BENEDITO RODRIGUES DA SILVA NETO
(ORGANIZADOR)

PROJETOS INOVADORES E PRODUÇÃO INTELECTUAL NA MICROBIOLOGIA

Atena Editora
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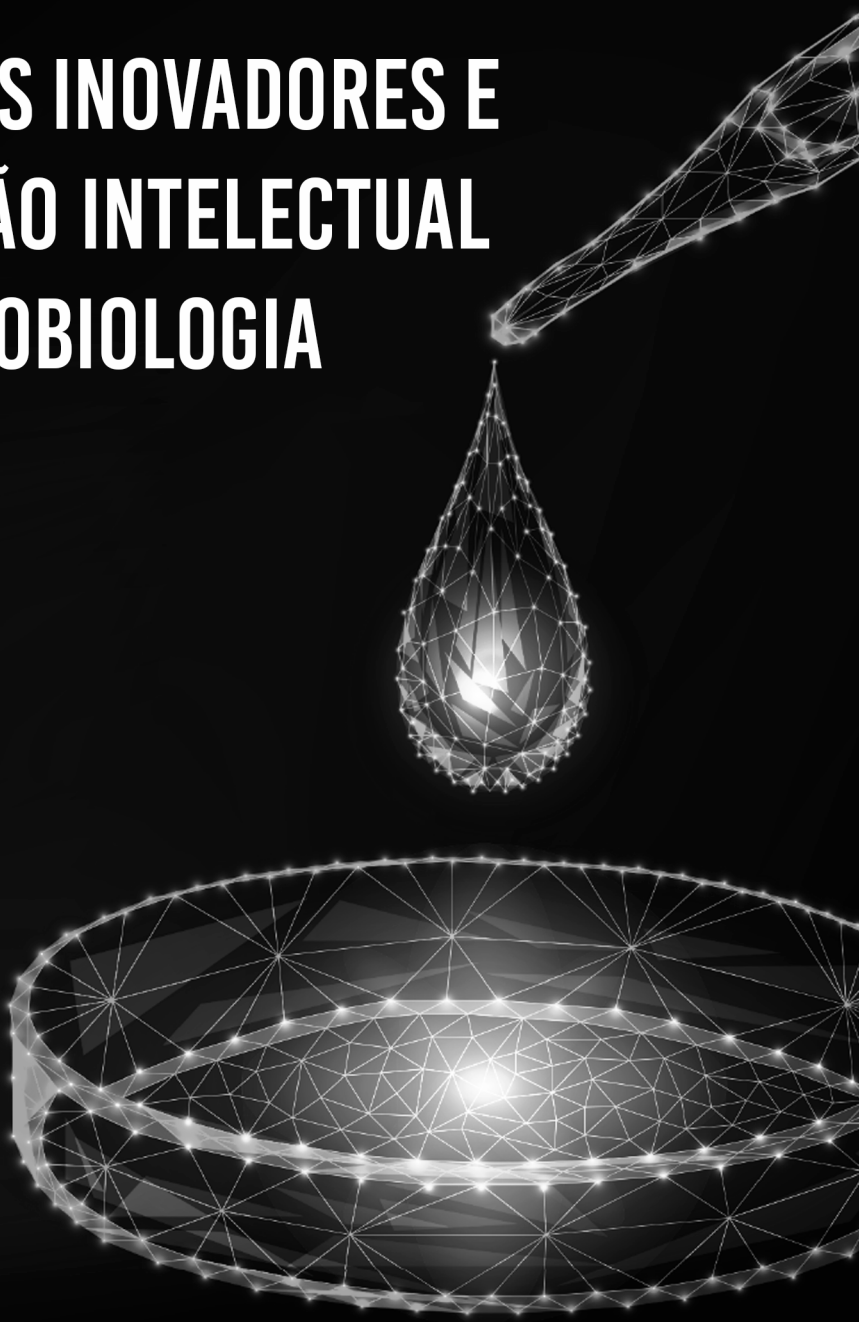

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Projetos inovadores e produção intelectual na microbiologia

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APRESENTAÇÃO

A microbiologia tem sido um assunto recorrente nos últimos anos, desde os corredores universitários aos locais informais, as conversas vão desde as bactérias multirresistentes, passando por novas espécies de fungos descobertos até chegar no atual momento de pandemia viral que marcará na história o ano de 2020. Esse campo de estudo amplo inclui o estudo dos seres vivos microscópicos nos seus mais vaiados aspectos como morfologia, estrutura, fisiologia, reprodução, genética, taxonomia, interação com outros organismos e com o ambiente além de aplicações biotecnológicas.

Como ciência, a microbiologia iniciou a cerca de duzentos anos atrás, e tem passado por constantes avanços graças a descobertas e inovações tecnológicas. Sabemos que os microrganismos são encontrados em praticamente todos os lugares, e a falta de conhecimento que havia antes da invenção do microscópio hoje não é mais um problema no estudo, principalmente das enfermidades relacionadas aos agentes como bactérias, vírus, fungos e protozoários.

