Review

3.0 Strategies for Yeast Genetic Improvement in Brewing and Winemaking

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Abstract

Yeast genetic improvement is entering a transformative phase, driven by the integration of artificial intelligence (AI), big data analytics, and synthetic microbial communities with conventional methods such as sexual breeding and random mutagenesis. These advancements have substantially expanded the potential for innovative re-engineering of yeast, ranging from single-strain cultures to complex polymicrobial consortia. This review compares traditional genetic manipulation techniques with cutting-edge approaches, highlighting recent breakthroughs in their application to beer and wine fermentation. Among the innovative strategies, adaptive laboratory evolution (ALE) stands out as a non-GMO method capable of rewiring complex fitness-related phenotypes through iterative selection. In contrast, GMO-based synthetic biology approaches, including the most recent developments in CRISPR/Cas9 technologies, enable efficient and scalable genome editing, including multiplexed modifications. These innovations are expected to accelerate product development, reduce costs, and enhance the environmental sustainability of brewing and winemaking. However, despite their technological potential, GMO-based strategies continue to face significant regulatory and market challenges, which limit their widespread adoption in the fermentation industry.

Keywords: brewing; genome editing; synthetic biology; synthetic genomic; machine learning; adaptive laboratory evolution

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1. Introduction

Yeasts have been used in biotechnology since 6000 BCE for beer and bread production, though their role in fermentation was only discovered in 1876 by Pasteur [1]. The isolation of pure yeast strains by Hansen in 1881 marked a major advancement, establishing yeasts as essential model organisms in microbiology and biotechnology. Today, yeasts are widely exploited as cell factories for producing food, bioethanol, chemicals, and recombinant proteins, including approximately 15% of all protein-based biopharmaceuticals currently available for human use [2]. In the era of next-generation sequencing (NGS), yeast remains a prominent system in population genetics, microbial ecology, and synthetic biology [1–3].

Beer is the third most consumed beverage worldwide after water and tea and it holds the largest market segment within the global alcoholic beverage sector [4]. The global beer market is projected to reach USD 692,840.1 million by 2025, with a compound annual growth rate (CAGR) of 3.50% from 2025 to 2033 [5]. Craft beer is the fastest-growing

segment of the beer market industry, driven by diverse flavor profiles and appeal for local brands [5,6]. The European craft beer market size reached USD 40.2 billion in 2024, exhibiting a growth rate (CAGR) of 6.24% during 2025–2033 [6]. Craft beer drinkers are highly engaged and willing to pay a premium for unique beers, presenting opportunities for further market growth [7].

In alcoholic fermentation, Saccharomyces sensu stricto species (currently including Saccharomyces arboricolus, Saccharomyces cariocanus, Saccharomyces cerevisiae, Saccharomyces kudriavzevii, Saccharomyces mikatae, Saccharomyces paradoxus, Saccharomyces jurei, Saccharomyces eubayanus, and Saccharomyces uvarum (Saccharomyces bayanus var. uvarum)) are widely employed due to their superior resistance to high sugar concentrations (200–300 g/L), low pH (3–4), and elevated ethanol concentrations compared to other yeasts [8]. Brewing yeasts can be categorized into two main types: ale yeast and lager yeast. These two yeast groups primarily differ in their optimal growth temperatures. Ale yeasts, represented by Saccharomyces cerevisiae, are primarily used for ale beer production. They grow best within an optimal temperature range of 15–26 °C. In contrast, lager yeasts, classified as Saccharomyces pastorianus, are cold-tolerant, thriving in temperatures ranging from 8 to 15 °C [9]. They are employed in the production of cold-fermented, lager-style beers, which dominate the market and account for approximately 90% of global beer production.

Lager beer yeasts are natural hybrids derived from the crossing of *S. cerevisiae* and *S. eubayanus* [10]. Hybridization provides several competitive advantages, including cryotolerance, inherited from *S. eubayanus*, and efficient maltotriose utilization, a trait of *S. cerevisiae*, both of which are particularly beneficial for low-temperature brewing [11,12]. It is hypothesized that the hybridization between *S. cerevisiae* and *S. eubayanus*, leading to the formation of *S. pastorianus*, occurred in the Munich Hofbräuhaus between 1602 and 1615, during a period when both wheat beer and lager were being brewed simultaneously [13]. All *S. pastorianus* strains have an approximately diploid *S. eubayanus* subgenome, but some strains (referred to as Group 1 or Saaz strains) have a generally incomplete and haploid *S. cerevisiae* subgenome, while other strains (called Group 2 or Frohberg) have a diploid or higher *S. cerevisiae* subgenome [13,14]. Other notable natural interspecific hybrids include those between *S. cerevisiae* and *S. kudriavzevii* [1], which have been used in brewing of Belgian-style ales. These hybrids illustrate the diverse evolutionary trajectories within the *Saccharomyces* genus, highlighting their central role in beer innovation [14].

In addition to alcoholic fermentation, yeasts significantly contribute to the flavor and taste of beer and wine, which are the major determinants of beer and wine quality and consumer preferences [15]. Beer flavor research focuses on specific metabolites and genes directly influencing quality and taste [16]. For instance, genes like *EEB1*, *ETR1*, and *ATF1* affect ester production, while *ARO9* and *ARO10* are essential for higher alcohol synthesis [16,17]. However, the complete metabolic pathways and regulatory mechanisms of flavor compounds in beer and wine remain incompletely understood [15].

There is an increasing demand from the brewing and winemaking industry for novel and more diverse options for fermentation which can offer significant advantages in industrial demands. Current efforts focus on improving production processes to ensure high-quality beer, expanding flavor profiles, and developing beer with reduced sugar and alcohol content to promote human health [18]. Despite innovative techniques, genetically modified (GM) yeasts are not yet permitted in many countries, including in the European Union, for ethical and regulatory reasons. Classical techniques that do not produce GMOs, thereby avoiding stringent regulations, include mutagenesis and sexual breeding [19]. These methods aim to enhance and combine traits under polygenic control, such as fermentation capacity, ethanol tolerance, or the absence of off-flavors. While these techniques do not produce GMOs, they pose challenges in adding or removing characteristics without

altering strain performance [20]. When considering genetic improvement techniques, it is crucial to first define whether the target trait is monogenic or polygenic to select the optimal approach. Generally, the most important oenological traits, such as yield, ethanol tolerance, or temperature-dependent growth profiles, depend on multiple loci (QTLs) distributed across the genome and are thus poorly characterized [19,20].

