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Evolutionary adaptation of yeasts for lignocellulosic ethanol production: A review

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ABSTRACT
Evolutionary adaptation is a process where an organism undergoes physical or chemical selective pressure, either continuously or intermittently, to promote mutations or adaptive changes that enable the selection of individuals suited to a predetermined biotechnological objective. This review focused on sources that explore the evolutionary adaptation of pentose-fermenting microorganisms to establish resilience or tolerance to inhibitory or cytotoxic compounds that arise during the pre-treatment or processing of lignocellulosic biomass. The study considered articles published within the past 10 years and accessible through Science Direct, Google Scholar, and PubMed databases. The data indicates that yeasts' evolutionary adaptation is a reliable and frequent procedural technique due to its success in enhancing alcoholic fermentation of pentoses from lignocellulosic hydrolysates. This process enhances the genetic makeup of microorganisms in the challenging conditions of hydrolysates, boosting their ability to withstand cytotoxic substances and fermentation inhibitors. This, in turn, leads to an increase in ethanol yield and volumetric productivity.

Keywords: Pentose fermentation, 2G ethanol, Evolutionary engineering, Acclimatization.

1 INTRODUCTION

Evolutionary adaptation or evolutionary engineering consists of subjecting a microorganism to certain selective pressures that induce genotypic modifications and result in individuals or populations capable of generating desirable responses, even in the presence of stressors (MENEGON; GROSS; JACOBUS, 2022). Moremi, Rensburg and Grange (2020) and Ndubuisi *et al*. (2023) mention that this

technique can be employed to increase the efficiency and productivity of alcoholic fermentation by adapting the microorganism to different cultivation conditions. Over the years, researchers have been studying adaptation as a strategy capable of minimizing cytotoxic effects and improving the yield and productivity of the lignocellulosic ethanol production process.

Lignocellulosic ethanol or second-generation ethanol (2G) is produced from the monomeric components (hexoses and pentoses) of the structural polysaccharides present in the cell wall of plant biomasses. Lignocellulose, the main structural unit of plant walls, is formed mainly by cellulose, hemicellulose and lignin and its use for biotechnological purposes requires pre-treatment that decomposes hemicellulose, removes lignin and reduces the recalcitrance of cellulose to enzymatic saccharification (SELVAKUMAR *et al.*, 2022). The Chemical and physicochemical pretreatments are recognized for providing pentoses and hexoses that make up hemicellulose, but they usually produce inhibitors of alcoholic fermentation and cytotoxic substances for the fermentative agent, Studies are necessary for the detoxification of the hydrolysate or the use of microbial strains resilient to the conditions of the hydrolysate. (ZHAO; SHAO; CHUNDAWAT, 2020).

In this review work, the results of scientific research were collected and organized, whose purpose has been the evolutionary adaptation of naturally occurring yeasts capable of fermenting pentoses for the purpose of producing 2G ethanol. Google Scholar and *Pubmed*, considering publications that have addressed the topic in the last decade.

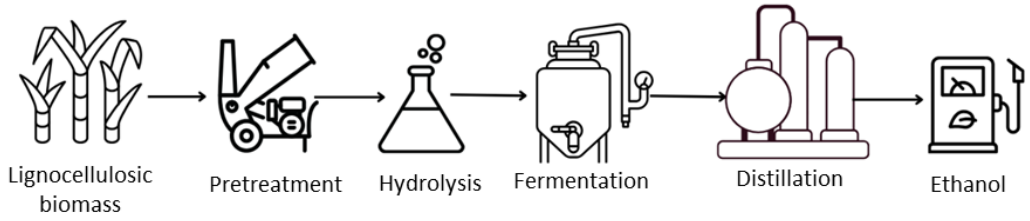
2 SECOND-GENERATION ETANOL: FACTORS THAT INFLUENCE THE CONDUCTION OF THE BIOPROCESS

Biofuels have been explored as an alternative to fossil fuels, and first-generation bioethanol (1G) has been the most relevant biofuel for fueling vehicles with otto cycle engines, originally fueled only with gasoline (JAYAKUMAR *et al*., 2023). However, the production of 1G ethanol conflicts with the food supply when it competes for inputs for food production, arable land and water (CHENG; WHANG, 2022). In this context, second-generation bioethanol (2G) presents itself as an interesting alternative, as it is produced from lignocellulosic biomass, often considered agricultural or agro-industrial residues, and still has a low cost and does not compete with the food market (RAMESH; SELVAN; BABU, 2022; CHENEBAULT; PERCHERON, 2023).