A grande importância dessa temática se reflete no material de qualidade já publicado na Atena Editora e mais uma vez recebe os nossos holofotes com o tema “Projetos Inovadores e Produção Intelectual na Microbiologia” contendo trabalhos e pesquisas desenvolvidas em diversos institutos do território nacional contendo análises de processos biológicos embasados em células microbianas ou estudos científicos na fundamentação de atividades microbianas com capacidade de interferir nos processos de saúde/doença.

Temas ligados à inovação e tecnologia microbiana são, deste modo, discutidos aqui com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela saúde em seus aspectos microbiológicos. Deste modo, propomos aqui uma teoria bem fundamentada nos resultados práticos obtidos em diferentes campos da microbiologia, abrindo perspectivas futuras para os demais pesquisadores de outras subáreas da microbiologia.

Desejamos a todos uma excelente leitura!

Benedito Rodrigues da Silva Neto

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CAPÍTULO 9

INDUSTRIAL YEAST STRAINS RESISTANCE TO NATURAL BIOACTIVE COMPOUNDS

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ABSTRACT: This chapter briefly provides an overview about industrial yeast strains, more specifically about yeast strains applied in the fermentation processes in Brazilian fuel-ethanol production industry. In the first section, historical aspects of fermentation and the use of yeast strains in Brazil are discussed and the concepts about selected yeast and native autochthonous, yeast strains are lightly addressed. Subsequent sections present the molecular differentiation among yeast strains and a brief explanation about the industrial application of natural bioactives, especially essential oils. Finally, the resistance profile of 20 industrial yeast strains is reported for 40 plant-derived essential oils, extracted from Brazilian and exotic medicinal and aromatic plants.

KEYWORDS: *Saccharomyces sensu stricto* group*;* yeast strains; Essential Oils; Industrial applications

RESISTÊNCIA DE LINHAGENS DE LEVEDURAS INDUSTRIAIS A COMPOSTOS BIOATIVOS NATURAIS

RESUMO: Este capítulo fornece resumidamente uma visão geral sobre cepas de leveduras industriais, mais especificamente sobre cepas de leveduras aplicadas nos processos de fermentação na indústria brasileira de produção de etanol-combustível. Na primeira seção, aspectos históricos da fermentação e do uso de cepas de levedura no Brasil são discutidos e os conceitos sobre leveduras selecionadas e cepas de leveduras autóctones nativas são levemente abordadas. As seções subsequentes apresentam a diferenciação molecular entre cepas de leveduras e uma breve explicação sobre a aplicação industrial de bioativos naturais, especialmente óleos essenciais. Finalmente, é relatado o perfil de resistência de 20 cepas de leveduras industriais para 40 óleos essenciais de origem vegetal, extraídos de plantas medicinais e

aromáticas brasileiras e exóticas.

PALAVRAS-CHAVE: *Saccharomyces sensu stricto;* linhagens de levedura; Óleos essenciais; Aplicações industriais.

1 | FERMENTATION AND YEASTS – HISTORY AND BRIEF OVERVIEW

Humans have adopted fermentation practices to produce fermented foods and alcoholic beverages since ancient times, although the protagonist of this process stayed "invisible" for centuries, being used through empirical observations without knowing their chemical and microbiological bases (AMORIM et al., 2005).

Inventions and innovations using fermentation and yeasts were done along the history based on observations and almost no knowledge about microorganisms, including the presence of yeasts as the main element in the fermentation processes. As a matter of fact, it would be several centuries of years before it was even known that microscopic organisms existed.

Antonie van Leeuwenhoek was the first to observe cells using a primitive microscope in 1680, when the seeds of Microbiology were sown.

Since then, scientific information has grown at an exponential rate. In some fields such as Chemistry and Biology, especially in Microbiology and Biotechnology this explosion of knowledge is more evident. This progress has been impacted by some of the most significant names in the history of sciences, including van Leeuwenhoek, Lavoisier, Gay-Lussac, Pasteur, Buchner and Koch.

More recently, important names in modern science argued that "*the most important test tube in the birth and growth of the modern life sciences is the fermenter*", and "*the most important model organism has been the yeast Saccharomyces cerevisiae*", commonly known as baking yeast, brewing or wine yeast (CHAMBERS; PRETORIUS 2010).

2 | YEASTS

Yeasts have been widely studied and used to produce value-added products by different industrial segments. One clear example is the origin of the word enzyme - 'en' meaning within and 'zyme' meaning leaven. Yeasts have been an important part of pioneer works in several fields, particularly in life sciences as Microbiology and Enzymology (BARNETT, 1998, 2000; BARNET & LICHTENTHALER, 2001).