Ongoing research is focusing on identifying novel natural yeast strains to enhance the quality of wine and beer. For instance, yeasts found in Patagonia have demonstrated high ethanol tolerance [21]. Similarly, yeasts isolated from the American Midwest, such as *Hanseniaspora vineae*, *Lachancea fermentati*, *Lachancea thermotolerans*, *Schizosaccharomyces japonicus*, and *Wickerhamomyces anomalus*, are of interest due to their ability to produce both ethanol and lactic acid, allowing them to potentially replace lactic acid bacteria (LAB) in sour beer wort fermentation [22]. However, further genomic and phenotypic studies are required, as certain strains within the same species have shown divergent metabolic behaviors, challenging the hypothesis of a conserved heterolactic fermentation pathway among these yeasts [23]. Clonal selection in this context refers to the isolation and identification of specific yeast strains from natural populations, while maintaining traits desirable for winemaking and brewing applications [19,24]. Yeasts originating from other fermented food such as cacao, kombucha, and sourdough are being explored for their potential in brewing [25].

In 1998, the so-called 1.0 era of yeast genetic manipulation was initiated through genetic engineering, which allowed for small modifications of the yeast genome [8,26]. Subsequently, numerous advances in sequencing, genome editing, and DNA synthesis paved the way for the Synthetic Yeast Genome Project (Sc2.0), the world's first synthetic eukaryotic genome initiative, which aims to construct a novel, rationally designed version of the *S. cerevisiae* genome [27,28]. This project was recently completed with the synthesis of the final synthetic chromosome, synXVI [29]. Currently, we are entering the 3.0 era of yeast improvement, characterized by the integration of artificial intelligence (AI), big data analytics, and synthetic microbial communities. These advances have significantly enhanced the potential for precise fermentation carried out either through single-strain culture or synthetic microbial consortia. In this work, we outline a range of both GMO and non-GMO techniques employed for the genetic optimization of yeast, focusing on how the integration of diverse strategies will increase the design of high-quality products. We emphasize that there is no universal strategy for yeast improvement; rather, the choice of technique must be tailored to specific objectives, depending on the desired phenotype and the extent of available knowledge regarding its genetic basis (Table 1).

Table 1. Overview of different genetic techniques for genetic improvement of yeast.

Technique	Pros	Cons
Mutagenesis Non-GMO	Generates a wide variety of potential phenotypes; relatively inexpensive and straightforward; suitable for monogenic phenotypes.	Random outcomes make it difficult to predict results; can introduce harmful mutations or undesired traits; not suitable for polygenic or complex phenotypes.
Hybridization Non-GMO	Simple method to combine beneficial traits from different yeast strains; heterosis compared with parents; well-established and cost-effective.	May result in sterility or instability of the hybrid offspring; difficult to obtain for poorly sporulating strains; undesirable characteristics may emerge.

Table 1. *Cont.*

Technique	Pros	Cons
Adaptive Laboratory Evolution (ALE) Non-GMO	Mimics natural selection, leading to improved fitness-related traits over time; suitable for phenotypes that can be directly selected under controlled environments.	Time-consuming and labor-intensive; Unintended side effects may occur, as it is difficult to control specific outcomes; May not be suitable for traits without clear selection markers.
Multi-Omics and AI Integration Non-GMO	Provides a comprehensive view of yeast metabolism and gene expression; unintended side effects may occur, as it is difficult to control specific outcomes.	Requires large datasets and significant computational resources; interpreting the data can be complex and requires expert knowledge.
Synthetic Microbial Communities (SMC) GMO or non-GMO (depending on strains used)	Enables creation of yeast strains consortia with complementary traits that work together; Can improve metabolic networks.	Requires compatibility between strains, including nutrient requirements; If GMO strains are used, there are ethical concerns around genetic modifications.
CRISPR-Cas9 GMO	Precise and efficient genome editing; Allows for modifications of multiple genes at once; highly versatile across different yeast strains.	Ethical concerns around gene editing; Requires optimization for different yeast species; Requires advanced understanding of yeast genetics.

2. Non-GMO Genetic Improvement Techniques

2.1. Classical Genetic Improvement Techniques for Non-GMO Yeasts

2.1.1. Random Mutagenesis

One conventional genetic improvement technique is random mutagenesis, which aims to eliminate undesirable traits. Since the spontaneous mutation rate in *S. cerevisiae* is generally low, approximately 10^{-6} per generation, and even lower than that of meiotic genetic conversion [8], mutagens such as UV radiation or chemical agents, including ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), and nitrous acid (NA) [20], are employed. The resulting library of mutagenized strains is submitted to either positive or negative selection processes by replica plating, as shown in Figure 1 [8]. The main constraint of this technique is that mutants can only be isolated in selectable phenotypes that in one way or another can be selected under specific conditions.

The technique is further limited by the genomic architecture of industrial yeasts, which are often diploid or polyploid. Although mutations frequencies are comparable across haploid, diploid, and polyploid cells, mutations are more readily identified in haploids, as diploid and polyploid genomes typically retain unmutated alleles. This can obscure the phenotypic expression of dominant mutations, making them more difficult to detect [20]. Additionally, while mutations may enhance specific traits, they can also occur in off-target genes, potentially compromising other relevant industrial phenotypes.

