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# Yeast Strain Optimization for Enological Applications

*David José Moreira Ferreira and Jessica Noble*

## Abstract

In the world of winemaking, tradition and innovation have always been side by side, on the one hand a culture of several centuries and on the other the need to constantly improve and answer new challenges. Consumers' preferences, climate changes, and fermentation efficiency are some of the modern questions that winemakers have to consider. Yeast, at the center of the fermentation, has revealed itself as the perfect platform to answer many of these challenges. By understanding the metabolism and the genetic basis that modulate specific phenotypes of yeast during fermentation, an era of yeast optimization has surfaced in the last decades and pushed research even further. In this chapter we will focus the attention on two of the most successful techniques to that end, quantitative trait locus (QTL) and evolutionary engineering. QTL relies on a highly precise identification of the genome regions that control a phenotype of interest. The transfer of these regions to selected wine yeasts is then possible by a technique called backcrossing. Evolutionary engineering induces the yeast itself to modify its genetic background to adapt to a selective pressure and improve its fitness. The right choice of pressure leads to the improvement of its performances in enological conditions.

**Keywords:** winemaking, yeast optimization, QTL, backcrossing, evolutionary engineering

## 1. Introduction

Nowadays, most of the enological fermentations are performed using selected wine yeast strains. Historically, and to some extent to this day, the selected wine yeasts have been found exploring the microbial flora present on the grapes, in the cellar, or in the vineyards. Next, a long process of characterization and selection is conducted in order to identify the yeast strain corresponding to a specific demand [1–5]. Since many years, the knowledge about wine yeasts has exponentially increased thanks to numerous scientific studies as well as the immense gain in the understanding of their metabolism. Consumption and requirements in nutrients (sugars, lipids, nitrogen, sulfur, vitamins, minerals), synthesis and production of biomass and metabolites (ethanol, glycerol, acids, alcohols, esters, sulfur compounds), resistance to stresses, and deficiencies have been well characterized. At the same time, the market trend and consumers' preference evolution results in a growing demand for new wine yeast strains combining different properties of interest or adapted to specific winemaking conditions and to global climate change. Consequently, meeting those steadily increasing requirements started to be a challenge. It becomes harder to find a strain combining all the properties

of interest [3, 6]. The development of wine yeast strain optimization strategies provided then a possible way out [7–9].

Optimization strategies of wine yeasts can be divided in two categories: the first exploits the existing diversity inside the *Saccharomyces cerevisiae* genus that has been recently demonstrated to be immense [10, 11] and the second allows to go further creating new phenotypes.

The exploitation of the natural diversity can be done by breeding. Breeding strategies of wine yeasts to combine properties of the parental strains have been implemented for many years [12, 13]. This can be done by sexual breeding or protoplast fusion for strains impaired in sporulation or mating. However, breeding without prior knowledge of the genetic basis of the properties of interest may lead to aleatory results, as most of the phenotypes are governed by complex regulations and often involve interactions. Additionally, a major drawback is that wine yeast strains are particularly difficult to mate due to the low spore viability and homothallism typical of this group [7]. Nowadays, more rational and powerful methods supported by the rise of the “omics” (genomic, transcriptomic, metabolomic studies, etc.), such as directed hybridization, can be carried out. Directed hybridization takes advantage of the knowledge of gene(s) of interest to follow and direct their transfer from one wine yeast strain to another [14].

On the other hand, going beyond the existing phenotypes can be performed by inducing new mutations. Mutagenesis by chemical or physical ways can be used to induce aleatory mutations inside the genome of wine yeasts [9]. Although simple to perform, this approach delivers quite random results with potential deleterious effects and requires massive clone screening, which can be unpractical depending on the phenotype being tested [15].

Evolutionary engineering also allows to go further the common phenotypes by continuously applying specific stressful conditions to a population of yeasts and selecting natural mutants presenting a higher fitness under those conditions [16–19]. This strategy is particularly powerful when the genetic bases of the phenotypes are not known.

