interestingly, when comparing the number of studies, a greater amount was dedicated to characterize contaminating yeasts from cachaça production than from ethanol fermentation. The contaminating yeast species for ethanol and cachaça fermentation belonging to these genera are described in Table 1.

Contamination by wild *Saccharomyces* yeasts raises significant concerns in industries, as there is no way to control these microorganisms without affecting the strains selected for fermentation. Furthermore, contaminating yeasts can interfere with the fermentation outcome. Wild strains of *Saccharomyces* can present flocculation, slow fermentation, promote lower yields, and generate a higher concentration of residual sugar (Reis et al., 2013). Within the group of non-*Saccharomyces* yeasts, an important contaminant is *Dekkera bruxellensis*. This yeast is associated with contamination in the final stage of fermentation as well as the production of acetic acid. *D. bruxellensis* can also use the ethanol produced by the industrial yeast as a carbon source, directly interfering with the yield of the produced ethanol. In the production of beverages, *D. bruxellensis*, when consuming ethanol, produces phenolic compounds that contribute to unwanted sensory characteristics in the final product (Branco et al., 2019).

In the case of cachaça fermentation, which uses natural fermentation and mixed fermentation, the microbial community can present different compositions throughout the fermentation cycles (Alcarde, 2017). It is believed that the adverse conditions of fermentation, coupled with the diversity of yeasts present in the process, promote competition between microorganisms in the hope that they will present unique characteristics and stand out (Conceição et al., 2015). However, microorganisms in spontaneous fermentation can, in addition to interfering with the quality of cachaça, promote inconstant fermentations and difficult-to-control populations of unwanted contaminants. "Caipira" or natural yeast can also generate unwanted acidity in the beverage as well as increase its higher alcohol content. These unselected yeasts also have low ethanol tolerance, lower growth rates, and low productivity (Alcarde, 2017).

3.2. Bacterial contamination

Bacterial contamination in the alcoholic fermentation of sugarcane must is a well-known issue in the industry and is widely studied in order to solve the problems that these microorganisms can cause to the final product. There is a range of bacteria from different genera with distinct metabolisms that coexist since the entry of the raw material into the industry and perpetuate until the fermentation stage (Basso et al., 2014; Bonatelli et al., 2017).

There is a consensus in the literature that the group of bacteria in greater numbers in the fermentation of cachaça and ethanol belong to the *Lactobacillaceae* family, including members of several genera, such as *Lactiplantibacillus*, *Limosilactobacillus*, *Leuconostoc*, *Scheleiferilactobacillus*, *Weissella*, *Oenococcus*, *Liquorilactobacillus*, *Lactobacillus*, and *Lacticaseibacillus* (Badotti et al., 2014; Bassi et al., 2018; Carvalho et al., 2015; Carvalho-Netto et al., 2015; Costa et al., 2018; Gomes et al., 2010; He et al., 2021; Lacerda et al., 2011; Torres-Guardado et al., 2022). However, microorganisms, such as *Bacillus*, *Pseudomonadaceae*, *Streptococcaceae*, *Ruminiciccaceae*, *Propionibacterium*, *Lachnospiraceae*, *Stenotrophomonas*, *Acetobacter*, *Sphingobacterium*, *Citrobacter*, *Thermus*, *Pediococcus*, *Anaerosporobacter*, *Enterobacteriaceae*, and *Streptococcus*, belonging to genera of other families may also be found (Brexó et al., 2018; Bonatelli

Table 1. Main contaminating yeasts in the alcoholic fermentation of ethanol and cachaça.