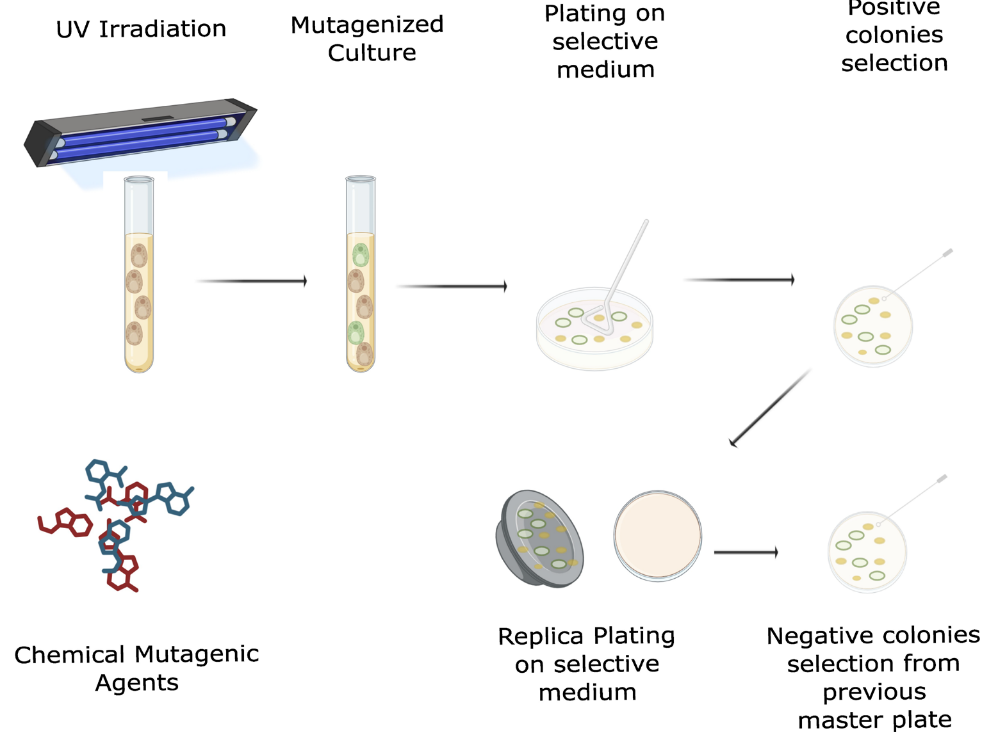

Figure 1. Schematic representation of the mutagenesis process. The process begins with induced mutagenesis achieved through UV irradiation or exposure to chemical mutagenic agents. The mutagenized culture is then plated on a selective medium, where the use of selection markers or readily distinguishable phenotypic traits is essential. Mutant clones exhibiting the desired phenotype can then be identified and selected. In the case where the target phenotype is a loss of function, replica plating is necessary to facilitate identification. This figure was created with BioRender (www.Biorender.com, accessed on 8 June 2025).

Random mutagenesis has been extensively employed to improve wine yeasts, particularly with respect to the production of amino-acid-derived aroma compounds [30]. To render this phenotype selectable, a toxic analogue of the target amino acid can be used to isolate mutants with reduced feedback inhibition in amino acid synthesis pathways, leading to overproduction and metabolic overflow into higher alcohols [31]. In brewing, random mutagenesis has been applied to eliminate phenolic off-flavor (POF) phenotypes and, more recently, to modulate the production of other flavor-active compounds [32,33]. Chemical mutagenesis has also been used to induce chromosome copy number variation in lager yeast, resulting in mutants with increased ethanol tolerance [34]. A summary of key mutagenesis-based yeast improvements in winemaking and brewing is provided in Table 2.

Table 2. Summary of wine and beer yeast strain improvements through random mutagenesis.

Product	Objective	Work
Wine	Increased ethanol tolerance	[35]
	Enhanced nitrogen source utilization	[36]
	Increased mannoprotein release	[37]
	Reduced volatile acidity	[38]
	Increased aroma compound concentration (esters)	[39]
	Increased higher alcohols via leucine auxotrophic mutants	[40]
Beer	Elimination of phenolic off-flavors (POFs) in *S. eubayanus*	[32]
	Enhancement of ethanol tolerance in lager yeast	[34]
	Increase in maltotriose utilization	[41]

2.1.2. Sexual Breeding

Another conventional genetic improvement technique is sexual breeding, which leverages genetic diversity arising from meiosis and mating to develop new strains with a combination of beneficial alleles [20,23]. According to Giudici et al. [19], mating of genetically distinct parents either of the same species (outcrossing) or of different closely related species (hybridization) allows polygenic and/or QTL traits, such as ethanol tolerance, to be enhanced, as well as obtaining heterosis [42]. Figure 2 shows the spore-to-spore method, where spores from two parental strains are placed in proximity to facilitate mating. This technique provides high mating frequencies and generates individuals with improved genetic stability; however, it requires that the parental strains can produce viable spores, and desirable phenotypes may be lost through meiotic recombination [19]. To mitigate this last limitation, homozygous monosporic *MAT*a/*MAT*α cultures exhibiting the desired phenotype can be generated through haplo-selfing from homothallic yeasts and, subsequently, used as parental lineages, thereby fixing the desired phenotype in the resulting zygote [19,42].