Among the yeasts, *Saccharomyces* genus should be highlighted because microorganisms inside this group were the first domesticated microorganisms. Besides that, they usually are related to several fermentation processes for industrial purposes.

In Brazil, as much as all over the world, the relevance of *Saccharomyces* genus covers most important biological systems, since it is highly employed as an eukaryotic organism model as well as widely used in industrial processes like bakery, wine industry

and brewery due to its key role in fermentative process.

However, in Brazil these yeasts go beyond the application in conventional industrial segments and have a very important role in the biofuels industry. That is the reason why it is so necessary to know the characteristics of these microorganisms and their behavior in different situations.

3 | YEASTS AND FERMENTATION PROCESSES IN BRAZILIAN FUEL-ETHANOL PRODUCTION INDUSTRY

Brazil has taken a notorious place in the biofuels worldwide scenario.

Nowadays, Brazil is the largest sugarcane´s bioethanol producer in the world, producing over 30 billion liters in 2019 (UNICA, 2019; UDOP, 2020).

Although this country has been producing ethanol since the1930s through fermentation processes, at that time serendipity presumably played an important role in the process since fermentations resulted from empirical observations of natural events. Indeed, the experience with the processes were generated in harnessing repeat 'experiments' in which the improvements were made by trialling modifications to practices and were communicated down through the generations, retaining the results that were beneficial and discarding those that failed (ALBA-LOIS; SEGAL-KISCHINEVZKY, 2010).

It was just in the 1990s that Brazilian distilleries started to use yeasts referred to as pure, purchased by manufacturers with required characteristics for the fermentation processes to industrial production of fuel-ethanol (AMORIM et al., 2011).

One might argue that the early use of yeasts in bioethanol production was based on little or no knowledge of microbiology that drives fermentation. In fact, it would be several decades before it was even known population dynamics of microorganisms in the fermentative processes.

Since then, several scientific groups have dedicated efforts to unravel the fermentation process in order to understand the major actor in this process - the yeasts.

The power of change which the understanding about these simple, unicellular microorganisms have caused in life science related to the fuel-ethanol industry could be described as a revolution.

This scientific revolution in bioethanol industry, range from prospection and identification methods, as done by Basso et al. (2008) until the potential modification for different applications described by Argueso et al. (2009) and Gu and Oliver (2009), passing through the characterization of the biodiversity of strains, as studied by Steckelberg (2001), Basso et al. (2004) and Tosetto (2008).

Nevertheless, until nowadays it is a challenge to understand completely the fermentation processes and all the dynamics related to this useful microorganism which has played a pivotal role in this process - *Saccharomyces cerevisiae*.

One of the reasons why it is difficult to reveal all the answers regarding the difficulties to be overcome in Brazilian fermentation processes could be explained by the operational conditions.

Most industrial processes installed for biofuel production in that country, use yeast cell recycling. However, this strategy promotes operations with high cell concentration, and dynamizes the fermentation, making it faster. On the other hand, these choices allow conditions favorable to grow contaminating bacteria and yeasts.

Besides that, fuel-ethanol industrial fermentations processes are subject to huge selective pressure considering oscillation in several attributes as temperature, pH, ethanol and sugar concentrations along all the process and also during the harvest season period, resulting in the growth and persistence of yeasts adapted to stress conditions.

For this reason, the fuel-ethanol industry has been incessantly trying to overcome the problems with the main bottleneck in the process – fermentation and, consequently, yeasts.

Saccharomyces cerevisiae has shown remarkable attributes and plays an important role in fermentation processes due to its versatility and capacity to act in different kinds of substrates (STRATFORD, 2006).

However, there are many differences inside the universe of *Saccharomyces* sp. This understanding just surfaced because of the explosion of knowledge sparked by the advent of gene technology which drove a convergence of several fields of life sciences, under the banner of molecular biology (CHAMBERS; PRETORIUS 2010).

Molecular biology brought light to several parts of biology, clarifying several processes and explaining the development of some microorganisms, including the yeasts. This newest approach enabled us to understand them and explain why yeasts inside of the same species might present a completely different characteristics and behavior in fermentation. So, the molecular biology tools allowed us to know different strains inside the group of *Saccharomyces sensu stricto.*

Therefore in the transition from the 20th to 21st century, researchers dedicated to unveil the Brazilian fermentation processes on fuel-ethanol production and selected some yeast strains, belonging to *Saccharomyces sensu stricto* group, from different industrial units. From that, five strains (BG1; CAT1; FT858; PE2 and SA1) are being produced and commercialized to be used as starter yeast inoculum at the beginning of the harvest season period.

According to Andrietta et al. (2011) the names of these strains are related to their original industrial unit from which they were isolated. Thereby, BG1 strain correspond to a yeast strains isolated from Usina Barra Grande de Lençóis S/A; whereas CAT1 strain was isolated from Catanduva unit, belonging to Virgolino de Oliveira S/A Açúcar e Álcool Group; PE2 strain was selected from Pedra Agroindustrial S/A unit and SA1 strain was originally isolated from Usina Santa Adélia S/A unit. Differently from these four strains, FT858 strain

was named after its isolation by Fermentec Ltda, a biotechnology Brazilian company. All these industrial units, as well as Fermentec, are located in the state of São Paulo in Brazil.

