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Growth Kinetics for the Selection of Yeast Strains for Fermented Beverages

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Abstract

Criteria to select autochthonous yeast strains for their use in fermented beverages include their ability to dominate the media and to enhance desired sensorial characteristics and their inability to produce undesired compounds such as biogenic amines or off-odors. One of the key features in yeast selection is its Implantation, surpassing different stresses, and its fermentation performance, which requires setting up the process and monitoring it, involving important amount of resources. Methods to evaluate the tolerance of yeast strains are usually based in the qualitative measure of the growth of the microorganism in a medium containing the limiting compound after a specific time of incubation. However, studying strain growth through optical density measurements permits to estimate quantitative and comparable parameters providing an insight into the fitness of the cell to certain environment, lag phase duration, growth rate, and maximum population, among others. In the last decades, culture-independent methods have been used to evaluate the dynamic of microbial populations during fermentative process. In this chapter, a review of recent advances in the selection of fermentative yeasts as well as the utilization of kinetic evaluation and molecular strategies in conditions associated with fermented beverage for selecting yeast strains is presented.

Keywords: yeast selection, fermentative process, growth evaluation, kinetic parameters, culture-independent methods

1. Introduction

The production of alcoholic beverages is one of the most ancient food traditions. Their elaboration relays on a fundamental stage: the alcoholic fermentation (AF), which is a biochemical

conversion of sugars into ethanol through the action of yeasts. The AF can occur in three ways [1, 2]: (i) spontaneously, from the microbiota naturally present in the musts or on surfaces of equipment; (ii) by adding commercial yeasts; and (iii) by inoculating selected native strains. Nowadays, the demand for autochthonous strains has increased worldwide for it is accepted that this option allows preserving the unique and typical character that native microorganisms provide.

Yeasts intervening in AF are considered "fermentative yeasts," and they are divided broadly in two groups: *Saccharomyces* and non-*Saccharomyces* (nS). The *Saccharomyces* possess a high efficiency in the conversion of sugars and tolerate high concentrations of ethanol and SO_2, being the fermentative genus for excellence [3]. Most of the nS are low tolerant to ethanol and include different genera such as *Candida, Kloeckera, Hanseniaspora, Zygosaccharomyces, Schizosaccharomyces, Torulaspora, Brettanomyces, Saccharomycodes, Pichia,* and *Williopsis* [4]. In beverages like wine, the importance of these yeasts lies on their metabolic features, as the wide set of enzymes they offer can improve the typicity and enhance the sensory profile [4–7]. Conversely, in other beverages as Mexican tequila and mescal, as well as Brazilian cachaça, nS are considered the main responsible for the ethanol production [8–10]. Yeast cells are exposed to several stress conditions from the beginning to the end of the fermentation process [11], resulting in the reduction of their growth and survival rate causing a decrease in fermentation efficiency. Yeasts capable of overcoming these conditions with low viability loss are best suited for these purposes [12].

2. Stress tolerance of fermentative yeast

The primary goal of fermentative yeast is to rapidly and efficiently convert simple sugars into ethanol without developing unpleasant flavors [13]. Several factors affect the yeast ability to grow in the fermentative media related with the type and style of beverage produced; therefore, the ability to adapt and to cope with this hostile environment is considered the main feature to select fermentative yeast [12]. Some of the most relevant inhibiting conditions are summarized in this section.

2.1. Limiting conditions associated with musts

2.1.1. Carbohydrates

Carbohydrates are the most important nutrient since they are metabolized to form biomass, ethanol, and different by-products such as volatile compounds, glycerol, and others that will develop the sensorial characteristics [14]. However, they also produce the first stress due to osmotic pressure in the cells after their inoculation. Therefore, tolerance to high sugar concentration is one of the main criteria for yeast selection, especially for those designated for their use in the elaboration of liquorish beverages such as "Sauternes" wine. Yeast cells have developed mechanisms to adjust to high external osmolarity and maintain or reestablish an inside-directed driving force for water. Adaption to this stress usually

takes several hours in which yeast cells accumulate glycerol and trehalose [15] and change their cell wall composition [16] to counter loss of water by the osmotic pressure. The stress level will depend on the type and concentration of sugars found. Concentrations range from diluted juices to the high gravity worst containing 16–18% of dissolved solids, rice mash for sake production with 20% of solids, and grape juice with 200 g L^{-1} of sugar content or even more.

Sugar composition of the media strongly impacts on yeast metabolic physiology [17]. Glucose and fructose are widely found in nature as free sugars or as polysaccharides, also in different proportions. Grape juice contains approximately 1:1 of glucose-fructose [18] apple juice 5:8 [19], and agave juice for the elaboration of tequila and mezcal, after thermal processing of inulins from the plant, contains 1:20 ratio [20]; in *pulque* production from raw agave juice, inulin is also predominant [21]. For beer fermentation maltotriose and maltose comprise 50% of sugars; in rum, cachaça, and tafia, produced by sugar cane juice or molasses, mainly sucrose is present [22].