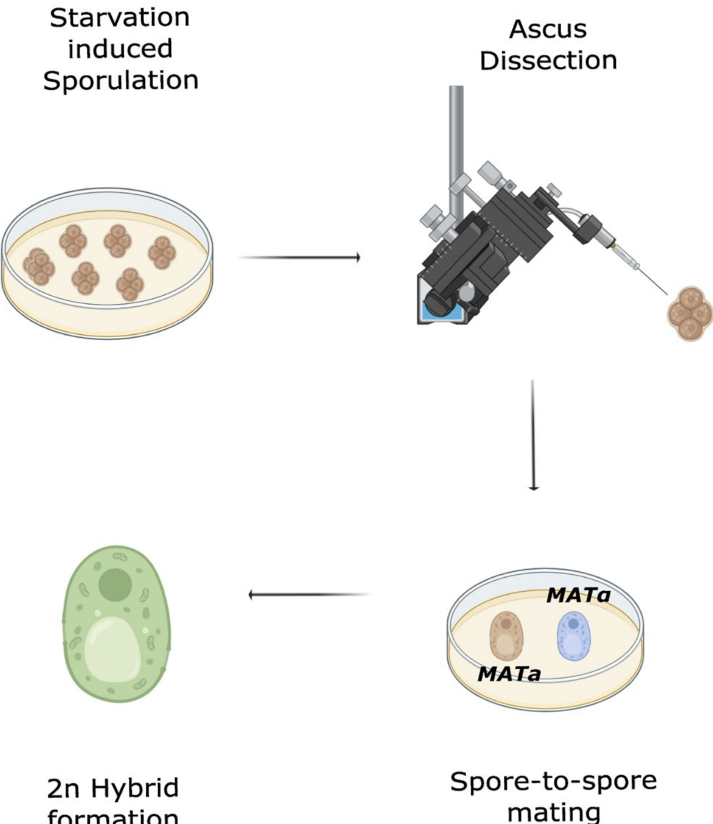

Figure 2. Schematic representation of spore-to-spore mating for yeast sexual breeding. Two meiosis-competent parental strains undergo sporulation under stress conditions, such as carbon and nitrogen starvation. The resulting asci, containing the haploid spores, are then disrupted to obtain monosporic cells. These haploid monosporic cells can be directly submitted to mating or, alternatively, left in sugar-rich medium to induce in homothallic strain germination and haplo-selfing, resulting in homozygous *MAT*a/*MAT*α monosporic clones. Haploid spores of either parental strains or their monosporic *MAT*a/*MAT*α derivatives are then placed in close proximity to induce mating. The fusion of spores with compatible mating types results in the formation of a diploid (2n) *MATa/MATα* zygote. This figure was created with BioRender (www.Biorender.com, accessed on 8 June 2025).

Mating is possible only between haploid cells with compatible mating type; however, commercial yeasts often sporulate poorly or do not produce viable haploid gametes. Given

the challenges associated with using spores, rare mating can also be employed, especially when spore viability is low or mating frequencies are insufficient. Rare mating relies on the occasional occurrence of an erroneous mating type switch, where some individuals in a diploid or polyploid cell population become either *MATa/MATa* or *MATα/MATα* homozygous and can mate with a haploid cell of the opposite mating type or even with a homozygous diploid cell of the opposite mating type [25].

A summary of key hybridization-based yeast improvements in winemaking and brewing is provided in Table 3.

Table 3. Schematic overview of yeast strain improvements through sexual breeding.

Product	Cross	Objectives	Work
Wine	*S. cerevisiae* × *S. cerevisiae*	Elimination of undesirable traits (e.g., SO_2 formation, excessive foam production)	[43]
	S. cerevisiae × *S. cerevisiae*	Increase in flocculation	[44]
	S. cerevisiae × *S. uvarum*	Increase optimal temperature range; modulation of by-products	[45–47]
Beer	*S. cerevisiae* × *S. eubayanus*	Improved fermentation and aroma production in lager hybrids	[12,48–50]
	S. mikatae × *S. eubayanus* and *S. jurei* × *S. eubayanus*	Improved fermentation and aroma production in lager hybrids	[50]
	S. cerevisiae × *S. mikatae*	Enhancement of beer flavor under cold temperatures	[51]

Interspecific hybridization has been successful in improving wine and beer yeasts. In winemaking, *S. cerevisiae* was mainly crossed with *S. uvarum* [45–47]. In brewing, *S. cerevisiae* was mainly crossed with *S. eubayanus* [48,49], even if other *Saccharomyces* species could serve as valuable alternatives [50,51]. The resulting de novo lager hybrids displayed enhanced sugar utilization and aroma compound production compared to parents [49]. However, these diploid interspecies hybrids are often sterile due to post-zygotic sterility barriers, limiting the selection process to the F1 generation and reducing the potential for further recombination [19,25].

*MAT*a/*MAT*a/*MAT*α/*MAT*α tetraploids, which arise through rare mating events, are fertile and capable of producing mating-competent *MAT*a/*MAT*a and *MAT*α/*MAT*α diploid gametes. These gametes can participate in a subsequent round of mating to generate F2 hybrids [52]. Alternatively, return-to-mitotic-growth (RTG) is a non-OGM approach that has been successfully employed to introduce genetic and phenotypic diversity in diploid, sterile interspecific hybrids. In this strategy, genetic recombination is promoted between parental subgenomes by inducing F1 hybrid cells to enter meiosis, followed by a shift to a nutrient-rich medium before meiosis completes. This interruption generates a recombinant population exhibiting novel phenotypes [53].

Genome shuffling is another approach that addresses the issue of sterility in diploid interspecific hybrids. It involves iterative rounds of protoplast fusion between different parental lineages using fusogenic agents such as polyethylene glycol. This strategy was used to improve yeast performance in high-gravity fermentation [54]. Unlike rare mating and RTG, genome shuffling generates improved strains which fall under the GMO classification according to the European Union [55].

2.2. Innovative Genetic Improvement Techniques in Fermentation

2.2.1. Adaptive Laboratory Evolution (ALE)

Among the innovative non-GMO techniques for strain improvement, adaptive laboratory evolution (ALE) exploits spontaneous mutations and selective advantages under specific environmental conditions. ALE was first applied by Dallinger in the late 19th century in an experiment aimed at adapting protozoa to high-temperature conditions [56]. Since then, ALE has been employed across a wide range of organisms, including *Escherichia coli*, viruses, and microalgae [56]. Yeasts are particularly well suited for ALE due to their short generation times—which can be further enhanced using mutagens, transposons, or mutator strains [57]—and their ease of cultivation under laboratory conditions [56].

Figure 3 shows a schematic representation of the ALE method, which enhances desirable yeast traits and reduces undesirable ones by subjecting a strain population to either a constant or an increasing selective pressure [11]. During ALE, natural selection is steered toward a defined environment, with the goal of improving the fitness traits of microbial populations under specific conditions, including growth rate, vitality, and substrate consumption. However, complex traits not directly linked to fitness require other genetic improvement strategies than ALE [19,25].