Finally, the GMO strategy, also called genetic engineering, can be considered. This strategy exploits a set of molecular tools in order to manipulate the genetic characteristics of yeasts. In comparison to conventional improvement strategies that can transfer a large number of both specific and nonspecific genes to the recipient or may be responsible for some nontargeted variations in the genome, genetic engineering only transfers a small block of desired genes. Thus, this strategy is less time-consuming and yields more reliable products. However, the use of GMOs in food is strictly regulated in the EU and requires a heavy declaration, traceability procedures, and mandatory labeling even if no trace of the GMO can be found in the final product [20]. Although in some countries, the use of GMOs in food applications can be more easily allowed, the lack of background and studies to assess their impact on food safety, public health, and environment led to the creation of strict regulations and legislation during the 1990s. Several European regulations (e.g., EC258/97, EC1829/2003, 65/2004) have been issued to regulate every aspect of GMO use in the EU [21]. In enology, different strains were genetically modified, for instance, to obtain better aromatic profiles [22–24] or to overproduce glycerol and reduce ethanol yield [25, 26]. However their use in the EU and other countries is far from simple: long and costly administrative procedures, international and local regulations, consumer distrust, and the desire to keep the process within traditional boundaries point to a future in the wine industry without GMO [15]. More recently, the development of a marker-free, high-throughput, and multiplexed genome editing approach, the clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (Cas9) (CRISPR-Cas9) immune system, an easier and

traceless method of genome editing, has also been classified by the European Court of Justice as a GMO and is subject to the same controls [27]. It becomes clear that this kind of approaches cannot be reasonably developed for wine applications in the current context.

In this chapter, we will develop more in depth the two most widely used approaches for wine yeast improvement, directed hybridization through quantitative trait locus (QTL) mapping combined with backcrossing cycles and evolutionary engineering. These approaches currently provide very efficient, GMO-free strategies that have been greatly contributing for yeast optimization, particularly in winemaking.

## 2. QTL mapping and backcrossing

### 2.1 Identification of the molecular basis of technological properties: QTL mapping

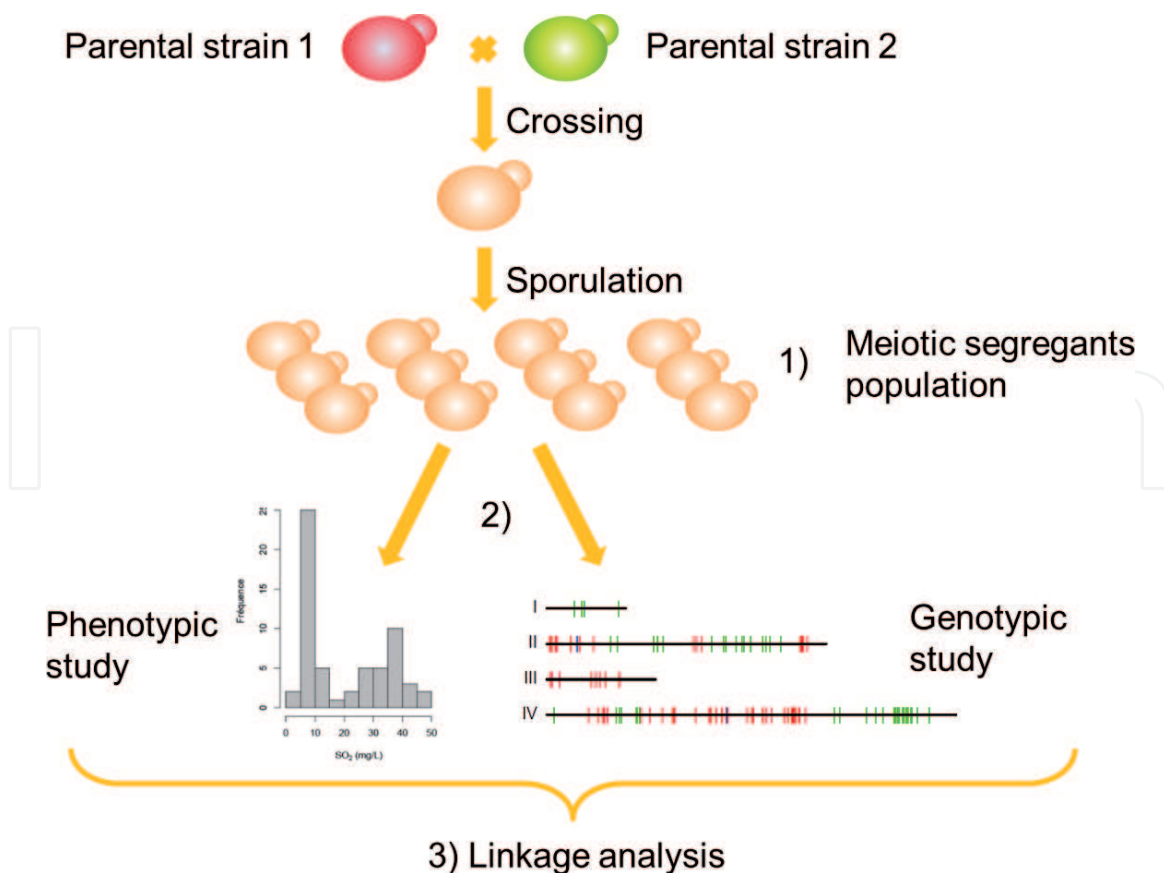
Numerous properties and phenotypes of wine yeasts are quantitative traits. These present continuous variations among individuals, in opposition to qualitative ones showing discrete variations. Those quantitative traits are due to complex genetic mechanisms, often linked to interactions between several loci. It is possible to identify the genetic determinants of such phenotypes using a QTL mapping. A quantitative trait locus is defined as a region of the genome, often scattered, associated with the phenotypic variation of a quantitative trait. The first study using the principles of QTL was done almost 100 years ago [28]. At the time, Sax [28] performed a genetic analysis correlating the size of beans with the color of pigmentation. Shortly after, the concept was applied to agriculture and since then has been widely used in many different organisms such as *Drosophila melanogaster* [29] and *Arabidopsis thaliana* [30], in crops [31], and in yeast [32, 33].