Lignocellulosic biomasses are composed of about 15% to 30% lignin, 20% to 35% hemicellulose, and 30% to 50% cellulose (BASAK *et al.*, 2023) which form a three-dimensional, complex and rigid matrix of the cell wall of plants (BHARADWAJ *et al.*, 2023). Hemicellulose and cellulose are polysaccharides that can be hydrolyzed into monomeric sugars, thus becoming subject to fermentation to obtain ethanol (ALMEIDA; NASCIMENTO, 2021). The conversion of lignocellulosic biomass into bioethanol goes through different stages: pretreatment, which involves changing the

structure of the cellulose-hemicellulose-lignin matrix, ensuring the separation of these fibers into independent fractions, in order to make them accessible to chemical or enzymatic attack that will decompose the polysaccharides (SINGH *et al.*, 2022); saccharification or hydrolysis of glycosidic bonds, which aims to depolymerize holocellulosic components (cellulose and hemicellulose), releasing monosaccharides, pentoses, and hexoses (MACHINENI, 2019); fermentation, a microbiological process that ensures the conversion of all monomeric sugars into ethanol and carbon dioxide (CO_2); and distillation, the process of separating ethanol from the rest of the fermented wort. Figure 1 shows the steps of the 2G ethanol production process.

Figure 1. General scheme of the stages of the lignocellulosic or 2nd generation ethanol production process.



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The yield of the conversion of biomass into free sugars depends on the choice of operating conditions employed in the pretreatment and hydrolysis steps (SHRIVASTAVA; SHARMA, 2023). These steps, although necessary for the consolidation of second-generation ethanol, can generate a variety of chemical compounds with undesirable effects, such as furan derivatives, aliphatic acids, and phenolic compounds, which can promote cell death or inhibition of microbial metabolism (ZHAO; SHAO; CHUNDAWAT, 2020). The presence of these inhibitors interferes with the consolidation of lignocellulosic bioethanol, since the yield and productivity values, which are essential requirements, are negatively affected. In this scenario, the metabolic characteristics of the microorganism are fundamental for the good performance of ethanol production, and it is important to search for species and strains that overcome the presence of inhibitory compounds formed during the pre-treatment process.

The species *Saccharomyces cerevisiae*, a yeast known for its robustness and considered a reference in first-generation industrial ethanol production processes for converting hexoses to ethanol efficiently, does not ferment the pentoses made available after the process of hydrolysis of lignocellulosic biomass. Saxena *et al*., 2023 report the fact that this yeast is not able to multiply or grow when the only source of carbon is xylose, because although its genome has a metabolism pathway for this sugar, there is a low level of expression of the genes responsible for the pathway's enzymes. Therefore, one of the challenges for the consolidation of the 2G ethanol process is the adoption of an

organism that is capable of efficiently fermenting both glucose and xylose, from the process of saccharification of cellulose and hemicellulose, respectively, to ethanol.

To be successful, bioethanol production must also rely on fermentative agents that are resilient to osmotic stress, high temperatures, and the presence of high concentrations of the product formed, ethanol (ELIODÓRIO *et al.*, 2019).

Sugars, although important sources of energy for yeast cells, can become toxic when in high concentrations in the wort (ITTO-NAKAMA *et al.*, 2023). The increase in the osmolarity of the fermentative medium affects cell growth and viability (SAINI *et al.,* 2018). Exposure to high concentrations of sugars results in rapid intracellular water loss by osmosis, followed by cell shrinkage (AUESUKAREE, 2017). In order to counteract these effects, yeast cells increase the production of glycerol, thus increasing internal osmolarity (HOPPERT; KÖLLING; EINFALT, 2022).

In the fermentation process, during the conversion of sugars into ethanol, exothermic reactions occur and heat exchange of the microorganisms with the fermentative medium, raising the temperature in the fermentation vats (RIVERA *et al.*, 2017). If there is no temperature control, yeasts can suffer heat stress, affecting cell growth, viability, and metabolism, as it can destabilize proteins, enzymes, plasma membrane, and cytoskeletal structures, leading to protein dysfunction, metabolic imbalance, and cell collapse (AUESUKAREE, 2017). Ribeiro *et al.* (2019) when fermenting with *Pichia membranifaciens* LJ4 at different temperatures (32°C, 37°C and 40°C) found that, among the treatments tested, the temperature of 40°C showed an increase in the cell death rate of yeast cells, as well as a decrease in the total concentration of ethanol.