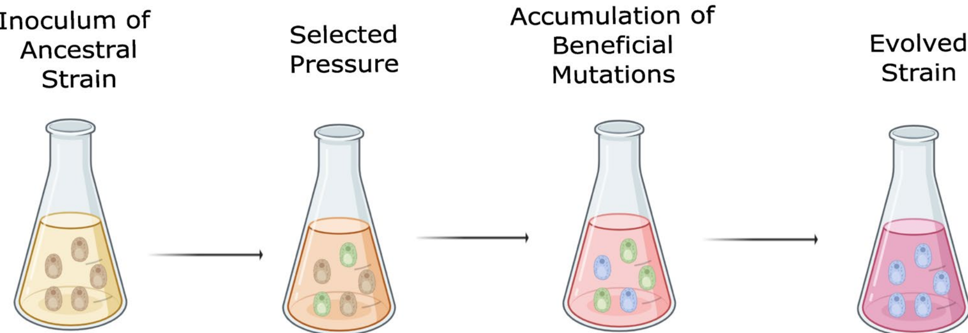

Figure 3. Graphical representation of adaptive laboratory evolution (ALE). The ancestral strain is exposed to an environment with either constant or progressively increasing selective pressure. This process promotes the selection of evolved populations exhibiting phenotypic traits that confer enhanced fitness under the applied selective pressure. After a certain number of generations, individual evolved clones can be isolated for the identification of beneficial mutations underlying the adaptive phenotype. This figure was created with BioRender (www.Biorender.com, accessed on 8 June 2025).

In brewing, ALE mimics the natural evolutionary process that led to the domestication of strains currently used in commercial breweries. Most domesticated beer strains exhibit traits such as ethanol tolerance and the lack of the POF phenotype, which emerged through long-term selection driven by humans [58,59]. Early brewers intuitively practiced a rudimentary form of strain selection by using a portion of a completed fermentation to inoculate the next batch—a process known as backslopping—resulting in faster and more consistent fermentation [60].

The genetic modifications that randomly occur during ALE typically include single nucleotide mutations (SNPs), frameshift mutations, insertions, or deletions, which may alter open reading frames, regulatory sequences, chromatin structure, protein folding, and gene expression [61]. Structural variation (SV) events, such as copy number variations (CNVs), also contribute to adaptive evolution by causing changes in gene dosages. For instance, increased copy numbers of genes involved in glycolysis, nutrient transporters, and stress resistance are commonly observed adaptations in *S. cerevisiae* [62]. Furthermore, continuous cultivation in chemostats has proven effective for improving traits such as maltotriose consumption in lager yeasts [63]. A summary of key yeast strain improvements achieved through ALE is provided in Table 4.

Table 4. Summary of yeast strain improvements through adaptive laboratory evolution (ALE).

Product	Objective	Ref.
Wine	Increased glycerol production for low-ethanol wines	[64,65]
	Development of yeast strains producing higher levels of glutathione (GSH)	[66]
	Increased ethanol tolerance	[67]
	Enhanced sulfite resistance in *B. bruxellensis*	[68]
Beer	Improved beer yeast performance via UV mutagenesis and high-gravity wort fermentations	[69]
	Enhanced ethanol tolerance in evolved de novo lager yeasts	[12]
	Genomic adaptations linked to chromosomal duplications and mutations in *IRA2* and *UTH1*	[70,71]
	Adaptation of *Saccharomyces* variants to overcome premature yeast flocculation (PYF)	[72]
	Increased flocculation to favor yeast removal	[73]
	Reduction in phenolic off-flavors via *PAD1* and *FDC1* mutations	[74]
	Improvement of fermentation efficiency	[75]

Despite its advantages, ALE is a time-consuming process that requires intensive monitoring and strict aseptic conditions to prevent contamination [57,61]. Recently, automation technologies have been introduced to enhance the efficiency and reproducibility of ALE experiments, addressing challenges associated with manual handling. For example, eVOLVER is a modular tool which optimizes multiple cultures management by enabling simultaneous execution of multiple independent experimental versions [76]. The VERT system employs fluorescent markers to track adaptive events in isogenic strain populations, allowing real-time monitoring of evolutionary dynamics [77]. In addition, AI models can analyze results from ALE experiments to guide further selection cycles and predict future evolutionary trajectories. One such tool, ALEsim, is a simulator platform which optimizes the design of ALE experiments in organisms such as *E. coli* [78].

In inverse metabolic engineering approaches, evolved clones are sequenced to identify adaptive mutations, and techniques such as backcrossing or QTL analysis can further support the identification of genetic bases underlying complex traits [57]. When implemented effectively, these strategies expand the genotype–phenotype correlation landscape, enabling the rapid generation of novel strains for diversification and innovation in the beverage industry.

2.2.2. Big Data, AI, and Omics

Accurate phenotype prediction from genotypes is a cornerstone of modern biology. Omics technologies, including genomics, proteomics, metabolomics, and transcriptomics, are revolutionizing our understanding of complex biological systems. The integration of diverse omics datasets offers unprecedented opportunities to reconstruct genome-scale metabolic networks with information on stoichiometry, compartmentalization, biomass composition, thermodynamics, and genes responsible for each reaction. Genomics and transcriptomics represent the foundation of omics research, focusing on genome sequencing and RNA transcript analysis, respectively. While genomics provides a comprehensive blueprint of an organism's genetic architecture, it does not inherently establish genotype-to-phenotype relationships. This underscores the necessity for complementary omics approaches [79].

Proteomics and metabolomics fill this gap by examining proteins and metabolites, which are directly linked to biological function and phenotypic traits. Metabolomics captures rapid responses to genetic and environmental changes, providing a snapshot

of the cell's metabolic and physiological state. However, the chemical complexity and diversity of proteins and metabolites make proteomic and metabolomic analyses more challenging than genomics and transcriptomics. These challenges highlight the importance of integrating datasets for a comprehensive understanding of biological systems [80].

Extracting actionable knowledge from large, high-dimensional omics datasets remains a significant challenge. A "knowledge-driven" approach analyzes omics datasets with constraints-based mathematical models. The resulting genome-scale models (GEMs) are computational representations of metabolic networks that enable in silico prediction of biological functions and facilitate precise manipulation of biological systems [80].

Currently, GEMs are publicly available for over 100 species (e.g., via www.optflux.org/models; accessed on 3 June 2024 and http://systemsbiology.ucsd.edu/InSilicoOrganisms/OtherOrganisms; accessed on 4 June 2025). As the first eukaryote to have its genome sequenced, *S. cerevisiae* has been extensively modeled. Yeast-GEMs define the gene–protein–reaction associations for all known genes and are continually improved by the research community [81].