Thanks to those approaches, chromosomic regions, genes, or even mutations, responsible for several wine yeast properties, have been deciphered. These include traits like acetic acid production, sporulation, ethanol tolerance, growth at high temperature, flocculation, wine aroma production, amino acid consumption, nitrogen requirement, fermentative performances, and sulfur compound production [34–45]. These studies have shown some phenotypes to be particularly complex.

The QTL mapping method is divided into three steps. First, a recombinant population is constituted, second, this population is then phenotyped and genotyped, and, lastly, a statistical analysis to link the regions of the genome to the phenotypes is performed (**Figure 1**).

The recombinant population is usually constituted from a hybrid obtained by crossing two parental strains, selected based on their phenotypic diversity. We can note that it is also possible to start directly with a highly heterozygous diploid parental strain, e.g., selected after evolutionary engineering. The hybrid is induced to sporulate to generate a population of meiotic segregants. The meiotic segregants passed through recombination so that each segregant possesses a random distribution of the alleles of the two parents. As the recombination rate is a crucial point in the accuracy of the final mapping of the QTL, it is also possible to generate an F2 segregant population to increase the allelic mixing. In that case, the initial meiotic segregant population, F1, is submitted to random crossing before a second sporulation round to constitute the F2 haploid segregant population [42, 43].

The phenotyping of the segregant population is a crucial step that can be limiting in the QTL approach. Each segregant has to be phenotyped individually for the trait of interest. The higher the number of segregants that are phenotyped, the



**Figure 1.** Schematic representation of the QTL mapping strategy divided in three steps: (1) constitution of the meiotic segregants population, (2) phenotypic and genotypic study, and finally (3) linkage analysis.

better the precision will be in the mapping of the QTL. Some phenotypes can be measured on plates, such as ethanol tolerance; however, numerous phenotypes of interest for wine yeasts require to perform enological fermentations.

The next step is to create a genetic map constituted by molecular markers differentiating the two parental strains. The aim is to obtain the most homogeneous and dense distribution of the markers throughout the genome. The better the coverage is, the more accurate and precise the QTL mapping will be. Then, genotyping of the segregants attributes a parental origin to each marker. Nowadays, the development of sequencing approaches and the reduction of their costs allow to genotype the strains using whole-genome re-sequencing implementing next-generation sequencing technology [46]. This is done for parental strains as well as for the selected segregants.

Different approaches of QTL mapping can be carried out, using individual genotyping or bulk segregant analysis (BSA). For the individual genotyping, each segregant is genotyped, and a linkage analysis identifies the regions that are more likely to be involved in the phenotype. The powerful method of interval mapping is often used [47]. This method is based on the distances between markers. For each marker, the probability that this locus is a true QTL is calculated by a model. A significant threshold can be established by permutation testing. This approach is based on the hypothesis of a single QTL, but it is possible to identify other QTLs by a composite interval mapping that will iteratively scan the genome and add known QTL to the regression model as QTLs are identified.