Thus, Brazilian distilleries, in the early 2000s, started to use these selected industrial yeast strains in their fermentation processes.

In 2006, on the study presented by Basso et al. (2008), it was mentioned that 190 bioethanol plants among the 329 Brazilian distilleries were using industrial selected yeast strains.

In the last decade, our group compiled the information about the yeast strains used as inoculum to start up the alcoholic fermentation at the beginning of harvest season by 125 Brazilian industrial units, from 2011 to 2018, as shown in Figure 1.

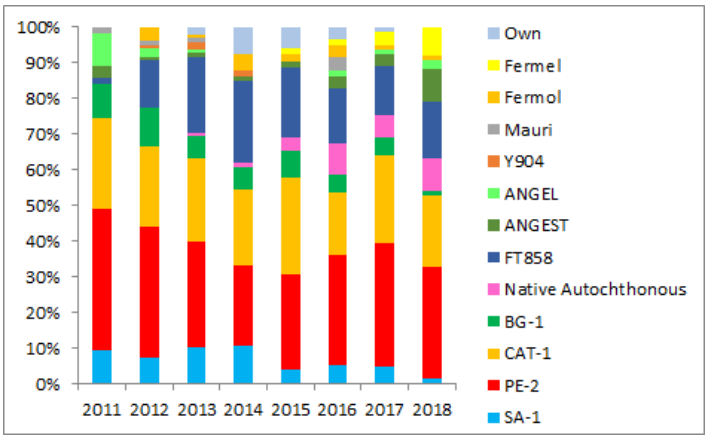

Fig.1 Historical use of starter yeasts strains in Brazilian fuel-ethanol production processes in the harvest season periods from 2011 to 2018. All the terms used to refer to the yeasts are abbreviations or commercial names, except to “Native autochthonous” and “Own”. The expression “own” yeast is used to refer to a mixture of yeasts stored at the end of one harvest period and used in the next harvest season period as inoculum, without any identification or characterization. All these information was sourced by personal communication with 125 the Brazilian distilleries during the period evaluated.

Figure 1 shows the historical use of yeast strains to start up the alcoholic fermentation in 125 Brazilian fuel-ethanol production processes, along the harvest season periods from 2011 to 2018. The names of the yeasts inside the figure refer to its commercial names. We referred to “Native autochthonous”, yeasts which were isolated from each industrial fermentation process, identified and characterized previously. The expression “Own” is used to refer to a mixture of yeasts stored at the end of one harvest period and used in the next harvest season period as inoculum without any identification or characterization. In this way “own” does not refer to one specific strain, it is a mixture of undetermined strains. All this information was based on personal communication with 125 the Brazilian distilleries

during the period evaluated.

As mentioned above, generally, the hard conditions in fermentation processes in the industrial context lead to a huge selective pressure over the microorganisms, especially yeasts, originating a driven natural selection of strains.

Despite of the continuous use of selected yeast strains in the industry, several researches related to yeasts population dynamics in Brazilian fermentation processes along the harvest season, have shown the replacement of these strains, as put forth by Steckelberg et al. (2017).

New approaches of yeast strains use have arisen from this deeper knowledge about the yeast strains population dynamics.

Recently, indigenous yeast strains, previously considered as a problem in the fermentation processes, once they replaced selected yeast strains during the harvest season period, were reclassified as native autochthonous yeast lineages with high potential to be used after identification and characterization analysis.

So the fermentation processes became a huge source of biodiversity and possible solutions to improve their own processes.

For this reason, in this study we used twenty yeast strains with three different profiles: 6 baker's yeast strains; 6 selected yeast strains and 7 native autochthonous yeast strains in order to evaluate, comparatively, their resistance profile under the action of 40 different essential oils. We also evaluated one yeast strain named CLE isolated from one Brazilian industrial unit more than 20 years ago, characterized, stored in the Bioprocess laboratory and used as a fermentation standard.

All the strains were previously evaluated considering their genetic identification in a sub specific level using molecular fingerprint tools. In the next session we will present the yeast strains differentiation.

4 | YEAST STRAINS DIFFERENTIATION

Our study involved twenty microorganisms belonging to *Saccharomyces sensu stricto* group, divided into three different classes: 1) baker's yeast strains, which were used exclusively in the beginnings but they are also used nowadays; 2) commercial yeast strains (named and described above as selected yeast) including ANGEL yeast strain, which is a Chinese strain popularly used in Brazil as inoculum and 3) native autochthonous yeast strains.