In parallel, machine learning offers a "data-driven" statistical approach to phenotype prediction. As a sub-category of AI, machine learning algorithms learn statistical relationships between input features (e.g., protein sequence) and output response (e.g., function or structure), without requiring mechanistic insights into the biological process. Even if no biological information is encoded in the algorithm, careful curation of input biological data is essential to enable accurate learning of relevant biological relationships [82].

In this regard, the expanding availability of yeast omics datasets enables in silico screening for desired traits. The 1011 yeast genomes project [27] and its derivatives, which include genomic, transcriptomic, and proteomic data, provide a valuable resource to identify candidate genes responsible for desirable phenotypes and to predict strains with the desired phenotype [58]. This approach, known as a genome-wide association study (GWAS), evaluates the correlation between each genotypic marker and phenotypic traits across a population [83]. GWASs successfully predicted brewing aptitude in *S. cerevisiae* strains not associated with beer fermentation, based on genotype–phenotype associations [84]. Compared to in vitro experiments, this in silico approach offers faster results and reduced costs.

The application of multi-omics approaches in industrial biotechnology is exemplified by their role in optimizing wine and beer fermentation processes. For instance, the *MAL* loci are positively correlated with maltose consumption [58,84,85], while the *STA1* gene is associated with maltotriose and dextrin utilization [86]. Genes such as *IRA2* and *INA2* are implicated in ethanol tolerance [87,88]. In addition to GWASs, QTL mapping has been used to identify genes, including *LEU1*, *BAT1*, *EHT1*, and *IRA1*, involved in flavor modulation, as well as key loci involved in regulatory mechanisms of nitrogen consumption [83]. In some cases, candidate genes underlying industrial phenotypes have then been validated through CRISPR-Cas9-based overexpression and knockout experiments [15].

Omics research into non-conventional yeasts such as *Pichia kluyveri* and *Hanseniaspora* has uncovered unique aroma production pathways and gene regulation mechanisms, expanding the toolkits for engineering these specialized fermentative strains [89,90].

Recently, GEMs, high-throughput biosensors, and machine learning have been combined to effectively direct metabolic engineering efforts and identify mutant strains capable of producing high titers of tryptophan [91]. In another study, fluxomic predictions generated by GEM simulations were integrated with experimental transcriptomic data to accurately predict growth phenotypes across multiple *S. cerevisiae* strains [92]. Machine learning and probabilistic modeling techniques have been employed to identify bioengineering strategies for developing yeast strains tailored to produce hoppy beer aromas [93].

Other studies used AI to accelerate synthetic biology. In a pivotal work, Nguyen et al. (2020) developed Evo, a type of AI that treats DNA much like a language and is capable of both predictive modeling and novel sequence generation across scales, from single molecules to full genomes [94]. Evo successfully generated EvoCas9–1, a Cas9 variant with an increased double-strand break capability. More recently, Bunne et al. (2024) proposed the concept of an AI virtual cell (AIVC), which integrates multi-omics with AI to create a multi-scale, multi-modal, large-neural-network-based model that can represent and simulate the behavior of cells across diverse states [95].

Despite these advances, integrating multi-omics data with AI remains challenging due to cell complexity. Cells operate on multiple scales across space and time; diverse processes have massive numbers of interacting components, and in each process a multitude of biomolecules are present in diverse configurations and states; in addition, most cellular processes are nonlinear, such that small changes in inputs can lead to complex changes in outputs.

2.2.3. Synthetic Microbial Communities

Humans have long utilized natural microbial consortia to produce fermented products, often without fully understanding the microbial roles. Advances in metagenomics have shown that genotypically diverse microbial consortia are more resilient and can outperform isogenic cultures. Additionally, polymicrobial consortia can exhibit novel functionalities, which are absent in single-strain culture isolated from the consortium [96]. This knowledge has driven the development of artificial binary co-cultures and, more recently, of synthetic consortia composed of multiple cultivable strains designed for a specific bioproduction task [97]. These systems offer several advantages, including reduced metabolic burdens, prevention of toxic by-product accumulation, and improved thermodynamic efficiency, leading to higher yields and greater resilience. Konstantinidis et al. (2021) described a coevolution experiment using ALE in an obligate mutualistic microbial community, composed by *Lactiplantibacillus plantarum*, which requires amino acids from *S. cerevisiae*, and an auxotrophic *S. cerevisiae* strain dependent on vitamin B produced by *L. plantarum* [98]. Under selective pressures, the *L. plantarum* strain enhanced the production of B vitamins, supported by its yeast partner.

In natural microbial consortia, cells need to engage in synergistic and antagonistic ecological interactions. Synthetic microbial consortia have been used as laboratory models to study these dynamics during wine and beer fermentation, focusing on factors such as inoculum ratios, inoculation timing, and metabolite inhibition [99,100]. Tools such as Automated Ribosomal Intergenic Spacer Analysis (ARISA) and flow cytometry have been employed to monitor population dynamics [99,100]. Although limitations persist, e.g., inability to distinguish between live and dead cells, Bagheri et al. (2017) have used ARISA to show that *S. cerevisiae* can suppress certain non-*Saccharomyces* species while promoting others during wine fermentation [99].

Table 5 shows that studies have explored how microbial consortia can enhance the sensorial profile of wine and beer. Synthetic consortia in brewing are an emerging concept: current implementation mostly uses co-cultures of different yeast strains to achieve cooperative flavor production. Co-culture of *Lactiplantibacillus plantarum* or *L. brevis* with *S. cerevisiae* has been proposed to better control souring during sour beer fermentation [101]. Mixed cultures of *Saccharomyces* and non-*Saccharomyces* yeasts with complementary metabolisms have been proposed to improve flavor complexity, reduce ethanol, or modulate acidity. For instance, combining *S. cerevisiae* with *Torulaspora delbrueckii*, *Lachancea thermotolerans*, or *H. uvarum* can increase ester content, glycerol production, or reduce volatile acidity [102,103].

A recent study used a synthetic consortium of non-*Saccharomyces* yeasts strains to produce low-alcohol beers without physical dealcoholization [104]. Microbial consortia have also been applied in brewery wastewater treatment, improving environmental sustainability [105].