2.1.2. Assimilable nitrogen

Deficiencies in the supply of assimilable nitrogen will lead to sluggish fermentation. The amounts of total nitrogen in musts for the production of fermentative beverages vary from 40 mg L^{-1} in agave juices [23], 50–150 mg L^{-1} in apple juice, 80 mg L^{-1} in sugar cane for cachaça [24], more than 150 mg L^{-1} in beer malt wort [25], and from 100 to 500 mg L^{-1} in grape must [26]. A minimum of 66 mg L^{-1} to sustain the growth of yeasts and to finish fermentation with a total consumption of sugars, and high ethanol yield is considered [26].

In the fermentative media, after yeasts are inoculated, a rapid uptake of nitrogen compounds used for the biosynthesis of macromolecules and storage in vacuoles is carried out if sufficient amount is present in the must. As yeasts have high preference for ammonium ions, it is used as exogenous nitrogen source in fermentations. Glutamate, aspartate, and glutamine are the first amino acids uptaken if they are present in the must. When a favorite nitrogen source is depleted, yeast will use a less preferred, resulting in reduced growth and fermentation rates [12].

A target for the improvement of fermentation is then the selection of yeasts with low nitrogen requirements, which will reduce the necessity of adding supplements to the must that will make the process expensive.

2.1.3. Sulfur dioxide (SO₂)

During the beginning of wine fermentation, native microbiota mainly nS yeasts and bacteria dominate the media with low ethanol production that could negatively affect the quality of the final product. To inhibit them and to favor native or inoculated *Saccharomyces* strains to develop, sulfur dioxide (SO₂) is added at levels ranging from 20 to 50 ppm [27]. *Saccharomyces* also produces SO₂ during metabolism of sulfate ions [28]. If the amount of SO₂ added and produced by *Saccharomyces* strain are high and remain until the end the process, then safety

problems arise as this compound causes certain level of toxicity in human population that consume it. The selection of yeast strains resistant to SO_2 and low SO_2 producers is then desirable.

2.2. Fermentative-derived conditions

2.2.1. Interaction with other microorganisms

Different microorganisms interact during the elaboration of fermented beverages. The variety of these interactions and their impacts on efficiency and product quality should be individually determined, as they will depend on the fermentative strains, the native associated microbiota, and the type of beverage. One of the main metabolites exerting a clear effect on yeast growth and performance is ethanol, mainly produced by *Saccharomyces cerevisiae*. Other metabolites as medium-chain fatty acids and high amounts of acetic acid can negatively affect the growth of a co-fermenting yeast species [29]. Cell-to-cell contact as well as oxygen availability appears to be also involved in the interactions between *S. cerevisiae* and other nS species [30].

One special aspect is the "killer phenotype," which refers to those yeasts able to secrete polypeptide toxins which kill sensitive cells and which is believed to be a potential mechanism to prevent a competitor from gaining access to a resource [31]. Killer toxins differ between species and strains, thus varying the modes of action, from changing membrane permeability in sensitive cells to inhibiting DNA replication or stopping cell division at G_1 phase. All killer toxins are usually active and stable at pH 4–5 and 20–25°C; nevertheless each toxin has an optimum pH and temperature at which it manifests its killer character more effectively [32, 33].

2.2.2. Ethanol

Ethanol produced during fermentation is known to inhibit yeast growth, resulting in a primary factor on yeast efficiency; in turn, the viability of yeast cells in the presence of ethanol constitutes a key feature on strain selection for fermentative purposes [34].

Ethanol affects many aspects of yeast survival, as the fluidity of the plasmatic membrane [35], the vacuole morphology [36], the activity of crucial glycolytic enzymes [37], and the mitochondrial DNA [38]. Ethanol also causes the denaturation of hydrophilic and hydrophobic proteins, affecting various transport systems such as the general amino acid permease and glucose uptake processes [18]. Regarding molecular response of *S. cerevisiae* in the presence of ethanol, it increases the expression of genes associated with glycolysis and mitochondrial function and decreases gene expression in energy-demanding growth-related processes; it also induces the production of heat shock-like proteins, lowering the rate of RNA and protein accumulation, enhancing the frequency of small mutations, altering metabolism, denaturing intracellular proteins and glycolytic enzymes, and reducing their activity [39].

Moreover, yeasts have developed diverse strategies to counteract the damages produced by ethanol [40], as the generation of fatty acid unsaturation of membrane lipids in *S. cerevisiae* [35]. Genes involved in intracellular pH homeostasis are also crucial for the resistance to

ethanol and other alcohols [41]. This entails in better adapted strains that show a better capacity to activate these mechanisms and endure in the hostile environment formed through alcoholic fermentation.