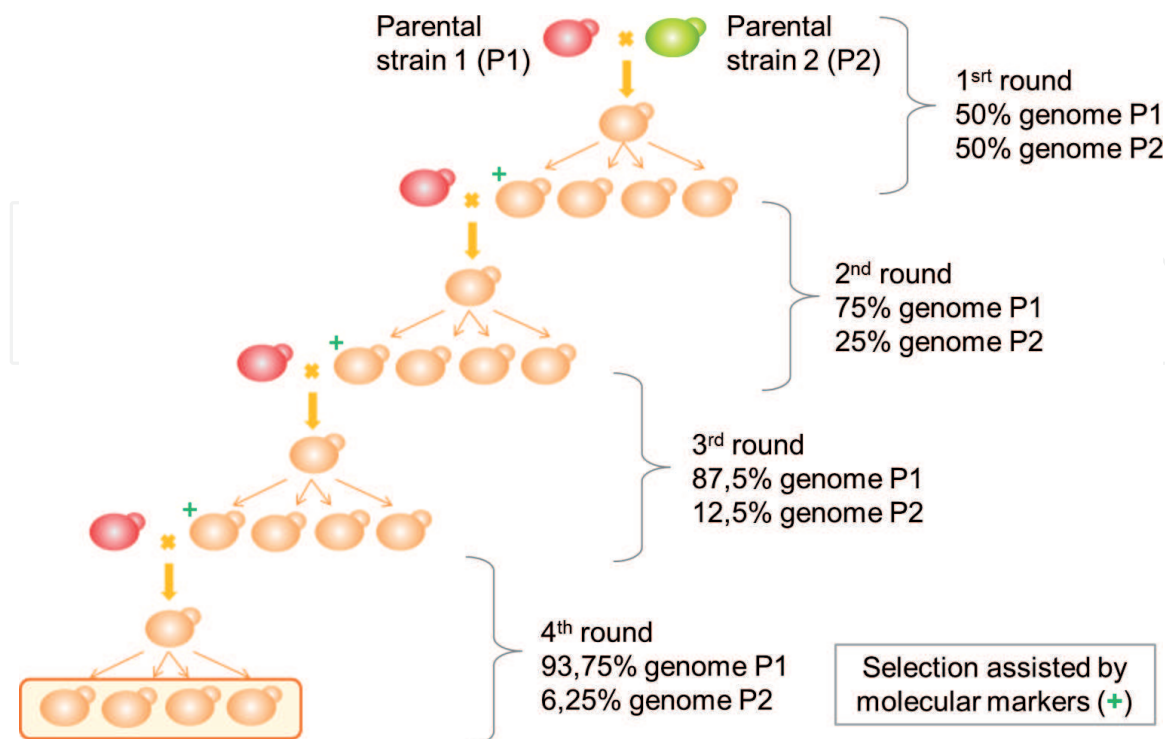
In the BSA approach, the segregants that present the same phenotype are pooled together [48, 49]. The aim is to identify the regions of the genome that are common to all the segregants presenting the same phenotype. The allelic frequency between the two bulks or with the control is studied and allows detecting gene variants involved in the phenotype.

A powerful extension of BSA has also been developed: extreme QTL (X-QTL) mapping [50]. This approach is based on the generation of segregating populations of very large size. Those populations composed of large numbers of progeny with extreme trait values can be constituted using selection for drug or stress resistance or by cell sorting. Pooled allelic frequencies are then determined.

The genomic regions identified by QTL mapping strategies can vary from few to 1000 kilobases. Inside those regions, the sequences of the genes are compared, and non-synonymous mutations between the parental strains are searched in the coding region and the promotor/terminator regions. A study of the function of the genes located in this region using databases allows identifying candidate genes. A functional validation of the candidate genes can then be performed. Allelic replacement and reciprocal hemizyosity analysis (RHA) are the common ways to validate the impact of an allele on the phenotype. Allelic replacement consists in deleting the candidate gene in a parental strain and replacing by the allele of the opposite parent. Hemizyotes are constructed using the hybrid of the parental strains and deleting only one copy of the gene. The obtained strains are tested for their phenotypes. Thanks to those approaches, genes, mutations, or even translocations have been validated for diverse wine yeast properties, such as lag phase duration, fermentation capacity under nitrogen starvation, and ester production [42, 51, 52].

## 2.2 Transferring properties of interest from one strain to another: backcrossing

Once markers or mutations have been identified thanks to a QTL mapping strategy, it is possible to manage their transfer from one wine yeast strain to another. Introgression, also called backcrossing, or selection assisted by molecular markers, consists in recursive hybridization between a strain possessing the allele of interest and a strain to improve (Figure 2).