Despite these benefits, maintaining population during fermentation and ensuring reproducibility and process control at industrial scale remain key challenges which hamper the application of synthetic consortia during beer and wine fermentation.

Table 5. Summary of microbial interactions in beer and wine fermentation and bioprocesses.

Product	Application	Involved Species	Work
Wine	Malolactic fermentation for flavor complexity in wine	*Oenococcus oeni*	[106]
	Adaptation to vineyard microbial terroir	Various LAB and yeast species	[107]
	Optimization of yeast interactions for improved fermentation	*K. apiculate*, *C. stellata*, *C. pulcherrima*	[108]
	Influence of nutrient scarcity, oxygen availability, and ethanol on fermentation	*Saccharomyces cerevisiae* and other yeasts	[109]
	Ecological interactions driving fermentation outcomes	Multiple yeast species	[110]
	Persistence of certain non-Saccharomyces yeasts in vineyard ecosystems	*Starmerella bacillaris*, *Lachancea thermotolerans*	[111]
	Yeast ecosystem modulation by *S. cerevisiae*	Various non-*Saccharomyces* species	[99]
Beer	Bio-acidification and microbial control in beer	Lactic acid bacteria (LAB)	[112]
Beer	Reduction in final ethanol content	*Saccharomyces cerevisiae*, *Lentilactobacillus brevis*	[113]

3. GMO-Based Genetic Improvement Techniques

3.1. Synthetic Biology and CRISPR/Cas9

In recent years, synthetic biology has emerged as a key interdisciplinary field combining biological research with engineering principles to design biological circuits and elucidate system-level functions. This approach enables the creation of new biomolecular components, pathways, and regulatory networks to reprogram organisms into engineered cellular factories [114]. In the food industry, synthetic biology offers solutions to challenges in food safety, nutrition, and sustainability [114].

Efforts to improve the genetic makeup of wine yeast strains began in the 1990s with the advent of genetic engineering [8] and have accelerated with the introduction of CRISPR/Cas-based genome editing [115] (Figure 4). These tools enable precise, marker-free genetic modifications, enhancing process efficiency and substrate utilization [8]. Unlike traditional methods such as mutagenesis and sexual breeding, genetic engineering enables targeted improvements and reduces the risk of off-target effects. Advanced multiplex genome editing strategies, including delta and rDNA cluster integration using CRISPR-Cas tools, support multi-copy gene insertions, significantly facilitating strain development [113].

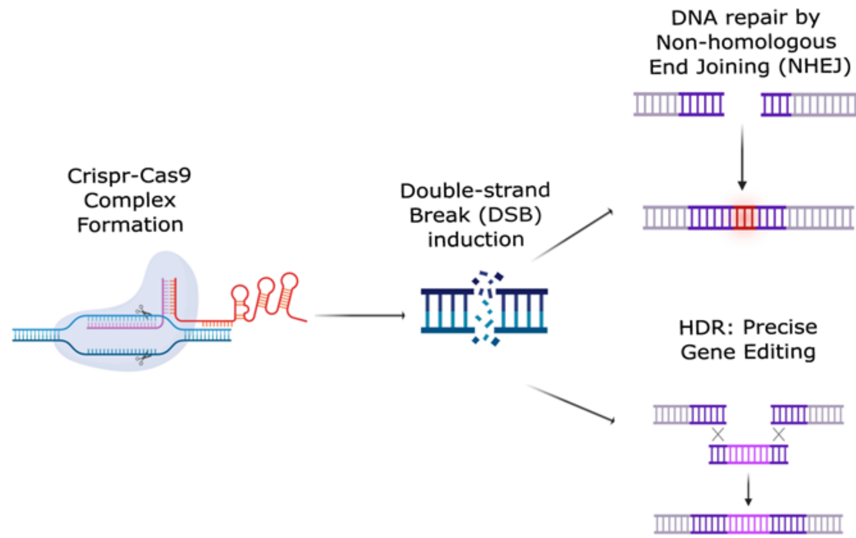

Figure 4. Schematic representation of the CRISPR-Cas9 genome editing mechanism. The process begins with the design of a guide RNA (gRNA) complementary to the target DNA sequence. The gRNA binds to the Cas9 protein to form the active CRISPR-Cas9 complex. This complex recognizes and binds to the target DNA site, where Cas9 induces a double-strand break (DSB). The resulting DSB is then repaired by one of two cellular pathways: non-homologous end joining (NHEJ), which may introduce mutations and result in gene knockout, or homology-directed repair (HDR), which enables precise gene editing when a homologous repair template is supplied.

However, CRISPR/Cas9 efficiency can decline with increased numbers of sgRNA due to competition for Cas proteins. To overcome this, auxiliary elements like ribozymes, tRNA, and Csy4 have been used to optimize sgRNA levels [116]. High-copy gene integrations may also impose metabolic burdens, which can be mitigated by synthetic repetitive sequences or DNA "landing pad" such as the "wicket" cassette, to fine-tune gene expression levels [113].

For example, Di-CRISPR, combining CRISPR/Cas9 with delta integration, facilitates large gene cassette insertions by leveraging delta sequences [113]. This method was successfully used to integrate a 2,3-butanediol biosynthesis pathway into the *S. cerevisiae* genome [117]. Additionally, inserting genes near autonomously replicating sequences has been shown to improve both integration efficiency and gene expression [113].

Further research is needed to optimize gene expression systems, reduce off-target effects, and improve scaling-up for industrial applications. A deeper understanding of CRISPR components in vivo will be essential for enabling more predictable, efficient, and sustainable large-scale engineering [118].

Table 6 provides an overview of the major genetic modifications in wine and beer yeast biotechnology. Wine and beer GM yeasts are commercially available in North America. In contrast, their adoption in Europe has been limited, primarily due to regulatory and market constraints. To date, two GM *S. cerevisiae* wine strains have been commercialized, one engineered to convert malic acid into lactic acid and another to reduce urea release during arginine metabolism.