2.2.3. Organic acids

As previously stated, microorganisms interacting with yeasts during fermentation produce organic acids, and some of them can affect the growth and fermentative efficiency. Lipophilic weak acids, such as acetic, may accumulate inside yeast cells in their undissociated form diffusing into the yeast cells where it dissociates, inducing an acidification of the cytosol [42]. Fatty acids of medium-chain length, as hexanoic, octanoic, and decanoic acids, and also their respective ethyl esters have shown to negatively affect the survival and growth capacity of yeast, being partly responsible for the premature stoppage of fermentations carried by *S. cerevisiae* [43]. These metabolites can be absorbed by the cell membrane; its toxic effect increases in the pH range of 3.0–5.4. This apparent disadvantage of permeability to fatty acids has been exploited, using the denominated "yeast ghosts," which are a commercially available product that can be added when a sluggish or stuck fermentation occurs in order to absorb this kind of inhibitors [44].

2.2.4. pH

During alcoholic fermentation, pH in the media tends to reduce [45], which is known to be a limiting factor on growth of microorganisms, including yeasts. The optimal pH value for yeast growth is around 4.5, and fermented beverages range in pH from 2.5 to 5.5, which by itself does not imply a restrictive condition, but a low pH (<3.5) combined with ethanol, as it usually occurs on fermented beverages, can prematurely inhibit yeast growth and/or fermentation rate [42].

3. Yeast selection workflow

The aspects previously described define the medium in which yeasts will be developed, and they must be kept in mind onward. On the other hand, the selection of fermentative yeasts involves several sequential steps in which the final objective is the identification of strains capable to efficiently ferment the must and obtain a product with optimum sensory qualities [3]. The framework for selection usually starts determining the diversity of yeasts present during the spontaneous fermentation of the beverage. The isolation of yeasts usually takes place along with that stage; afterward, the aspects of interest ranging from tolerance conditions to sensory impact are evaluated in each strain in order to identify the best suited to be selected.

3.1. Determination of yeast diversity

As recently reviewed, in every fermented beverage produced around the world, a particular microbiota will develop as a cause of the raw material from which it starts (fruits, grains, dairy products, parts of plants, etc.), its characteristics (nutrients, pH, type of sugar, etc.), the

geographic region, and the modifiable options in each elaboration process, including pre-fermentative manipulations, temperatures, and added substances, among others [45]. Furthermore, yeast diversity that develops will largely determine the sensory profile of the final product; therefore it is first necessary to become acquainted with the species present, their dynamics, and the possible role each one plays during the fermentation. For these studies, advances in molecular techniques have been exploited, being nowadays the area with most scientific contributions on fermented beverages worldwide. Culture-independent methods are the preferred, since they are neither affected by the viability of the microorganism nor by low populations of less abundant species [46]. Some of the preferred techniques are high-throughput or next generation sequencing (HTS or NGS, respectively), denaturing gradient gel electrophoresis (DGGE), and quantitative PCR (qPCR), as it is summarized in **Table 1** [47–49].

In spite of the multiple advantages of the culture-independent techniques, it is necessary to become aware of possible gaps inherent to these approaches, as undetectability of minor populations, preferential amplifications, limited databases, and different effectiveness of lysis protocols on certain species are some of the principal. By applying both, culture-dependent and culture-independent techniques, these drawbacks can be surpassed, along with other benefits, as recovering the isolates needed to characterize individually and select possible starter cultures.

Beverage	Country	Technique	Year	Main yeasts found	Reference
Caxiri (cassava, corn, and sweet potatoes)	Brazil	DGGE	2015	*S. cerevisiae, P. kluivery, C. tropicalis, D. fabryi*	[50]
Wine (cv. "Grenache")	Spain	NGS	2015	*Hanseniaspora, Saccharomyces, Candida, Issatchenkia*	[51]
Tarubá	Brazil	DGGE	2015	*T. delbrueckii, P. exigua, P. manshurica, P. kudriavzevii, C. tropicalis, C. ethanolica*	[52]
Grape must	Spain	DGGE, qPCR, NGS	2015	*H. uvarum, S. bacillaris, S. cerevisiae*	[53]
Fuzhou Hong Qui (Rice wine)	China	DGGE	2015	*Saccharomycopsis fibuligera, P. guilliermondii, S. cerevisiae, Wickerhamomyces anomalus, C. glabrata*	[54]
Cold soak (wine)	Spain	qPCR, DGGE	2016	*H. uvarum, S. bacillaris, S. cerevisiae*	[55]
Xaj-pitha (rice wine)	India	Whole genome shotgun sequencing	2016	*Meyerozyma guilliermondii, Wickerhamomyces ciferrii, S. cerevisiae, C. glabrata, D. hansenii, Ogataea Parapolymorpha, D. bruxellensis*	[56]
Taberna (palm wine)	Mexico	DGGE	2016	*H. guillermondii, S. cerevisiae, P. kudriavzevii, C. tropicalis, K. exigua*	[57]
Pitmud used in strong-flavor liquor	China	DGGE and NGS	2017	*Candida, Wickerhamomyces, Debaryomyces, Saccharomyces, Pichia*	[58]

Table 1. Recent studies on the diversity of yeasts present during the elaboration of fermented beverages.