The baker's yeasts strains were isolated from baker's yeast samples commercially used in Brazil. Likewise, the commercially selected yeast strains were isolated from commercial yeast samples produced in large scale and commercialized in Brazil.

On the other hand, the native autochthonous yeast strains were isolated from yeast samples from fermentation processes in different industrial units in Brazil. The fuel-ethanol

production industrial plants which provided the samples are located in four Brazilian states: São Paulo, Minas Gerais, Mato Grosso do Sul e Tocantins, the former three belonging to the main Brazilian biofuel production axis.

The ARO and CVG strains were isolated from two different industrial units, located in Minas Gerais; whereas the SM strain and RV strain were isolated from two different industrial units located in São Paulo; SF and TFT strains were isolated from fermentation processes located in Mato Grosso do Sul and finally BPA strain were isolated from fuel-ethanol production industry located in Tocantins. All of these yeast strains were previously evaluated in fermentation assays by the Bioprocess Laboratory team at Unicamp and were classified as satisfactory or superior performance in fermentation tests.

To proceed the differentiation protocols, firstly all yeast strains were grown using spread-plating technique, in which a small sample is spread over the surface of an agar plate in order to evaluate the biotypes, i.e., we evaluated the morphology of discrete colonies formed across the surface of the WL Nutrient medium agar (WLN, DIFCO 242420).

After that, we used a streak-plating technique to check the purity of the culture of yeast. Then, single colonies, which are comprised of millions of cells growing in a cluster on an agar plate, were transferred to another plate using spread-plate technique. Thus, the pure microorganisms were grown in petri dishes with Potato Dextrose Agar medium (PDA, DIFCO 213400) to be submitted to DNA extraction protocol (BIDENNE et al., 1992 modified; OAKLEY-GUTOWSKI et al., 1992 modified; ARGUESO, et al. 2008).

All isolates were distinguished by electrophoretic karyotyping profile obtained from pulsed field gel electrophoresis (PFGE). This technique consists in the separation of intact chromosomal DNA according to its size on a gel matrix of agarose. According to the number and size of the chromosomes present in each strain, specific banding patterns were obtained.

In a different approach, considering conventional DNA electrophoresis which is able to separate molecules of up to 50 kilobases, PFGE is able to do the separation of large DNA molecules, as yeast chromosomes, which range from several hundred to several thousand kilobases (ZIMMERMAN; FOURNIER, 1996). This separation is possible because this technique uses an electric field that periodically changes direction in a gel matrix of agarose.

Unquestionably, nowadays there is an immense variety of molecular techniques for the identification of microorganisms. Nonetheless, not all of them are able to have sufficient sensitivity to distinguish some microorganisms at the sub-specific level, i.e., by distinguishing among different strains.

According to VILANOVA et al. (2007), PFGE has a greater discriminatory power when compared to mtDNA restriction analysis for *Saccharomyces cerevisiae* yeast clones. Therefore, this greater resolution in the power of differentiation among strains makes it better suited for the detection of genetic diversity in yeasts.

For this reason, we used PFGE to do the differentiation of all yeast strains. Below

we present the DNA electrophoretic profile for two groups of yeast strains evaluated: commercial selected yeast strains and native autochthonous yeast strains (Figure 2). The terms used to name commercially selected yeast strains were discussed above. The native autochthonous strains were named according to the protocol used in Bioprocess Laboratory, at Unicamp, which names yeast strains considering their original place of isolation.

The DNA electrophoretic profiles of all baker's yeast strains evaluated were presented by Kitaka et al. (2020) in the study "*Sturdiness of baker's yeast strains to natural bioactive compounds"*. The expressions used to name the baker's yeast groups were established based on the commercial description: (ADY: Active dry baker's yeast; SDY Sweet dough baker's yeast; SY strong baker's yeast).

The Figure 2 presents the genomic DNA electrophoretic profile of all industrial yeast strains, which allows the differentiation among yeast strains belonging to *Saccharomyces sensu stricto* group. From the (A) to (F) were demonstrated the genomic DNA profiles of commercially selected yeast strains, whereas from (G) to (M) present DNA profiles of native autochthonous yeast strains and in (N) the DNA profile of CLE standard strain, stored in laboratory for more than 20 years.