**Figure 2.** Schematic representation of backcrossing cycles or recursive hybridization between the receptor strain (parental strain 1, in red) and the donor strain (parental strain 2, in green). The molecular markers (green cross) are followed at each step by PCR. The final strain (in orange) possesses a major part of its genome coming from the receptor strain and a small part transferred from the donor and containing the region of interest.

The first step is the selection of a “receptor” strain. This strain possesses a good genetic background and presents numerous properties of interest, except the one aimed to be enhanced. This strain will be crossed with a “donor” strain that possesses the property of interest. A first cross results in a hybrid possessing 50% of the genome of each parental strain. This hybrid is induced to sporulate, and a population of meiotic segregants is constituted. A segregant with the right marker or allele of interest is selected using a simple identification by PCR. This segregant is crossed again with the receptor strain. The second hybrid possesses 75% of the genome of the receptor strain and 25% of the donor strain. Several cycles of breeding/sporulation are performed to regenerate the genome of the receptor strain and to recover its good properties. Generally, four cycles are sufficient and lead to a strain possessing more than 93% of the genome of the receptor strain and less than 7% from the donor, including the genes of interest.

This approach has been implemented in plants for many years [53, 54]. Its application to the improvement of wine yeasts has started more than 10 years ago [14] and since then it has been applied to generate numerous wine yeast strains. The production of H<sub>2</sub>S, lag phase, and POF character [14], volatile thiol release [55], or SO<sub>2</sub>, H<sub>2</sub>S, and acetaldehyde [56] have been improved using this approach.

### 3. Evolutionary engineering

#### 3.1 Evolutionary engineering as a simulation of nature

Evolution is one of the most important processes present in nature to which all living beings are submitted. After traveling around the world collecting much data, Charles Darwin published the book *On the Origin of Species* on the mid-nineteenth century explaining his theory of evolution based on natural selection. To this day, aside from minor revisions, this theory is the one broadly accepted within the scientific community to best explain evolution. In short, the theory bases itself on the fact that genetic variation occurs among individuals of the same species in a given population leading to phenotypic variations as in morphology, physiology, and behavior traits. In each specific environment, different traits confer different survival rates and different reproduction chances. Upon natural selection, advantageous traits can be passed from generation to generation in a stable heritability. By combining these principles, the progeny of the fittest (best adapted) in a given environment will gradually replace the members of a population and take over. In the case of adverse conditions or sudden environment change, this is one of the main mechanisms on which species rely to keep thriving and avoid extinction.

Evolutionary engineering, also designated as adaptive, directed, or experimental evolution, is an approach where these very same principles of evolution are applied to a selected population in a known and controlled environment [57]. The main difference from nature's evolution is the orientation of the natural selection toward specific selective pressures, the ones which best represent a given environment where we look for an evolution. Over time, individuals initially not optimally adapted may evolve and gain a higher fitness with the accumulation of natural and positive mutations for that specific environment. As the fittest, these individuals will be able to better utilize the available resources, grow faster, and multiply faster in higher number. The natural course is then for their progeny to gradually become dominant within the population, leading to the evolved individuals initially sought. Multiple evolutionary engineering experiments have been performed with different organisms such as *Drosophila* [58], domestic mouse [59, 60], the unicellular

algae *Chlamydomonas* [61], *Pseudomonas fluorescens* [62], *Escherichia coli* [63], and *S. cerevisiae* [64] demonstrating how the evolution principles can be successfully applied to different living beings and contexts.

### **3.2 Evolutionary engineering applied to yeast: why and how?**

Yeast has been the focus of many evolutionary studies due to its potential to generate academic knowledge as well as its broad range of applications. Its success in the evolutionary context is related to different advantages such as the high number of individuals that can be obtained within the same population, the easiness of maintenance/growth of populations with relatively low costs, and a fast generation time. Additionally, evolutionary engineering is a non-GMO technique. As explained before, no direct human manipulation occurs since the yeast itself improves and evolves its genetic background with natural mutations. Thus, evolved yeasts are perfectly safe and can be used in any food and/or beverage context without restrictions. Finally, another positive feature is the simplicity and empirical way on how the evolutionary engineering can be performed [21]. Contrary to other approaches, no genetic characterization or deep knowledge about the selected yeast is required. Nonetheless, when planning a yeast evolutionary engineering, key parameters need to be defined.