3.2. Isolation of strains

Although the sources of yeasts can be quite diverse, isolates are preferably obtained from the initial must and along the fermentation process, intending that this system itself directs to select the best adapted strains. Two possibilities arise at this stage: (1) to isolate random species using a general solid media (PDA or YEPDA) or with an agent leading to add visual differentiation in colony morphologies (WL nutrient agar) or (2) to focus on a specific group using selective media (lysine media for nS or nutrient medium supplemented with sodium metabisulfite and ethanol for *Saccharomyces*) [3, 81]. If one of the purposes is to complement the community study, the first would be the best choice, but if the selection is directed to certain species, the second would be best. After isolating several strains, characterization and selection will take place.

3.3. Characterization (qualitative approach)

The desirable aspects of fermentative yeast are quite variable; preference is given to one or the other, depending on each case [75]. Some of the most important and various studies in which they were applied are summarized in **Table 2**. Among this characteristics, it can be considered essential to evaluate: resistance to high concentrations of sugar and ethanol, high fermentation performance (this may not apply when selecting nS yeasts), low production of sulfurous compounds, and volatile acidity and implantation aptitude [79–82]. This last trait is not always performed, even though it is almost imperative, as it requires the design and implementations of molecular methods, such as pulsed field gel electrophoresis (PFGE), mini- or microsatellite markers, qPCR, or others [65, 66]. Once the survival capacity of the strains is determined, the next interesting feature is the impact they exert on sensory aspects. Olfactory qualities of yeast are usually assessed by determining their enzymatic activity, including glucosidases, lyases, proteases, or reductases, which can release or produce active odorant compounds [75]. Also, visual aspect can be affected by yeasts through their enzymatic activity (hydroxycinnamate decarboxylase) or their ability to excrete pyruvate and acetaldehyde, which can lead to the formation of highly stable colorant compounds as pyranoantocyanins that help to improve the visual quality of red wines [77, 83]. Besides, it is quite useful to analyze the nitrogen requirements of yeasts, if they possess the killer phenotype and flocculation ability (particularly important in beer and sparkling wines). Furthermore, some features can only be qualitatively studied such as the presence or absence of certain enzymes, as well as which killer phenotype they possess. Some other are by definition quantitative, as fermentation performance, and several characteristics can be determined both ways, qualitatively and quantitatively, which is the case of tolerance feature.

The methods to evaluate resistant traits of yeast strains are usually based in the qualitative measure of the growth of the microorganism in a synthetic medium containing the limiting compound after a specific time of incubation [82, 84–86]. In the case of ethanol tolerance, there are a broad number of tests that could define this characteristic [87]: (a) the ability to grow in the presence of ethanol, (b) the degree of survival after exposure to a certain concentration, or (c) the maximal ethanol production capacity. Some of these methods are

Characteristic of interest	References
Tolerance to inhibitors (ethanol, SO_2, sugar, pH)	Nikolau et al. [59]; Fiore et al. [60]; Arrizon et al. [61]; Capece et al. [62]; Tristezza et al. [63]
Implantation aptitude	Lopes et al. [64]; Capece et al. [62]; Perrone et al. [65]; Alonso del real [66]
Fermentation vigor (efficiency, ethanol yield, speed)	Tristezza et al. [63]; Ribeiro et al. [67]
Low nitrogen requirements	Arrizon et al. [61]; Julien et al. [68]; Gardner et al. [69]
Enzymatic activity	Fiore et al. [60]; Capece et al. [62]; Capece et al. [70], Csoma et al. [71]; Romano et al. [72]
Color and aroma enhancement	Belda et al. [75]; Steensels et al. [76]; Morata et al. [77]; Morata et al. [78].
Production of specific metabolites (sulfurous, glycerol, fatty acids, other alcohols)	Nikolau et al. [59]; Capece et al. [62, 70]; Tristezza et al. [63]
Killer phenotype	Zagorc et al. [73].
Flocculation	Silva et al. [74]

Table 2. Main aspects considered to select fermentative yeasts.

qualitatively measured, and some other imply the set up and monitor of a fermentation, requiring large amounts of resources and hindering the possibility to evaluate a high number of strains. However, fermentation parameters such as ethanol yield, productivity, and maximum specific velocity of cell growth must be measured during the traditional process [22].