Contaminating yeasts			
Cachaça	**Reference**	**Ethanol**	**Reference**
Dekkera	(Alcarde, 2017; Brexó et al., 2018)	*Dekkera bruxellensis*	(Reis et al., 2018; Bassi et al., 2018)
Torulaspora delbrueeckii		*S. cerevisiae*	(Pandey et al., 2019)
S. cerevisiae	(Conceição et al., 2015)	*Candida tropicalis*	(Costa et al., 2015; Lara et al., 2014; Pandey et al., 2019)
Candida	(Alcarde, 2017; Brexó et al., 2018)	*C. intermedia*	(Lara et al., 2014)
C. intermedia	(Brexó et al., 2018)	*C. glabrata*	(Pandey et al., 2019)
C. parapsilosis		*C. dubliniensis*	
Meyerozyma guilliermondii	(Alcarde, 2017; Brexó et al., 2018; Conceição et al., 2015; Portugal et al., 2016)	*Pichia manshurica*	(Costa et al., 2015)
		Wickerhamomyces anomalus	(Pandey et al., 2019)
Pichia fermentans	(Portugal et al., 2016)	*Meyerozyma caribbica*	(Limtong et al., 2014)
Pichia kudriavzevii	(Pongcharoen et al., 2018)	*Cyberlindnera fabianii*	
P. manshurica	(Brexó et al., 2018)	*Meyerozyma guilliermondii*	(Lara et al., 2014; Limtong et al., 2014)
Wickerhamomyces anomalus	(Alcarde, 2017; Brexó et al., 2018; Conceição et al., 2015)	*Pichia kudriavzevii*	(Chamnipa et al., 2018; Pongcharoen et al., 2018)
		Kodamaea ohmeri	(Chamnipa et al., 2018)
Hanseniaspora guilliermondii	(Portugal et al., 2016)	*Schizosaccharomyces*	(Lopes et al., 2016)
		Ogata thermophilus	(Pandey et al., 2019)

et al., 2017; Carvalho et al., 2015; Carvalho-Netto et al., 2015; Costa et al., 2015; Lacerda et al., 2011; Tiukova et al., 2014; Torres-Guardado et al., 2022). The species of contaminating bacteria that can be found in the fermentation of ethanol and cachaça belonging to these genera are described in Table 2.

The primary group of bacteria contaminating the fermentation of cachaça and ethanol belong to the *Lactobacillaceae* family, which are lactic acid bacteria (LAB). This group can be divided into homofermentative bacteria, which can transform hexoses into lactic acid from available sugar, and heterofermentative bacteria, which produce both lactic acid and acetate and carbon dioxide (Basso et al., 2014). Although most genera pertaining to the *Lactobacillaceae* family are the most abundant at this stage, the bacterial communities found in the fermentation process present a great diversity of genera, in addition to small differences in their composition from distillery to distillery (Bonatelli et al., 2017; Nel et al., 2019).

Among the species of the *Lactobacillaceae* family, the species *Limosilactobacillus fermentum* (formerly *Lactobacillus fermentum*) is one of the main contaminants in sugarcane juice must. *L. fermentum* preferentially consumes fructose from the breakdown of available sucrose, producing acetic acid, lactic acid, and mannitol. Contamination by this species directly influences the yield of ethanol production (Basso et al., 2014). In alcoholic fermentation, LAB compete directly with commercial yeast for available nutrients, producing organic acids instead of ethanol. It is estimated that 1% of total fermentable carbohydrates can be turned into organic acids by LAB, thus reducing the production efficiency and causing a loss of thousands of dollars per year (Firmino et al., 2020). The organic acid, along with other metabolites produced by these contaminants, can inhibit industrial yeast and slow or even stop fermentation. In some cases, it is necessary to interrupt fermentation to clean and disinfect the line or even add more substrate and yeast (Firmino et al., 2020).

One of the biggest challenges for the industry is the identification of spoiled sugarcane shipments before they go into processing, since spoilage microorganisms can follow throughout the entire process. However, a recent study demonstrated that the production of dextran, a polysaccharide produced by *Leuconostoc* spp. during fermentation, alters the fermenting must by increasing its viscosity, which can be monitored and used as an indicator of bacterial contamination in the process (Nel et al., 2019).

In cachaça fermentation, contaminating bacteria promote parallel fermentations and generate unwanted secondary components such as organic acids, aldehydes, higher-order alcohols, and gum. They are also responsible for promoting yeast flocculation and producing toxic substances in industrial yeasts, directly impacting the drop in fermentation speed and decreasing yeast viability (Alcarde, 2017). Studies on the interaction of *S. cerevisiae* with *Liquorylactobacillus vini* demonstrated that the flocculation process also occurs in bioethanol fermentation with the interaction of the yeast *D. bruxellensis*. The gums are formed by a bacterial nucleus surrounded by yeasts, in which *D. bruxellensis* forms pseudohyphae that project themselves throughout the *S. cerevisiae* gums, resulting in a monolayer cap around the bacterial nucleus (Tiukova et al., 2014).