The accumulation of ethanol during the fermentation process can result in toxicity to the microorganism (SNOEK; VERSTREPEN; VOORDESCKERS, 2016). High concentrations of ethanol compromise several cellular functions, leading to reduced growth and loss of cell viability, ultimately promoting slow fermentation (BLEOANCA *et al.*, 2013), also hindering the transport of glucose (SALMON, 1989). The stress caused by alcohol also affects the fluidity and permeability of the cell membrane, since ethanol and membrane lipids are amphipathic molecules that interact directly, resulting in physiological changes of the membrane (MANSURE *et al.*, 1994; ITTO-NAKAMA *et al.*, 2023). The high concentration of ethanol can also cause the accumulation of Reactive Oxygen Substances (ROS), such as hydrogen peroxide (H_2O_2), superoxides (O_2) and hydroxyl radical (OH^-), which can cause damage to carbohydrates, lipids, proteins and DNA, macromolecules important for cellular metabolism (YANG *et al.*, 2019).

In the case of inhibitors, furfural (derived from furan) is generated from the dehydration of pentose by the action of acids and high temperatures (SJULANDER; KIKAS, 2020). The inhibition of enzymes such as hexokinase, glyceraldehyde-3-phosphate dehydrogenase, and alcohol dehydrogenase is one of the deleterious effects observed on microbial cells in the presence of furfural (WANG *et al.,*

2023). In general, cell-specific growth, viability, and exponential growth phase are directly affected and, consequently, there is a decrease in ethanol production and volumetric yield (WANG *et al.,* 2016). According to Bellido *et al.* (2011), this compound can also affect the consumption of substrates, as observed in his experiment with *Scheffersomyces* (*Pichia*) *stipitis* in wheat straw hydrolysate, in which there was a delay in sugar consumption rates with the increase in furfural concentration. This compound also induces the accumulation of reactive oxygen species (ROS), which denature proteins, damage the cytoskeleton and cause DNA mutagenesis (LIU *et al.*, 2021), damage mitochondria and vacuole membranes (SJULANDER; KIKAS, 2020; GENCTURK; ULGEN, 2022).

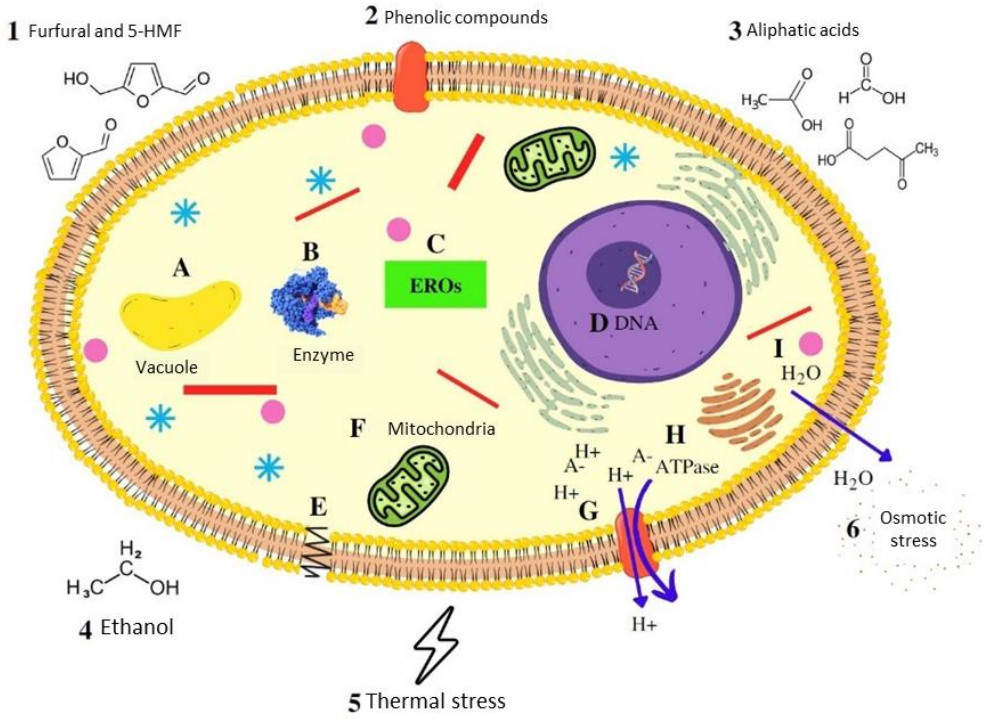
5-hydroxymethylfurfural (5-HMF), another furan derivative, is a product of hexose dehydration, and like furfural, it produces negative effects on cells, although it is less toxic (SJULANDER; KIKAS, 2020). Its effects are similar to those of furfural, mainly by causing a longer latency phase (or lag phase) during cell growth (TSAI *et al.,* 2021). The enzymes alcohol dehydrogenase, pyruvate dehydrogenase and acetaldehyde dehydrogenase are the most affected by 5-HMF and, because of this, high concentrations of this compound in the fermentative medium can completely stop the multiplication of cells (SEHNEM *et al.*, 2020).