Additionally, the fermentative traits are often individually evaluated, while in fermentation several stress factors intervene together and increase in number and magnitude along the process. Testing more than one inhibitor in a qualitative evaluation complicates the selection based only in the presence or absence of growth; thus, a more objective method should be used to compare between strains.

4. Quantitative methods used for the selection of fermentative yeast

An alternative to assess the tolerance of yeast strains to limiting factors is to study the growth kinetics of the strains exposed to the inhibiting condition. This can be achieved by traditional methods (plate counts), implying more resources but being more reliable, or by means of optical density (OD) measurements of the yeast in the media to test. Microbial growth data obtained by absorbance measurements permits to obtain kinetic parameters, which can be transformed into quantitative and comparable variables such as (a) detection time, the time to reach the detection level of Bioscreen (Automated OD reader equipment) and its period includes the lag time; (b) maximum growth rate could be estimated with the slope of the tangent of exponential phase; and (c) maximum population density, asymptotic level of OD at the end of exponential phase (**Figure 1**).
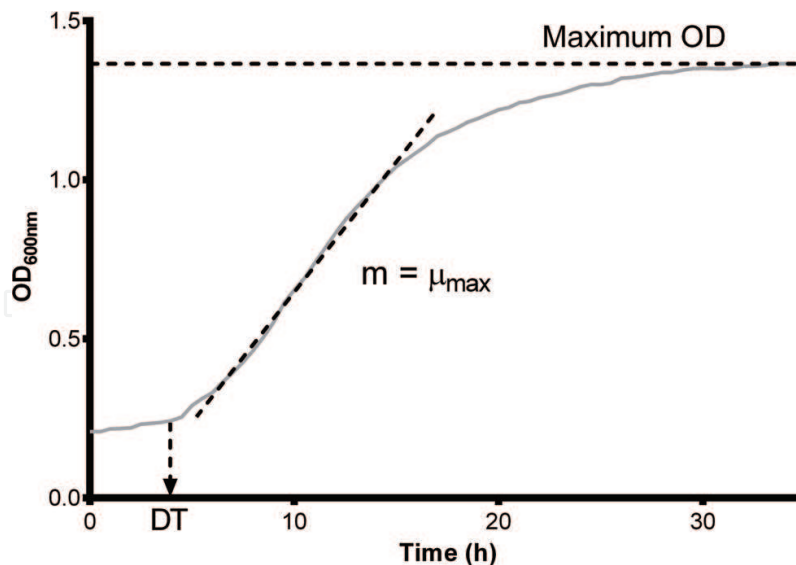
Figure 1. Kinetic parameters for microbiological growth using OD measurements. Detection time (DT), maximum growth rate (μmax), and maximum population density (maximum OD).

Measurement of microbial growth by using turbidity and cells' kinetic behavior description offer some benefits. A considerable amount of data could be generated faster and in real time. Nonetheless, this approach possesses some limitations such as the need to conduct the study in a high-transmittance liquid. Another factor to be considered is that cell concentration required to detect a shift in the measurement is at least 6 log CFU/mL.

4.1. Applications in food matrices

Analytical methods based on OD by assessing the final cell density have been used to evaluate the growth of foodborne pathogens in culture media, translucent liquid foods, and diluted food extracts [88–91]. Data from these studies have offered the possibility to obtain information to determine which food matrices and conditions could be inhibitory of pathogens growth. Also, this approach allows the comparison of strain variability of foodborne pathogens under several growth conditions, e.g., pH and salt concentration [92].

This method has allowed to rapidly evaluate the efficacy of antifungal compounds on the germination of fungal spores. The non-inhibitory concentration (NIC) and the minimum inhibitory concentration (MIC) for different environmental conditions can be calculated mathematically using the OD data [93]. MIC is considered the lowest concentration at which no growth is observed, while NIC is the lowest concentration at which any inhibitory effect is observed.

4.2. Potential applications for the selection of fermentative yeast

Quantitative measurement of the growth of microorganisms based in OD could be used to assess strain tolerance in fermentation-associated conditions. Some strategies and parameters have been proposed in base to OD data.

The MIC and NIC values are related to the susceptibility/tolerance of the microorganisms to stressful conditions. These parameters were proposed by Lambert and Pearson [94] to evaluate microorganisms not associated to fermentation processes, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Arroyo-López et al. [18] used MIC and NIC to compare the growth of 29 strains of yeasts (*S. cerevisiae*, *Saccharomyces paradoxus*, *Saccharomyces bayanus*, *Saccharomyces kudriavzevii*, *Saccharomyces mikatae*, *Saccharomyces arboriculus*, *Saccharomyces cariocanus*, and some nS) in a medium containing ethanol. Álvarez-Pérez et al. [95] used the MIC among the criteria to select *S. cerevisiae* strains to produce *Picudo Rosé* wines with different aromatic profiles in Spain.