Table 2. Main contaminating bacteria in the alcoholic fermentation of ethanol and cachaça.

Contaminating bacteria			
Cachaça	**Reference**	**Ethanol**	**Reference**
Lactiplantibacillus plantarum	(Carvalho et al., 2015; Torres-Guardado et al., 2022)	*Lactobacillaceae*	(Carvalho-Netto et al., 2015)
		Lactiplantibacillus plantarum	(He et al., 2021)
Limosilactobacillus fermentum	(Carvalho et al., 2015; Torres-Guardado et al., 2022)	Limosilactobacillus fermentum	(Bassi et al., 2018; Costa et al., 2018)
		Bacillaceae	(Carvalho-Netto et al., 2015)
Scheleiferilactobacillus perolens	(Gomes et al., 2010)	*Bacillus*	(Brexó et al., 2018)
Lactobacillus jensenii		*Leuconostoc*	(Costa et al., 2015)
		Liquorilactobacillus vini	(Tiukova et al., 2014)
Lacticaseibacillus casei		*Lacticaseibacillus casei*	
Lactobacillus ferintoshensis		*Pseudomonadaceae*	(Carvalho-Netto et al., 2015)
		Streptococcaceae	
Ligilactobacillus murinu	(Lacerda et al., 2011)	*Ruminiciccaceae*	(Bonatelli et al., 2017)
Liquorilactobacillus nagelii		*Propionibacterium*	
		Lachnospiraceae	
Lacticaseibacillus paracasei subsp. paracasei		*Weissella*	
		Stenotrophomonas	
Liquorilactobacillus satsumensis		*Acetobacter*	
Schleiferilactobacillus harbinensis		*Sphingobacterium*	
Streptococcus salivarius		*Lactococcus*	
Weissella	(Torres-Guardado et al., 2022)	*Citrobacter*	
		Thermus	
Lactococcus lactis	(Carvalho et al., 2015; Torres-Guardado et al., 2022)	*Anaerosporobacter*	
		Enterobacteriaceae	
Oenococcus alcoholitolerans sp	(Badotti et al., 2014)	*Oenococcus alcoholitolerans sp*	(Badotti et al., 2014)

Another negative effect caused by contaminating bacteria in alcoholic fermentation is the ability to produce biofilms and spores. Biofilm-producing microorganisms are predominant in the must in the early stages, probably due to the greater availability of sugar and the milder temperature. However, the spores formed by these microorganisms explain the reinfection of bacteria in subsequent fermentation cycles (Costa et al., 2015).

3.3. Control measures

Due to the different impacts of microbial contaminants that can arise at different stages of production, the cachaça and ethanol industries seek solutions that prevent yeasts and unwanted bacteria from compromising the yield and quality of their final product. In the cachaça industry, it is recommended that a good asepsis of the pipes and containers be carried out to control non-fermentative strains and generate recontamination outbreaks. Using selected yeasts is important to avoid losses or poor product quality. Moreover, the must should undergo heat treatment to inhibit the selected yeasts (Alcarde, 2017).

Other control measures, mainly aiming to reach the contaminating bacteria without affecting the industrial strains, include the use of acid treatment and antibiotics. Acid treatment consists of adding sulfuric acid at a pH of 2.0–2.5 to the fermentation cell mass for 1–2 h under agitation; thereafter, the cells proceed to a new fermentation cycle (Carvalho et al., 2020). However, acid control is not effective in eliminating the total bacterial population and does not affect the wild yeasts present in the must (Costa et al., 2018). Antibiotics, on the contrary, are used when acid treatment is insufficient. However, issues such as the amount of product needed and the possibility of leftover residues in yeasts that may be used for feed can affect the fermentative yeasts and are negative characteristics for their use (Costa et al., 2018).

4. Applications

Industrial yeasts present in the fermentation process are inserted in an adverse environment. They deal with the competition with other yeasts and with bacteria present in the process, as well as temperature fluctuations, low water activity, and high amounts of ethanol during this stage. All these factors can cause changes in both industrial strains and other microorganisms, which can lead to the emergence of new characteristics (Conceição et al., 2015). In this sense, several studies have been developed, not only to identify the microbiota present in the fermentation of cachaça and ethanol, but also to seek applications for these contaminating microorganisms in different biotechnological areas.