Acetic acid is a weak acid, generated from the deacetylation of hemicelluloses and considered one of the most common carboxylic acids found in lignocellulosic hydrolysates (RAJENDRAN *et al.*, 2018). Abud, Silva and Junior (2017) reported in their research that, in a fermentation process with the yeast *Scheffersomyces* (*Pichia*) *stipitis*, acetic acid was the main factor causing low fermentative yield, when compared to other inhibitory compounds such as furfural and 5-hydroxymethylfurfural. Almeida *et al.* (2023) mention that the toxic effects of acetic acid depend on the pH of the fermentative medium, which is generally lower than the intracellular pH, and that this effect is related to anions that, in an undissociated form, propagate through the plasma membrane of microbial cells, decreasing cytosolic pH. Hu *et al.* (2022) report that the decrease in intracellular pH induces the production of the enzyme adenosine triphosphatase (ATPase) of the cytoplasmic membrane associated with the pumping of protons necessary for the maintenance of membrane potential. The authors also report that this phenomenon requires the use of ATP, reducing the availability of this important molecule for the production of ethanol from fermentation. Therefore, the presence of this acid has negative effects on cell growth and ethanol production. According to Almeida *et al.* (2023), such effects are only observed in the presence of high concentrations of acetic acid.

The microorganism, when cultured in media that contain cytotoxic substances that inhibit the fermentation process, must respond quickly to protect its cellular machinery and repair the damage caused (SAINI *et al.,* 2018). The response of yeasts to stressors depends on the species of the microorganism, the type of stress and other conditions of the growing medium. Most of the time, it is not possible to completely elucidate the yeast response to these stresses, requiring omics tools that

allow the analysis of genetic variations, proteins and metabolites (ZHAO; BAI, 2009). Figure 2 illustrates the effects of the inhibitory compounds on the yeast cell.

Figure 1 - Inhibitory effects of furan derivatives, aliphatic acids, phenolic compounds on yeast cell structure and metabolism



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Legend: (1A) Damage to the vacuole membrane; (1F) Damage to mitochondria; (1B; 4B) Affect enzymes of the glycolytic pathway and the bioethanol conversion pathway; (5B) Denature proteins and/or enzymes; (1C;4C) Increase the generation of reactive oxygen species (ROS); (1D) DNA degradation; (3G) Decrease in intracellular pH through the accumulation of anionic species; (3H) Production of the enzyme adenosine triphosphatase (ATPase) that uses ATP to pump protons out of the cell, decreasing the availability of ATP for the fermentation process; (2E; 4E) Cell membrane degradation; (6I) Intracellular water loss by the osmotic process.

Zhao and Bai (2009) reported that the microbial capacity to tolerate various stressors is one of the most important criteria for selecting strains capable of performing a more efficient alcoholic fermentation. It should be noted that over the years, researchers have been studying strategies that can minimize these inhibitory effects in order to improve ethanol yield (ROQUE *et al.*, 2019). In this context, Menegon, Gross, and Jacobus (2022) mentioned that evolutionary adaptation is an interesting technique to improve the fermentation efficiency of ethanol, by yeasts, when in the presence of inhibitory compounds, high temperatures, and/or high concentrations of sugar and ethanol.

3 EVOLUTIONARY ADAPTATION

Natural selection, a mechanism of evolution proposed by Darwin in 1859, is a process in which the organisms most able to survive in certain environmental conditions are selected, as they reproduce

and transmit their characteristics to their descendants. The evolutionary adaptation of microorganisms, also called laboratory evolution, follows the principle of natural selection, but in a laboratory-generated environment and under controlled conditions (PAL; VIJ, 2022).

Evolutionary adaptation consists of exposing a given microorganism to a stressful environment for a long period of time, such as nutrient unavailability, presence of cytotoxic compounds, thermal, osmotic and/or oxidative stress (ZHU *et al.*, 2018). These stress conditions generate a rapid response from microorganisms that evolve to protect their cellular machinery and repair the damage caused (SAINI *et al.*, 2018). From this process, certain genotypic characteristics of the microorganisms can be improved, without the need to use synthetic biology protocols for the modification of the yeast genome (PAL; VIJ, 2022).