The area under the OD-time curve could be estimated, and it represents the population generated along the incubation period at a specific condition. The inhibitory effect could be estimated as a relative fraction of the area obtained under an optimal condition (control). **Figure 2** shows an example of this parameter, in which the growth of *S. cerevisiae* in synthetic medium is contrasted with the yeast behavior at the presence of ethanol and SO_2 [96].

When OD measurements are obtained with an automatized spectrophotometer, the detection time (DT) is reported for each tested condition. Low DT values are associated with the ability of microorganisms to adapt to a specific condition, and consequently, they could start their growth in short time.

Ortiz-Barrera et al. [97] reported DT for 90 nS strains grown in artificial medium containing 6% ethanol or 30 mg of total SO_2 (**Figure 3**). This parameter was used to select strains with the best ability to grow under stressful conditions (ethanol and SO_2) associated to wine (enclosed in the square in the figure).

In log growth phase, a close relationship between OD and cell concentration is apparent due to the fact that most of the cells are in an active multiplication process which in turn is used to estimate growth rate. In **Figure 4**, growth rate values of 12 *Saccharomyces* strains incubated in grape juice media with ethanol and SO_2 are shown (unpublished data).
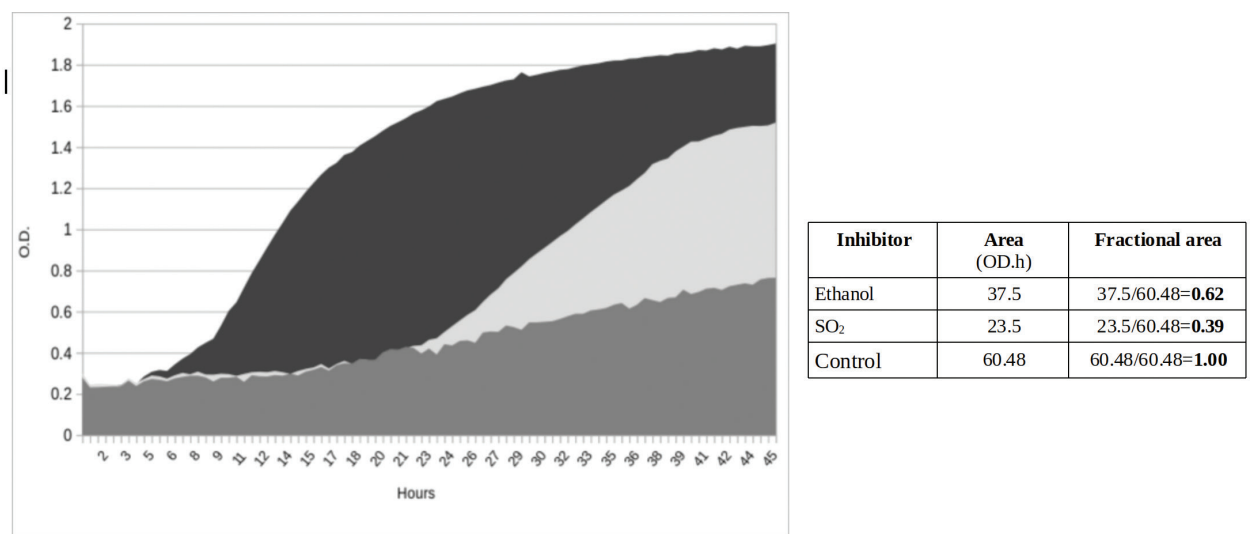

Inhibitor	Area (OD.h)	Fractional area
Ethanol	37.5	37.5/60.48=**0.62**
SO_2	23.5	23.5/60.48=**0.39**
Control	60.48	60.48/60.48=**1.00**