#### *3.2.1 Selective pressures*

During an evolutionary experiment, the mutations and consequently the diversity generated are completely random and cannot be predicted or controlled [65]. Therefore, it is crucial to identify the selective pressures that will best conduct the selection of positive mutations toward the desired phenotype. Once they are clearly identified, a proper initial experimental characterization should be performed to identify the intensity that these selective pressures should have. If too low there will be no selection, and if too high, yeast will struggle to continue in culture. Additionally, if a further industrial application is predicted, evolutionary engineering should be performed in conditions as close as possible to the actual conditions in which the yeast will later perform. By doing so, yeasts will not only face the selective pressures chosen to drive evolution but also all the other constraints naturally present [66]. For instance, in wine fermentation conditions, yeasts need to cope with stress factors as diverse as low pH, nutrient deprivation, ethanol, osmotic, and oxidative stress which are commonly present [67]. Therefore, it is preferable to use natural or synthetic must as a media that closely mimics realistic conditions while allowing the control and modification of specific parameters [68].

#### *3.2.2 Strain choice*

Depending on the final objective, the choice of a yeast strain can vary. Different laboratory strains have been used in evolutionary approaches with the main goal of generating academic knowledge. On the opposite side of the spectrum, industrial yeast strains have also attracted major interest due to the possibility of improving their efficiency and resistance [9, 66, 69]. Ploidy can also influence strain choice. Haploid strains have the advantage of evolving faster, making it easier to later on identify the mutations that lead to the evolved phenotype [70]. However, they are more sensitive to deleterious mutations that could easily become lethal, whereas diploid strains, as most wine yeast strains, have an increased ability to buffer such mutations. This way diploid strains tend to be more stable and robust when submitted to evolutionary engineering.



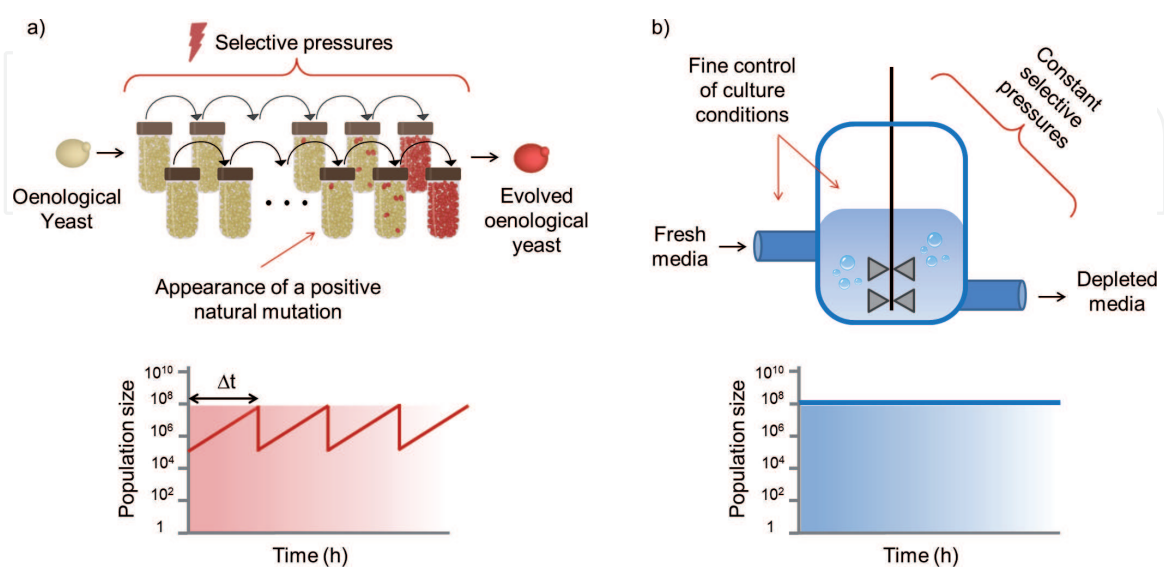
### 3.2.3 Cultivation

Microbial evolutionary engineering approaches are typically done in one of the two ways: serial cultivation (batch) and continuous cultivation (chemostat). Both are equally valid, and the choice will depend on the experimental conditions and objectives. With serial cultivation, the principle is to aliquot the culture into a new fresh medium at regular intervals (**Figure 3**). This is often used to select for microorganisms with shorter lag phase or higher growth rate, but certain regimes might also allow the selection for higher biomass formation or a better survival after nutrient depletion [18, 19, 69, 71]. Due to manipulation easiness and economic maintenance, this method allows several parallel cultures, often performed in shake flasks. On the downside, batch cultures are prone to some uncontrolled parameters and fluctuations in population density, growth rate, or dissolved oxygen [57]. In continuous cultivations bioreactor vessels are typically used. Here, all experimental parameters such as medium influx rate, temperature, oxygenation, and pH are continuously monitored leading to constant growth rates and population densities (**Figure 3**). Continuous cultures usually favor selection for higher substrate affinity [69]. The major disadvantages are the much higher costs and limitation in parallel experiments, depending on how many chemostats are available [57].