The adaptation strategies consist, in general, of cultivating the microorganism of interest in fermentative medium added with cytotoxic substances and/or subjected to stressful factors (temperature, pH, salinity, osmotic pressure). Such strategies are numerous and varied, and based on the gradual increase of the stressor during the fermentation process. Considering the various strategies, Mavrommati, Papanikolaou and Aggelis (2022) described some techniques that can be employed, such as batch processes using liquid medium contained in vials that are subjected to agitation and after a certain time interval, the cells are removed and inoculated into a new vial that may contain a greater or equal concentration of a toxic substance, as depicted in Figure 3. In this strategy, the steps are then repeated several times, according to the desirable number of generations. Wang, Sun and Yuan (2018) mentioned that this same procedure can be performed using a solid medium, contained in a Petri dish or culture tube, containing the stressor, in which the microorganism will be inoculated. Then, the grown culture will be transferred to a new medium containing the stressor in equal or greater concentration or intensity, which will promote selective pressure. Dragosits and Mattanovich (2013) mentioned that these experimental procedures, regardless of whether they are in liquid or solid media, are simple and low-cost, and may have controlled temperature and homogeneity conditions, however they may present some obstacles, such as: variable population density, nutrient scarcity, and variations in pH and dissolved oxygen. The variation of these factors may not be of great importance for many experimental settings and work objectives, thus confirming the efficacy of the method.

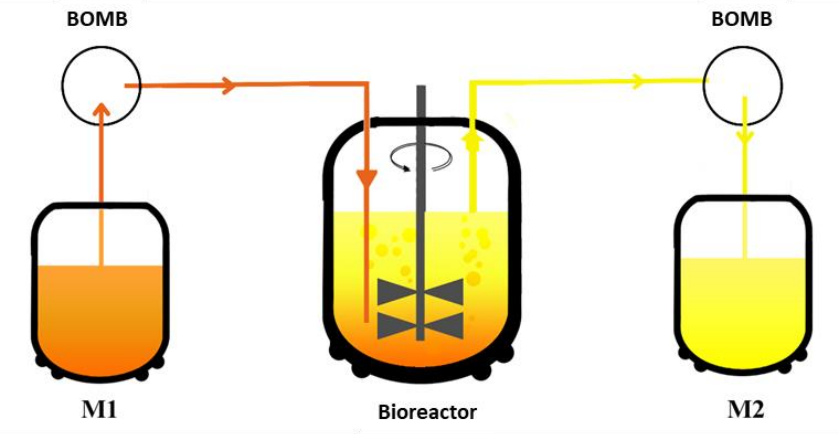
Figure 3 - Evolutionary adaptation procedure, by means of successive transfers in liquid medium containing increasing concentration or intensity of the stressor

Source: AUTHORED BY THE AUTHOR, 2023

Legend: M (fermentative medium); M1, M2, M3... Mn (fermentative medium containing increasing concentration of the stressor, such as inhibitory compounds, ethanol, sugars, temperature, pH, among others).

There are some adaptation strategies that require greater control, and one of the alternatives is the use of continuous cultures in bioreactors, which allows the manipulation of factors such as pH and temperature (MAVROMMATI; PAPANIKOLAOU; AGGELIS, 2022). During the continuous process, there is a gradual increase in the stressor factor, and adapted cells are obtained at the end of the process, as shown in Figure 4. However, the adaptation process presents obstacles, such as discovering the mechanisms and genetic alterations that led the cells to an evolved phenotype and, for this, the genomic sequencing of the evolved strains or a more in-depth analysis are usually necessary complementary processes (MOHEDANO; KONZOCK; CHEN, 2022).

Figure 4 - Evolutionary adaptation procedure by continuous process



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Legend: M1 (fermentative medium with a gradual increase in the concentration of the stressor, such as inhibiting compounds, ethanol, sugars, temperature, pH, among others); Bioreactor (equipment containing the microorganism, where fermentation will take place); M2 (fermented medium).

The selective modification of genotypic characteristics of microorganisms and the efficiency of the fermentation process are directly correlated, since the adapted fermentation agent can increase its specific growth rate, its tolerance to inhibitors and ethanol, and create stability against high temperatures and pH variations (TURANLI-YILDIZ et *al.*, 2017; SAINI *et al.*, 2018).

In this context, the use of adapted microorganisms minimizes the deleterious effects of fermentation, such as those caused by the presence of inhibitory compounds from the pretreatment stage of the lignocellulosic hydrolysate (NOURI *et al.*, 2020).