Figure 2. OD curves over time of *S. cerevisiae* growth in synthetic medium added with ethanol (12%), SO_2 (200 mg/L) and without inhibitors (control).
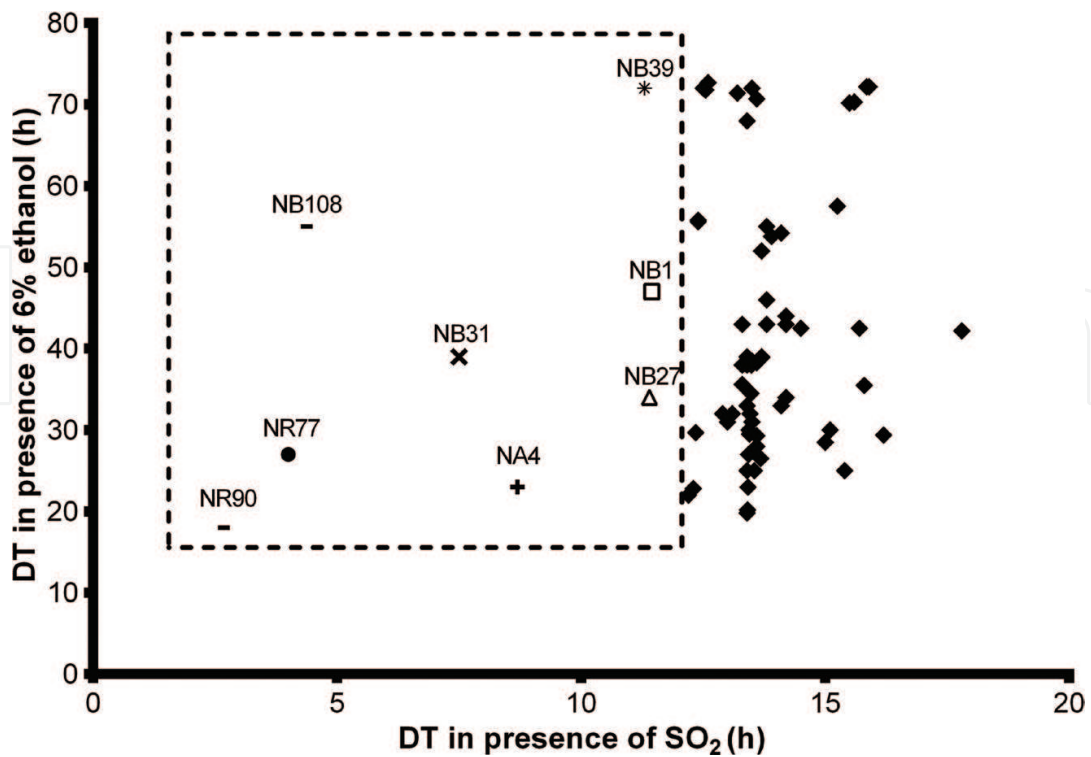
Figure 3. Detection times of 90 non-*Saccharomyces* strains in YPD (pH 3.5, 20°Bx) containing ethanol (6%) or SO$_2$ (30 mg·L^{-1}). Selected strains are shown in the inset.
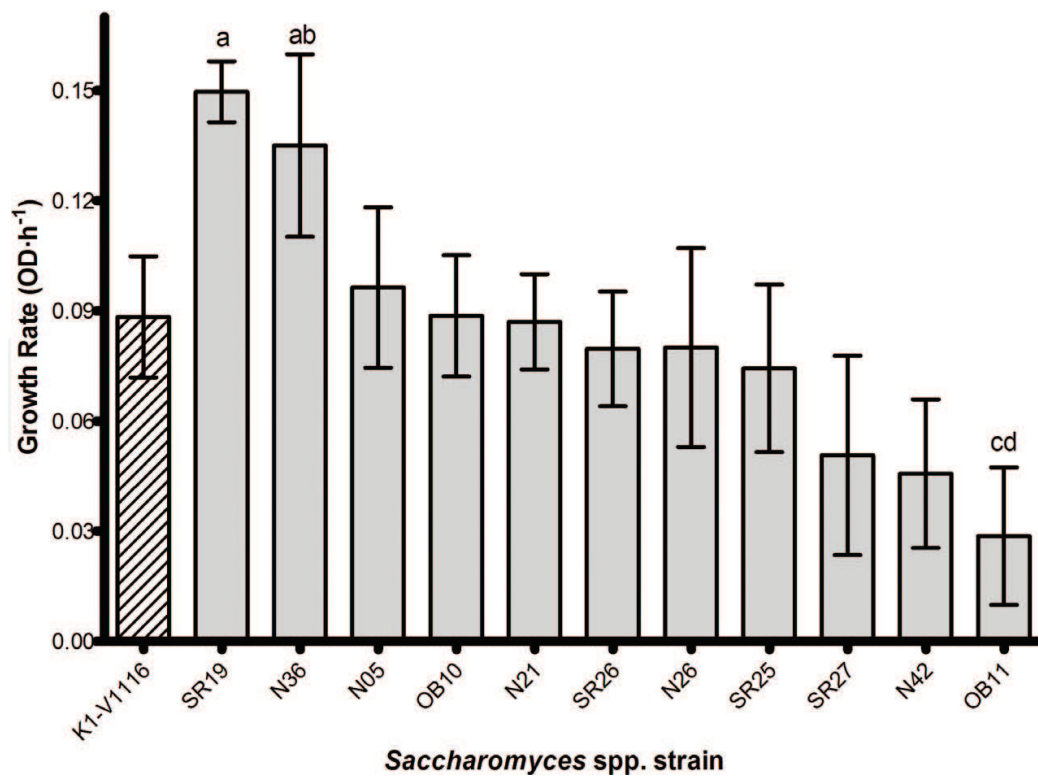

Figure 4. Growth rate of a commercial strain (stripped bar) and 12 *Saccharomyces* strains (solid bars) in grape juice media with 14% of ethanol, 50 mg/L of SO$_2$, and pH 3.5. Different letters denote statistical difference. Bars without letters correspond to ABCD.