Independent of the cultivation method used, running simultaneous evolutionary engineering approaches of the same condition is advised. Woods et al. [72], using *Escherichia coli* in 12 identical and parallel evolutionary engineering experiments, showed that different random mutations can be fixed in different populations. As a consequence, the final outcome of each evolutionary process can vary. Identical phenotypes can be obtained with equivalent or different mutations; however different phenotypes can also be obtained despite the same exact conditions. Having parallel experiments increases the chances of success.

### 3.2.4 Duration

How long an evolutionary engineering approach lasts is highly case dependent and somehow unpredictable due to the randomness of mutations. Rather than



**Figure 3.**

Illustration of both directed evolution strategies: (a) serial transfer and (b) continuous culture. (a) Done with regular inoculations/transfers to fresh media which makes it similar to a batch. Once inoculated, populations increase over time and interact with medium with no intervention until new transfer. (b) A continuous nutrient feed that allows a constant population over time, permanently under the same conditions. Similar principles as a fed batch.

absolute time, duration is often measured by the number of generations. Natural mutations mostly occur when microorganisms divide, and since experimental conditions can modulate cell division from a few hours to several days, measuring the number of generations is a more accurate evolution timescale. If selective pressures are effective, in yeast a positive evolution is frequently observed between the 50th and the 200th generation. However, it might be the case that after many more generations, no evolution is observed. In this scenario, it might be the case that the approach setup, conditions, or parameters such as the selective pressures need to be reviewed. The best strategy to optimize duration is to regularly screen the evolving populations. By early detecting a positive evolution, the approach can either be stopped at the right moment or pursued if the evolved phenotype is still not satisfactory.

### 3.3 From the bench to the cellar

Once a positive evolution is detected in a wine evolutionary approach, a thorough work of validation needs to be done before an evolved wine yeast can actually be used in a cellar. The first step, often at laboratory scale, is to submit evolved yeasts to the evolutionary conditions in direct comparison with the parental strain, separately or in competition, to evaluate the relative improvement of the phenotype [73]. If acceptable, this comparison should also be validated in different realistic conditions where the yeast might perform. Typically, natural or synthetic musts are used in order to better reproduce enological fermentation conditions [68]. Aside from the characterization itself, this first screening allows for the search of possible trade-offs. A trade-off occurs when a particular phenotypic trait gets improved at the expense of one or more other phenotypic traits that get worsen. This is well illustrated in a study by Wenger et al. [74] who successfully evolved *S. cerevisiae* for a higher fitness in anaerobic glucose-limited media. Despite this, when in aerobic, carbon-rich environments, the evolved clones performed less well than their ancestor due to a trade-off. Similarly, yeast cells evolved for efficient galactose consumption which presented trade-offs when grown on glucose as a carbon source [75]. In winemaking context it is fundamental for yeast traits such as aroma production or fermentation efficiency to be kept at high standards and free of trade-offs. To note that in an evolutionary approach, the higher the number of generations occurred, the higher the chances of unrelated mutation accumulation. This reinforces the fact that the approach should be stopped as soon as a positive evolution is detected to avoid the accumulation of potential trade-offs.

Another fundamental test is to propagate and dry the yeast under industrial conditions, often the method used to produce commercialized wine yeast strains. Propagation and drying represent as the major sources of stress for yeast including oxidative, osmotic, and desiccation stresses which the evolved strains need to endure at least as well as the parental strains [21, 76–78]. The final stage of validation is the scale-up to pilot and industrial fermentation volumes, often performed by cellars with tanks of several hectoliters. If the evolved wine yeast strain performance is satisfactory both for the evolved phenotype and the remaining important traits, the evolutionary engineering process is then a success from an industrial point of view, and the yeast can be commercialized. From an academic point a view, new knowledge can also be generated by studying the new genetic profile in correlation to the evolved phenotype and how the evolved strains differ from the parental one. Approaches to conduct this characterization include genome microarray hybridization and direct DNA sequencing [75, 79, 80].