Morales *et al.* (2017), studied the yeast strain *Spathaspora passalidarum* NRRL Y-27907, which underwent an adaptation process as follows: (i) a microbial suspension was exposed to UV light for 480 seconds, (ii) followed by inoculum in a solid medium containing 1 g L^{-1} of acetic acid for 72 h, (iii) later the colony was transferred to a liquid medium containing 1 g L^{-1} of acetic acid for 7 h and then (iv) inoculated in a bioreactor with different dilution rates, where acetic acid concentrations were gradually increased. Next, the adapted cells were inoculated in a fermentative medium containing *Eucalyptus globulus hydrolysate*. These authors mention that the native cells of *S. passalidarum* NRRL Y-27907 showed lower yield (0.22 g $_{ethanol}$ substrate-1) and volumetric productivity (0.09 g L^{-1} h^{-1}) when compared to the values obtained in the processes conducted with adapted cells (0.36 gethanol substrate-1 and 0.55 g L^{-1} h^{-1}), which increased by 63.6% and 511.1%, respectively. It is worth mentioning that 1 g of carbohydrate can be converted, theoretically, into a maximum of 0.511 g of ethanol (C2H6O) and 0.489 g of carbon dioxide (CO2). The ethanol yield value may be close to the theoretical value, but not higher than it.

Trichez *et al*. (2023) also worked with the evolutionary adaptation process of the yeast *Spathaspora passalidarum*, but with the aim of improving its ability to co-ferment glucose and xylose. The assay was initially conducted with a stage of mutagenesis by UV radiation and later with an experiment using as selection criteria the consumption of xylose sugars in the presence of 2-deoxy-D-glucose (2-DOG), a non-metabolizable glucose analogue. Considering the results, the authors observed that the mutant strain demonstrated an improvement in its ability to simultaneously assimilate glucose and xylose in culture media containing both sugars. In the experiment conducted with the control strain of *Spathaspora passalidarum*, glucose (20.44 g L^{-1}) was completely depleted and 7.55 g L^{-1} of xylose was consumed, resulting in the production of 6.80 g L^{-1} of ethanol in a 24-hour cultivation period. On the other hand, the cells of the Spc3 lineage, the adapted version *of S. passalidarum*, consumed 5.59 g L^{-1} of xylose, even in the presence of 2.82 g L^{-1} of residual glucose, and produced 5.57 g L−1 of ethanol, with a slight improvement in the co-fermentation process of glucose and xylose, compared to the control strain. The same authors also worked with the evolutionary adaptation of the yeast *Scheffersomyces stipitis*, under equal experimental conditions. A more notable effect on the co-consumption of glucose and xylose was observed in the evolved cells derived from *S. stipitis.* The control cells began to assimilate the xylose only after almost complete consumption of glucose. On the other hand, both the adapted strains A5-1 and A5-8 were able to co-assimilate glucose and xylose simultaneously, but with a compromise in the rate of sugar consumption. The wild-type strain of *S. stipiti* consumed 1.01 g L−1 of xylose and 15.95 g L−1 of glucose, in a ratio of 0.06 g of xylose per g

of glucose, within only 6 hours of cultivation. In this same situation, the evolved strains showed higher consumption of xylose/glucose. The adapted strain A5^{-1} consumed 1.08 g L^{-1} of xylose and 5.43 g L^{-1} of glucose, with a ratio of sugar consumption of 0.20 g of xylose per g of glucose, while A5-8 consumed 1.74 g L^{-1} of xylose and 4.45 g L−1 of glucose, resulting in a consumption in the ratio of 0.39 g of xylose per g of glucose. However, it is important to note that despite the higher xylose/glucose consumption ratios, the evolved cells (Spc3, A5-1 and A5-8) exhibited a reduced rate of glucose consumption compared to the parent strains.

Sharma *et al.* (2016) explored the strain of *Kluyveromyces marxianus* NIRE-K1 for lignocellulosic bioethanol production from xylose through an evolutionary adaptation approach. The method used was carried out in batch with synthetic YEP medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} glucose/xylose, 15 g L^{-1} phytagel, pH 5.5) and sequential transfers. Adaptation continued for 60 batches until the adapted culture was able to utilize more than 80% (w/v) xylose. The authors reported that the volumetric uptake of xylose was 3.45 times higher for adapted K. marxianus NIRE-K1 (0.38±0.03 g L^{-1} h^{-1}) when compared to the native strain (0.11±0.02 g L^{-1} $h^{-1)}$ and the ethanol yield of 0.11±0.02 gethanol substrate^{-1} using K. marxianus This result was 57.1% higher than the yield obtained for the native strain (0.07±0.01 gethanol substrate-1). The volumetric productivity of ethanol also improved, since the value found for the native strain was 0.008±0.002 g L^{-1} h^{-1} and for the adapted strain the value found was 0.040±0.003 g L-1 h^{-1}, a result 400% higher.