Growth rate and DT are complementary parameters to describe the ability of yeast to adapt and grow in adverse conditions, similar to those found in fermentative beverages. Thus, both parameters (growth rate and DT) could be simultaneously used to select fermentative yeast strains (**Figure 5**).

Based on OD data, Salvadó et al. [98] estimated kinetic parameters of yeast strains (*S. cerevisiae* and nS) grown in a medium with ethanol (0–194.45 g/l) or incubated at different temperatures (4–46°C) by using Gompertz equation modified by Zwietering et al. [99]

$$y = D \times \exp\{-\exp[((\mu_{max})/D) \times (\lambda - t))+1]\} \tag{1}$$

where $y = \ln(OD_t/OD_0)$, $D = \ln(OD_{max}/OD_0)$ is the asymptotic maximum value reached, OD_0 is the initial OD, OD_t is the OD at time t, μmax is the maximum specific growth rate, and λ is the lag phase duration (h).

Based in μmax these authors generated a secondary model as a function of ethanol concentration and temperature of fermentation. Model prediction pointed to the temperature fermentation as the principal factor to promote the dominance of *S. cerevisiae* over other yeast genera during fermentation process. Only a few authors have applied this modeling strategy in yeast comparison [66, 100].
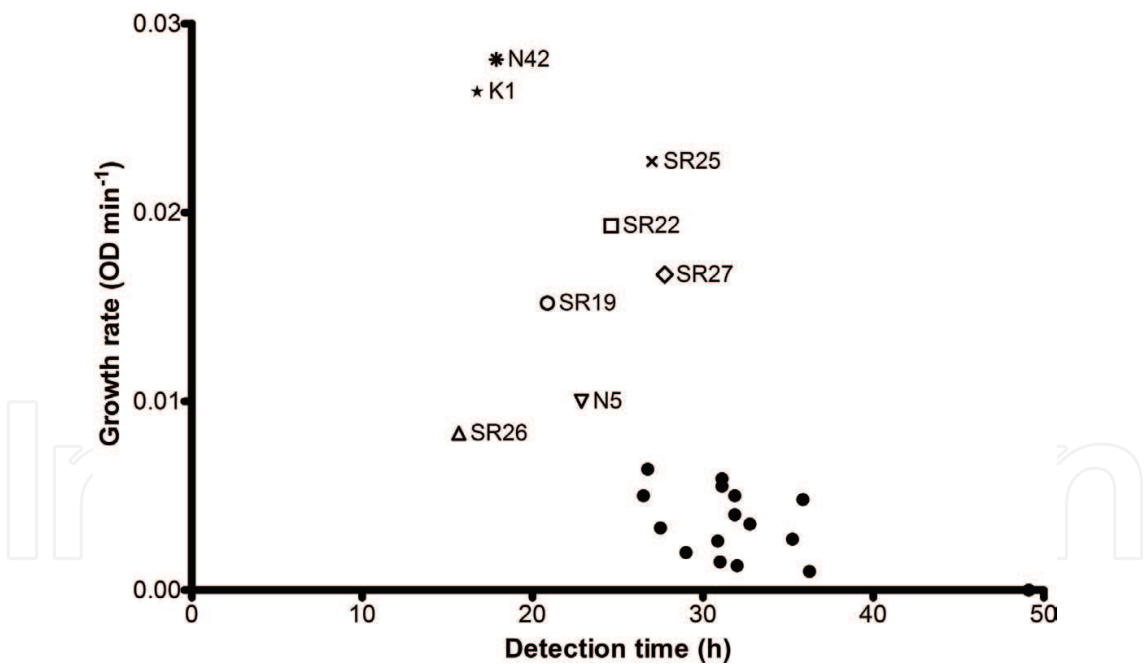

Figure 5. Detection times and growth rate of 29 strains of *Saccharomyces* grown in YPD medium with 12% of ethanol.

5. Conclusions

The vast variety of fermented beverages around the world and with them a great diversity of yeast present in each has encouraged the search for exceptional native yeast strains to be

selected in order to improve the quality of the products and the fermentation process itself. Molecular techniques have become a good strategy to elucidate yeast diversity and composition during any fermentation process. However, methods to evaluate the tolerance of yeast strains, developed during the last decades, are usually based in the qualitative measure of the growth of the microorganism in a medium containing the limiting compound after a specific time of incubation. Studying strains growth in limiting media through optical density measurements permits to estimate quantitative and comparable parameters fast and inexpensively, providing an insight on the fitness of each strain to certain environment, lag phase duration, growth rate, and maximum population and then performing a rapid initial selection of the strains.

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