### 3.4 Successful evolutionary engineering in winemaking

To illustrate the potential of evolutionary engineering approaches in winemaking, few examples can be used where technical or field problematics were successfully solved by using this approach with validated evolved strains.

While using a long-term batch culturing on gluconate (a carbon source poorly assimilated by *S. cerevisiae*), Cadière et al. [19] evolved a commercial wine yeast strain obtaining interesting results. Evolved clones presented a carbon flux through the pentose phosphate pathway which increased by 6% when compared to the parental strains. This also resulted in a higher fermentation rate, lower levels of acetate production, and increased production of aroma compounds. As the process was carried out at a laboratory scale but in realistic (enological) conditions, the same phenotypic improvements were verified when the evolved strain was used in pilot-scale trials [81]. It was identified that the evolved strain produced higher levels of phenyl ethanol, isobutanol, isoamyl alcohol, ethyl acetate, isoamyl acetate, and ethyl esters [82].

Other authors were able to obtain a stable wine yeast strain with slightly enhanced glycerol production. By employing sulfite as a selective agent in an alkaline pH, Kutyna et al. [83] obtained evolved clones with an increase of 41% in glycerol production, which can have a benefic impact in wine organoleptic properties. To reduce the final ethanol content in wine, Tilloy et al. [18] submitted a wine strain to hyperosmotic stress for 200 generations, which yielded evolved clones that grew better under osmotic stress and glucose starvation and produced markedly more glycerol but also succinate and 2,3-butanediol. The approach was then complemented with an intra-strain breeding strategy that further increased the glycerol yield and reduced ethanol production in wine by up to 1.3% (v/v).

More recently, López-Malo et al. [80] performed an evolutionary process aiming for a higher performance for low-temperature fermentations (12°C). It was discovered that inositol and mannoprotein limitations were responsible for an evolution toward shorter fermentation times and higher final populations. After genome sequencing, it was discovered that an SNP in the gene *GAA1*, fundamental in inositol and mannoprotein synthesis, was at the basis of the improvement.

## 4. Conclusions

For a long time, innovation in yeast applications was mainly based on empirical observation and selection of natural isolates. In wine fermentation, despite the hundreds of wine yeast strains well characterized and commercially available, this diversity started to become insufficient to effectively answer all modern problematics. Consumers' preferences (e.g., specific aromas), improvements in fermentation efficiency, or counterbalance climate change are examples of key challenges that winemakers currently face and to which they require rapid and viable solutions. The emergence in the last decades of the different techniques discussed in this chapter allowed major advances in that sense. QTL mapping/backcrossing and evolutionary engineering are particularly two techniques that excel in providing solutions to specific applied issues.

QTL mapping is relevant as most of the enological traits of interest are governed by multiple loci and present a continuous variation in a population. Thanks to recently growing genetic tools, the study of the genetic determinants is becoming easier, and QTL mapping can be performed using molecular markers or whole-genome sequencing. Once the alleles of interest are known, they can be transferred from one strain to another using introgression. This constitutes a powerful natural

approach to combine traits of interest of two wine yeast strains and/or to improve a strain conferring it a new property. On the other hand, some phenotypes and traits of interest can be hard to improve due to their complex regulation by different loci in the genome. If QTL mapping can precisely identify their genetic basis, evolutionary engineering is a solid alternative for a direct improvement of a trait to which low or no knowledge might be available. Often performed in the industrial context itself, this approach can provide both applied and academic outcomes with a relative simple and cost-effective methodology. Using this technique, most of the wine yeast traits of interest can be improved which leaves the future of winemaking with an immense potential in terms of innovation.

By combining relatively simple principles with high precision in addressing the problematics at their basis, QTL mapping and evolutionary engineering offer high rates of success. This justifies their initial success within the academia. In combination with their non-GMO status, this was quickly transferred to application and industry such as winemaking. Despite the precision that these techniques already offer, it is very likely that in the coming years their efficiency will continue to increase, while their cost will be reduced. Sequencing and whole-genome sequencing are following this exact trend and becoming more and more current. Identifying mutations or DNA regions responsible for specific phenotypic traits will then be more accessible with even more accurate results. In addition to other techniques that may emerge in the meantime, this suggests a bright future for wine yeast optimization and a continuous progress in winemaking.

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## Conflict of interest

The authors declare no conflict of interest.

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