The authors Du *et al.* (2022) studied the evolutionary adaptation with the yeast *Kluyveromyces marxianus* 1727, the same species previously studied, however, in the presence of multiple inhibitors and, for this, the cells were incubated in synthetic medium added with multiple inhibitors with concentrations of 0.2 g L^{-1} of formic acid, 0.5 g L^{-1} of acetic acid, 0.3 g L^{-1} of furfural and 0.2 g L^{-1} of 5-HMF. When the optical density of the suspension reached 2.0 D.O, the cells were collected and transferred to a higher concentration of inhibitors. The steps were repeated until the final concentration of inhibitors was 0.8 g L^{-1} formic acid, 1.2 g L^{-1} acetic acid, 0.8 g L^{-1} furfural, and 0.6 g L^{-1} 5-HMF. By fermenting the hydrolyzed corn stover inoculated with the adapted *yeast K. marxianus* 1727, the authors Du *et al.* (2022) reported observing a yield of 0.46 gethanol substrate-1, which was 12.2% higher when compared to the mother strain (0.41 gethanol substrate-1).

Hemansi *et al.* (2022) also carried out an adaptation process with the *strain Kluyveromyces marxianus* JKH5, which was conducted by the transfer of yeast into a synthetic medium containing a gradual increase in inhibitors (acetic acid/furfural/vanillin/inhibitor cocktail). The cells in logarithmic phase were collected for a subsequent inoculum performed by consecutive transfers for 60 batches. The authors observed that, when cultivated in synthetic medium with an initial concentration of 50 g L^{-1} of glucose and in the presence of a cocktail of inhibitors (acetic acid 3 g L^{-1}, furfural 1 g L^{-1} and vanillin 1 g L^{-1}), the adapted strain showed better yield (0.40 gethanol gsubstrate-1) and volumetric

productivity (1.11 g L^{-1} h^{-1}), when compared with the non-adapted strain (0.16 gethanol gsubstrate-1 and 0.45 g L^{-1} h^{-1}), which showed an increase of 150.0% and 146.7%, respectively. Still studying the same species, but of different lineage, *Kluyveromyces marxianus* CCT 7735, Silveira *et al* (2020) conducted an 85-day study carrying out the adaptation process, in which the microorganism was inoculated in SD medium (6.7 g L-1 of yeast nitrogen base - YNB - without amino acids and 20 g $^{L-1}$ lactose) with added ethanol 4% (v/v) (SDE). The collected data showed an improvement in ethanol yield (Yp/s) that was 0.32 g g-1 in the parent strain (P1) and 0.36 g g-1 in the adapted strain (ETS1). In addition, volumetric productivity also increased, from 0.56 to 0.66 g L^{-1} h^{-1}.

According to Nouri, Azin and Mousavi (2017), an adaptation process was carried out with the yeast *Barnettozyma californica* HNMA-5, using the adaptation procedure that consisted of using media with increasing concentrations of 25%, 50%, 75% and 100% (v/v) of sugarcane bagasse hydrolysate. When a vigorous growth of the crop was observed, transfers were made to a medium with a higher concentration of hydrolysate. The authors observed an increase in ethanol yield, which went from 0.166 gethanol to 0.216 gethanol substrate-1 with the adapted strain (30.1% improvement), as well as in volumetric productivity, which increased from 0.138 g L^{-1} h^{-1} to 0.158 g L^{-1} h^{-1} (14.5% improvement).

In the work of Fan *et al.* (2013), a yeast strain of *Pichia guilliermondii*, previously selected and adapted to hydrolysates from corn cob residues, was used to improve performance in lignocellulosic ethanol production. The authors reported that evolutionary adaptation was conducted in hydrolysates of corn cob residue without complementary addition of nutrients. The strains were transferred in increasing proportion (25-100%, in increments of 25%) of hydrolysate, where the colonies with the highest growth in solid medium containing 25% hydrolysate (v/v) were selected and transferred to a solid medium with even higher concentration of hydrolysate. The results showed that the adapted *yeast P. guilliermondii* showed better performance for ethanol production in non-detoxified hydrolyzed medium without the addition of nutrients. The adapted strain produced, in 120 h, 34.7±0.2 g L^{-1} of ethanol, with a volumetric productivity of 0.29±0.00 g L^{-1} h^{-1}, which corresponds to 84.6% and 81.3% higher than that of its non-adapted strain, respectively (18.8 g L^{-1} and 0.16 g L^{-1} h^{-1}). In addition, the adapted strain consumed all the glucose present in the hydrolysates of corn cob residues (about 74.9 g L^{-1}) in 120 h, with a consumption rate of 0.62±0.01 g L^{-1} h^{-1}, 87.9%, higher than that of the parental strain.