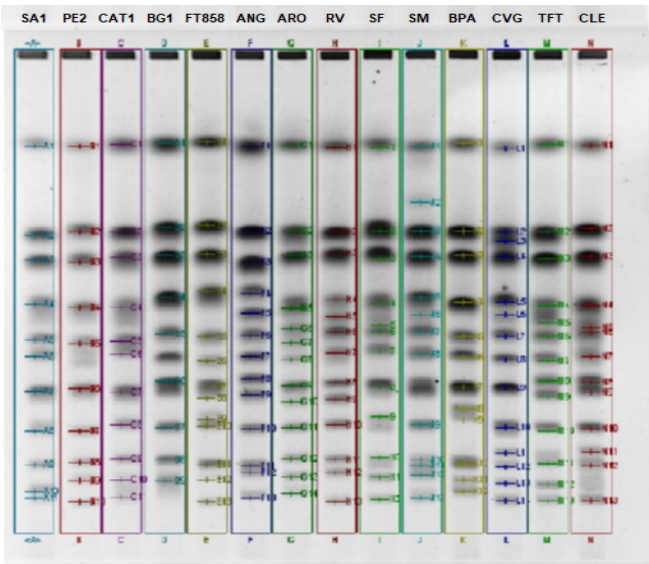

Figure 2: Genomic DNA electrophoretic profile of yeast strains. Differentiation among yeast strains (*Saccharomyces sensu stricto* group) obtained using PFGE (*Pulsed Field Gel Electrophoresis*) in agarose gel 0.8%. Image acquisition by UVP Vision Works LS system. (A) SA1 strain; (B) PE2 strain ;(C) CAT1 strain; (D) BG1 strain; (E) FT858 strain; (F) ANG strain; (G) ARO strain; (H) RV strain; (I) SF strain; (J) SM strain; (K) BPA strain; (L) CVG strain; (M) TFT strain; (N) CLE strain.

After the molecular differentiation test using PFGE analysis, twenty strains were

established as different yeast strains. All of them were tried with 40 plant-derived essential oils. The subsequent sessions explain why evaluate industrial yeasts strains in the face of essential oils. They also summarize some findings from our studies with yeast strains' resistance to these compounds.

5 | WHY NATURAL PRODUCTS AND INDUSTRIAL YEAST STRAINS

As mentioned above, yeasts, particularly yeasts strains belonging to *Saccharomyces sensu stricto* group, are widely used in different industrial segments.

Fuel-ethanol production industry, as well as other industrial segments, has positioned itself in the context of a more modern approach, generating a demand for natural products solutions and the development of eco-friendly technologies.

Throughout the history of Brazilian fuel-ethanol production, this industrial segment has been continuously using antibiotics in order to control bacterial contamination in their fermentation processes. Some of these antibiotics are the same consumed by humans and are posing a serious problem, since the promiscuous use of antibiotics increases the selective pressure over antibiotic resistance genes. This selective pressure caused by this practice facilitates the transference antibiotic resistance genes to pathogenic organisms and explains the growing concern about the effects of the indiscriminate use of antibiotics in human health (ALLEN et al., 2010).

In addition, traces of these antibiotics may be found in ethanol byproducts. Dry yeast is sold as a source of protein for animal feed preparation. Brazil, as a leading exporter of this protein, faces the customary rejection of this product by the European Community. This is because of antibiotics traces which are found in dry yeasts (GORDON, 2009).

Therefore, the replacement of antibiotics' use in the fermentation processes for bioethanol production is paramount. Hence, the replacement should entail the use of substances that do not turn into harmful residue to the environment and, as a consequence, a hazard to human health.

Essential oils (EO) are considered an important natural source of substances with antimicrobial activity, some of them are discussed by Burt (2004) and Kitaka et al. (2019).

Besides the antimicrobial activity, the fact that some EO are used commercially in several industrial segments, as food industry, pharmaceutical and / or cosmetic, demonstrates interesting technological characteristics of essential oils, as they do not become harmful residues to the environment and human health (BAKKALI et al., 2008). Thus, these oils may become a solution to do this replacement.

These facts motivated the investigation of *Saccharomyces sensu stricto* resistance in the face of EO, as a screening evaluation test in yeasts, in order to develop a technology to serve as an alternative solution to replace antibiotics to control contamination in alcoholic fermentation processes.

6 | RESISTANCE OF YEAST STRAINS TO ESSENTIAL OILS

This session presents some concepts about plant-derived essential oils and summarizes some findings from our studies about the resistance of industrial yeast strains to these compounds.

According to Roller (2003), plants produce a range of antimicrobial compounds. Some of them are always present in the plants, while others are produced as a response against microorganisms and physical injuries. The production of these phytochemicals as a stress condition response to combat pathogenic infections is mentioned by several authors (THEIS; LERDAU, 2003). Inside of this phytochemicals group are the essential oils (EO).

Essential oils are a mixture of several compounds, with different chemical origin. Their composition varies and could include terpenes, alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfides. These complex aromatic and volatile mixtures could be obtained from different plant materials such as leaves; flowers; buds; roots and barks (GUENTHER, 1948; BURT, 2004).

In this study, 40 plant-derived essential oils were used, which were described by Kitaka (2018; 2019). These essential oils were extracted from plants belonging to the Medicinal and Aromatic Plant Collection (CPMA) of Chemical, Biological and Agricultural Multidisciplinary Research Center (CPQBA) at University of Campinas (UNICAMP), in Brazil.

The resistance of the 20 different industrial yeast strains, previously identified and differentiated, was established considering their ability to grow in the face of different essential oils, in all concentrations evaluated.