Some lines of research are dedicated to isolating and studying the physiological characteristics of specific contaminating strains and evaluating the potential for application in different bioprocesses. Some contaminating yeast strains, such as the species *Candida parapsilosis*, *Candida intermedia*, *Wickerhamomyces anomalus*, *Torulaspora delbrueckii*, and *Pichia mashurica*, were isolated from artisanal cachaça fermentation facilities to be analyzed regarding their fermentative performance. Among them, the species *Torulaspora delbrueeckii* showed considerable biotechnological potential, standing out in productivity and biomass yield during the fermentation process (Brexó et al., 2018).

The yeast species, namely, *Pichia guilliermondii* and *P. anomala*, among others, were studied for their biotechnological application, aiming to understand their physiological characteristics in order to apply them in the bioethanol process and their residues in the biofuels sector and to improve the yield of the final product. Both *P. guilliermondii* and *P. anomala* showed promising results for second-generation ethanol fermentation using sugarcane bagasse (Conceição et al., 2015).

Other lines of research dedicated to isolating specific contaminants and evaluating their effects on industrial yeasts have been carried out. An example of this type of study was the one developed by Bassi et al. (2018), in which a co-culture of *S. cerevisiae* in association with *Dekkera bruxellensis* and *Limosilactobacillus fermentum* (formerly called *Lactobacillus fermentum*), the major contaminants of alcoholic fermentation, was performed. The authors intended to analyze the interaction between the industrial yeast and each contaminant separately and together to evaluate their effects during fermentation.

During alcoholic fermentation, *S. cerevisiae* can naturally secrete saccharomycin, a compound that is capable of inducing the death of other yeasts. This substance is composed of antimicrobial peptides derived from the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Branco et al., 2017). This perspective led researchers to test concentrations of saccharomycin against contaminating yeasts in the fermentation stage, genetically modifying strains of *S. cerevisiae* to produce higher levels of saccharomycin and evaluating its potential during alcoholic fermentation. Recently, it was demonstrated that genetically modified *S. cerevisiae* strains induced the death of *D. bruxellensis* present in alcoholic fermentation (Branco et al., 2019).

Since the fermentation conditions are extreme and make the environment hostile to microorganisms, some studies have been carried out using contaminating species in an attempt to cause positive effects on industrial yeast so that it may develop mechanisms to support extreme factors throughout the fermentation process. The bacterium *Lactiplantibacillus plantarum* (formerly known as *Lactobacillus plantarum*) was co-cultured with *S. cerevisiae* in order to influence the yeast's resistance to high levels of ethanol during fermentation. This association proved positive, as *L. plantarum* was able to regulate some metabolic processes of the yeast and increase its tolerance to ethanol, showing that the impact of contaminants on fermentation can also be beneficial (He et al., 2021).

In the same line of research, Ding et al. (2021) evaluated the inhibition factors associated with LAB that compete with *S. cerevisiae*. The authors also used the LAB *Lactiplantibacillus plantarum*, limiting the factors that influence the bacteria on the yeast, to evaluate them in detail and clarify whether unknown factors also play a role in this interaction. Their results indicated that there are aspects of the inhibition of *S. cerevisiae* by *L. plantarum* that were not previously known by the researchers, such as the inhibition of the yeast by direct contact with the bacteria during fermentation. In this case, when in contact with the cell wall of *L. plantarum*, the bacteria can reduce the yeast biomass;

however, the results also indicate that when in contact with the dead bacteria, this same contact can also stimulate the yeast to increase its tolerance to ethanol and its fermentation efficiency.

5. Conclusion

The present review indicated that microbial contamination in the fermentation stage during the production of cachaça and ethanol is relevant to different industrial processes. There is a great diversity of contaminating species, although the ones that are found in greater abundance are those belonging to the family *Lactobacillaceae*. Both contaminating bacteria and yeasts can cause significant interference in the fermentation stage and in the final product, cachaça or ethanol, which makes the control of these microorganisms necessary. However, several recent lines of research have clarified the physiological characteristics and different applications of contaminating yeast and bacterial species, elucidating their importance for the improvement of *S. cerevisiae* in fermentation conditions and for the discovery of new factors that can interfere with this critical process. New research has demonstrated that these microorganisms can present interesting biotechnological applications for several productive sectors, revealing the potential of this microbiota and the importance of studying them.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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