Pontes (2022) studied the evolutionary adaptation process of the yeast strain *Candida orthopsilosis* UFVJM-4G, employing the technique of successive transfers in media containing increasing concentrations of sunflower seed hydrolysate (0%, 25%, 50%, 75%, 100%). Subsequently, the strains from the different concentrations were isolated and inoculated in pure sunflower seed hydrolysate to define the fermentative parameters. The author observed that the adaptation process of

this strain was efficient, since there was an increase in the yield and volumetric productivity of the adapted yeast (0.50 gethanol gsubstrate-1 and 0.42 g L^{-1} $h^{-1)}$ when compared to the non-adapted yeast (0.34 gethanol $substrate^{-1}$ and 0.32 g L^{-1} h^{-1}). In addition, he reported that there was an improvement in the growth of this yeast, whose maximum specific growth rate (μmax) went from 0.07 h-1 to 0.28 h^{-1}.

Table 2 summarizes the results of this review. From the analysis of the results reported in the literature, it was possible to infer that the evolutionary adaptation technique positively influenced the fermentation parameters. The lowest percentage increase observed with the use of this technique was 12% ethanol yield for *Kluyveromyces marxianus* 1727 and 1.530% for *Scheffersomyces stipitis* Y-7124.

Table 2 – Compilation of data from the literature on fermentation processes using pentose fermenting yeasts submitted to the evolutionary adaptation technique for ethanol production

Yeast		**YP/S (gethanol substrate-1)**	**QP (g L-1 h-1)**	**Fermentative media**	**References**
Spathaspora passalidarum NRRL Y-27907	Native	0,22	0,09	Hydrolysate of *Eucalyptus globulus*	Morales *et al.* (2017)
	Adapted	0,36	0,55		
Kluyveromyces marxianus NIRE-K1	Native	0,07	0,008	YEPX Synthetic Medium	Sharma *et al.* (2016)
	Adapted	0,11	0,040		
Kluyveromyces marxianus 1727	Native	0,41	-	Corn husk hydrolysate	Du *et al.* (2022)
	Adapted	0,46	-		
Kluyveromyces marxianus JKH5	Native	0,16	0,45	Synthetic medium with inhibitors	Hemansi *et al.* (2022)
	Adapted	0,40	1,11		
Kluyveromyces marxianus CCT 7735	Native	0,32	0,56	SD Synthetic Medium	Silveira *et al.* (2020)
	Adapted	0,36	0,66		
Barnettozyma californica HNMA-5	Native	0,166	0,138	Sugarcane bagasse hydrolysate	Nouri *et al.* (2017)
	Adapted	0,216	0,158		
Pichia guilliermondii	Native	-	0,16	Hydrolyzed corn cob residues	Fan *et al.* (2013)
	Adapted	-	0,29		
Candida orthopsilosis UFVJM-4G	Native	0,34	0,32	Sunflower seed hydrolysate	Pontes (2022)
	Adapted	0,50	0,42		

Source: AUTHORED BY THE AUTHOR, 2023

4 FINAL THOUGHTS

The success of lignocellulosic ethanol production depends on the use of microorganisms capable of fermenting hexoses and pentoses with performance similar to conventional industrial yeasts, whether or not they are in the presence of a hostile fermentative environment, such as lignocellulosic hydrolysate.

Several alternatives have been studied to reduce the pressures to which yeasts are subjected during the fermentation of lignocellulosic hydrolysates. However, evolutionary adaptation aims to make unconventional yeasts, recognized for their ability to ferment pentoses, more resilient and efficient to the fermentative environment of lignocellulosic hydrolysates.

From the review carried out on the subject, it was possible to observe that the methodology of evolutionary adaptation, considered the alternative of intervention by genetic engineering, is a simpler and less costly tool of execution, with wide application and that covers most of the issues related to the intercurrences of the fermentation process with a direct answer about the increase of fermentative yield and volumetric productivity.

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