The resistance was inferred from the growing ability considering the inhibitory effect of the essential oils (EOs) using the microdilution test and determining Minimal Inhibitory Concentration - MIC (NCCLS, 2002) for each of the 40 essential oils. The Figure 3 summarizes the results from the experiments with the 40 EOs in different concentrations, ranged from 2mg/mL to 1µg/mL in which we catalogue the resistance profiles of all yeast strains studied.

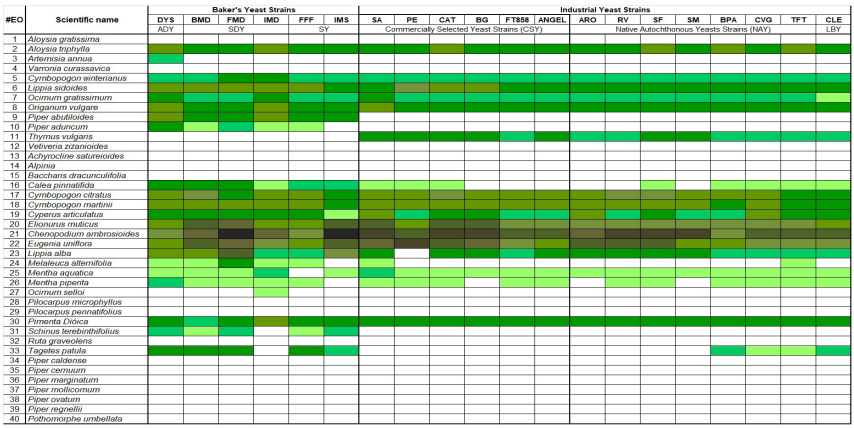

Figure 3 Minimal inhibitory concentration (MIC) - Effect of plant-derived essential oil on yeast strains growth

Considering all of the 20 yeast strains, they presented an average resistance of 57.7% to all oils tested. The Figure 4 illustrates the resistance profile of all the yeast strains evaluated. In (A) the profile of strains referred as baker's yeast strains shows an average resistance of 51.7%, the worst in comparison to the other groups of yeast strains. The lowest resistance inside this group achieved 45% in DYS strain. The comparative study in this group of yeast strains were discussed by Kitaka et al. (2020).

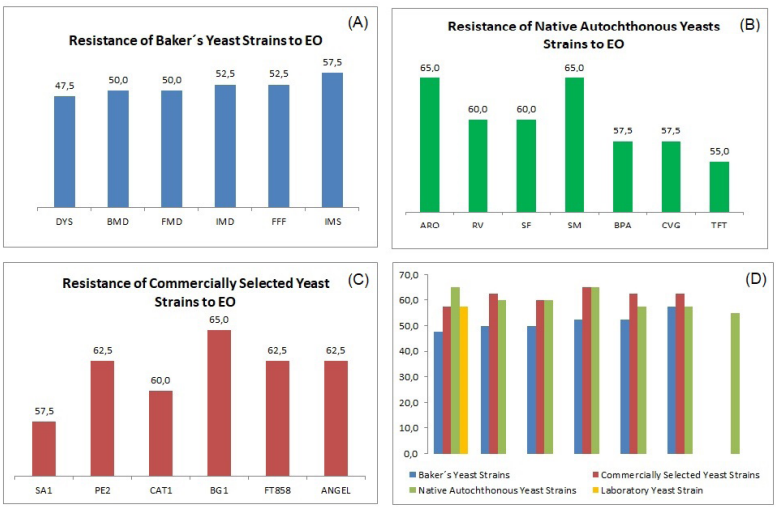

Figure 4: (A) Resistance of Baker's Yeast Strains to EO; (B) Resistance of Native Autochthonous Yeast Strains to EO; (C) Resistance of Commercilly Seleted Yeast Strains to EO; (D) Comparison of resistance profile to EO among all yeast strains evaluated.

Contrasting the baker's yeast strains group, for the other two groups, (commercially selected yeast strains (C) and native autochthonous yeasts strains (B)), there was no convergence among resistance or susceptibility profile, comparing the strains inside each group. For both groups the average resistance was similar, 61.7% and 60.0% respectively.

In the native autochthonous yeasts strains group (B), we should highlight ARO strain (isolated from fermentation process located in Minas Gerais) and SM strain (isolated from fermentation process located in São Paulo), both presenting 65% of resistance to all essential oils evaluated. Inside this group, the strain with the worst resistance performance was TFT, isolated from fermentation process located in Mato Grosso do Sul, which is resistant to 55% of all EO studied.

Similarly, in the group of commercially selected yeast strains (C), there was no uniformity with the resistance profile of the strains, although all of them have shown higher resistance profile. In this group BG1 strain should be highlighted with 65% of resistance, followed by PE2, ANGEL and FT858, all with 62.5% of resistance.