In brewing, CRISPR-Cas9 technology has been used to switch off the *FDC1* gene responsible for 4-vinul guaiacol off-flavor production in *S. eubayanus* [119], as well as to increase ester production by esterase encoding gene deletion [120]. Krogerus et al. (2021) have also applied CRISPR/Cas to induce double-strand breaks in *MAT* locus, facilitating the construction of yeast hybrid strains [121]. Denby et al. (2018) have developed a Cas9-mediated methodology for stable and marker-less integration of a monoterpene synthesis pathway in *S. cerevisiae* [122]. The resulting engineered strain displayed a "hoppy" phenotype, potentially reducing the reliance on hops in brewing. More recently, CRISPR/Cas9

technology has been employed to introduce the biosynthetic pathway for xanthohumol, a flavor-active compound naturally found in hops, enabling its production from glucose in *S. cerevisiae* [123].

Currently, three GM brewing yeasts are commercially available: *S. cerevisiae* YH178 and YH179 (developed by Lallemand in collaboration with Berkeley Yeast), engineered to produce thiol-releasing enzymes (tropical hop aromas), and *S. cerevisiae* YH72 (Berkeley Yeast), which was modified to downregulate acetolactate decarboxylation, thereby reducing diacetyl production (buttery off-flavor).

Table 6. Main genetic modifications in wine and beer yeasts obtained through synthetic biology tools.

Product	Process	Technique	Ref.
Wine	*GPD1* overexpression and *ALD6* deletion to reduce alcohol yield in wine yeast	Episomal vector; *KanMX* deletion cassette	[124]
	Expression of extracellular hydrolytic enzymes to improve juice extraction and release primary aromas	Episomal vector constructed by restriction cloning	[125]
	Reduction in urea and ethyl carbamate formation	CRISPR/Cas9	[126]
	Expression of malolactic enzymes to degrade malate and integrate malolactic fermentation	Episomal vector constructed by restriction cloning	[127]
Beer/Wine	Heterologous expression of pediocin to increase resistance to wild yeasts and bacteria	Episomal vector constructed by restriction cloning	[128]
Beer	Engineering yeast strains to produce methyl anthranilate with grape aroma	CRISPR/Cas9	[129]
	Expression of acetolactate decarboxylase (*ALDC*) to reduce diacetyl formation	Episomal vector constructed by restriction cloning	[130]
	Engineering yeast strains to produce hop monoterpenes	Plasmids obtained by Golden Gate assembly, CRISPR/Cas	[122]

3.2. Ethical and Commercial Challenges

GM foods, a key area of debate within biotechnology, have garnered significant attention due to concerns regarding safety, ethics, and environmental impacts.

Proponents of GM technology argue that the benefits of GM far outweigh the risks [131]. Opponents, however, contend that genetic modifications could lead to undesirable off-target or on-target effects which can affect the nutritional quality of food, as well as introducing health risks such as antibiotic resistance and allergenicity [131].

Regulations on GMOs differ from country to country. In the European Union, there are strict rules which consider organisms modified through either gene editing or genetic engineering as GM, even if they are free of any transgene [55]. The EU's approach focuses on the process itself, classifying something as a GMO simply because it has been genetically modified, without considering the final product [132]. Based on this assumption, products classified as GMO need to seek extensive regulatory and safety assessments before their approval for commercial use by the European Food Safety Authority (EFSA). This precautionary approach may have serious implications on research, development, and commercialization of CRISPR technology in Europe.

In the USA, the use of GM yeast in beverage production (including beer, wine, cider, and spirits) is regulated primarily by Food and Drug Administration (FDA) which applies to GM yeast the general food safety rules. There is no requirement for mandatory labeling of products made with GM yeast unless the modification results in a significant change in significant properties such as allergenicity and nutritional content [133]. After the demonstration of safety (performed as a voluntary consultation by the companies), GM yeasts can be commercialized. Consequently, companies like Berkeley Yeast and Omega Yeast have developed engineered yeasts that produce fruity esters or eliminate off-flavors, currently widely diffused in U.S. craft brewers and wineries.

Canada uses a "novelty-based" regulatory framework, not strictly a "GMO" framework. Beverages obtained with GM yeasts are labeled as "novel food" only if genetic modifications confer a significant novel trait [134]. Similarly to the USA, Canadian regulation does not require mandatory labeling of products made with GM yeast. In Latin America, several countries such as Brazil and Argentina have a permissive regulation of GMO in food, like the USA and Canada.

Based on these differences, there are several proposals to update GMO regulations in the European Union. One key suggestion is to shift the focus from regulating the process (how genetic modifications are made) to regulating the final product (the traits or phenotypes resulting from those modifications), with a more flexible and scalable regulation that matches the level of genomic alteration. There is also a growing recognition of the need for international cooperation and harmonization of regulations [132].

4. Conclusions

Today, yeast genetics is entering a "3.0 era", marked by the integration of conventional and innovative techniques to design tailored products, increase production efficiency, and reduce environmental impact, without losing the cultural and artisanal value of winemaking and brewing tradition.

The emergence of big data analytics and AI is accelerating yeast improvement by enabling a more accurate prediction of gene–function relationships, regulatory networks, and metabolic outcomes. Multi-omics integration is paving the way for a system-level understanding of yeast biology, while AI models trained on large-scale genotypic and phenotypic datasets can forecast the effects of specific genetic modifications and guide rational strain design. These innovations are expected to shorten development timelines and reduce costs for new beer and wine products, with positive impacts on environmental sustainability of fermentation process.

Advances in synthetic biology and engineered microbial consortia further expand possibilities, allowing the precise reprogramming of microbial communities, beyond traditional single-strain paradigms. However, genetic modification should not be considered a universal solution. Its full potential is realized when used in synergy with ALE and other traditional approaches such as breeding and random mutagenesis in improving complex, multigenic traits and quantitative trait loci (QTLs) relevant to brewing. Nevertheless, the time required to enhance brewing yeast using such methods is typically much longer compared to the CRISPR/Cas approach.

Ethical and safety concerns related to GMOs must also be carefully addressed. Public acceptance remains a major barrier, often hindered by misinformation and negative perceptions. Transparent, evidence-based communication is critical to build trust and empower both consumers and professionals to make informed decisions based on scientific data.

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