In Figure 4 (D) it is possible to notice the resistance performance of one laboratory yeast strain, named CLE. It was resistant to 57.5% of all essential oils tested. Despite of the fact that this yeast strain has been stored in laboratory conditions for more than 20 years, it seems to have kept, in general, the pattern of resistance observed in native yeast strains, since it was also isolated at one Brazilian industrial unit and characterized (in the same way of native autochthonous yeast strains).

Taking into consideration the activity of all EOs over yeast strains, as shown in the pie chart (Figure 5), it is possible to notice that, for 52% of the EO, there was no antimicrobial activity for all yeast strains tested.

Contrastingly, the remaining 48% EOs have shown activity on the yeast strains. Among these EOs, 35% presented activity for all strains while 13% acted differentially, presenting activity for certain strains.

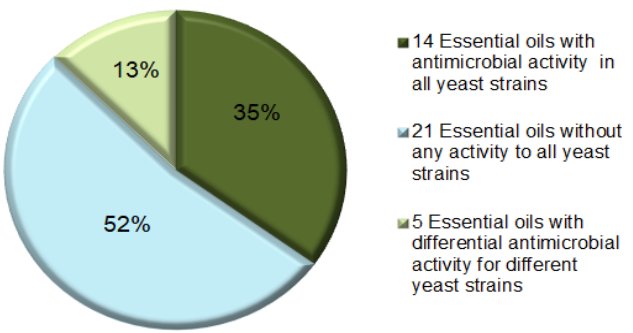

Figure 5: Antimicrobial activity of essential oils in industrial yeast strains

As the baker's yeast strains' robustness were previously discussed by Kitaka et al. (2020), herein, we will discuss the effect of the essential oils in the other strains, referred to as industrial strains. All these strains were affected by EO of *Aloysia tryphylla; Cymbopogon winterianus; Lippia sidoides; Ocimum gratissimum; Origanum vulgari; Cymbopogon citratus; Cymbopogon martini; Cyperus articulatus; Elyonurus muticus; Dysphania ambrosioides; Eugenia uniflora; Mentha aquatica and Pimenta dióica*.

Distinctly, most of the industrial yeast strains have presented slight inhibitory growth pattern in the face of *Melaleuca alternifolia*, *Mentha piperita* and *Tagetes patula* essential oils, in which concentrations can reach 0,5mg/mL.

The EOs under which all the industrial yeasts strains have shown resistance, were presented by Kitaka et al. (2018) and were screened to be evaluated in other microorganisms isolated from fuel-ethanol fermentation processes.

Comparison among the three groups of yeast strains shows that baker's yeast group presented the lowest resistance and the major uniformity in the resistance profile.

Both selected yeast group and native autochthonous yeast strains group presented strains highly resistant to most of the essential oils. As the strains inhabiting the fermentation processes are mainly composed of selected yeast strains and native autochthonous yeast strains, the use of some EOs could be a possibility, considering the previous screening, as we showed here.

7 | FINAL CONSIDERATIONS

This chapter provided the characterization of several yeast strains used as inoculum in Brazilian fermentation processes in the fuel-ethanol production industry. These strains include: baker's yeast strains; commercially selected yeast strains and native autochthonous yeasts. The characterization involved the genetic differentiation among strains and the establishment of resistance profile using essential oils obtained from native Brazilian plants and exotic ones.

However there are uncountable studies using essential oils as potential antimicrobials and antioxidant, extremely few show their application in processes using *Saccharomyces sensu stricto* as fermentation platforms.

This chapter discussed both the resistance of different yeast strains groups in the face of several EOs and the comparison among them.

Overall, the results of yeast strains resistance presented a convergence, i.e., the same EO inhibitory effect was observed in different strains for one specific essential oil.

Although there is not a general convergence of results regarding the resistance of all the strains to all the EO, it was possible to observe the convergence of findings in the resistant profile by the strains against one specific EO.

Furthermore, for some EO, the results of yeast strains resistance point toward a

similar pattern. This similarity might allow a prediction of the effect of EOs extracted from plants belonging to the same plant family or genus. Some convergent examples are related to the sensitivity profile of yeast strains in the face of the essential oils in which there was a high harmful effect.

One example is observed in the inhibitory effect of EOs 17 and 18, extracted from plants belonging to the *Cymbopogon* spp. genus (Poaceae family) which presented similar harmful effects against all the yeast strains.

In other cases, as *Elyonurus muticus*; (20); *Dysphania ambrosioides* (21) and *Eugenia uniflora* (22), although essential oils were not extracted from plants belonging to the same family or genus, the harmful effect in one specific strain could give us a clue about the higher toxicity effect for all the industrial yeast strains.

In short, not only the findings of resistance in industrial yeast strains but also the essential oils inhibitory effect has implications on technological applications for industrial segments that use *Saccharomyces sensu stricto* strains. Finally, these results open new perspectives for applications of bioactives as plant-derived essential oils, especially in the industrial context.

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